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SUPERINTENDENCIA Y COMERCIO
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Señora Jefe de la
DIVISION DE NUEVAS CREACIONES
SUPERINTENDENCIA DE INDUSTRIA Y COMEF
E. S. D.

Referencia : Expediente No. 00.093.237

Solicitante : **PHARMACIA CORPORATION.**

Asunto : Solicitud de patente para:
"FORMA CRISTALINA DE EPLERENONA"

Fecha de solicitud : 6 de diciembre de 2000

TRAMITE 002 EVENTO 001 ACTUACION 329

1. Yo, Emilio FERRERO, obrando como apoderado de la solicitante en el asunto de la referencia, atentamente, presento con este escrito las copias certificadas de las solicitudes de patente estadounidenses No. 60/169.556, 60/169.608, 60/169.639, 60/169.683, 60/169.707 y 60/169.807 del 8 de diciembre de 1999, junto con las respectivas traducciones de sus carátulas y capítulos reivindicatorios de los cuales se reclama prioridad en esta solicitud de patente.

2. Ruego a la Señora Jefe se sirva aceptar este escrito con sus anexos, y se continúe con el trámite de la solicitud.

Bogotá,
Señora Jefe,

EMILIO FERRERO
T.P.A. No. 31.929

COLO 12482-801-001-5
JJC-ID-12

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PTO 348031

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

January 05, 2001

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE UNDER 35 USC 111.

APPLICATION NUMBER: ***60/169,556***
FILING DATE: ***December 08, 1999***

By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

H. Phillips

H. PHILLIPS
Certifying Officer

12/09/99
JC#12 U.S. PTO

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A/PROV 27 & 23

60/169556
1-811 U.S. PTO
12/08/99

Express Mail Label Number EL523767085US
Attorney Docket Number SRL 6173 (C-3256)

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

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[x] Additional inventors are being named on the 3rd & 4th separately numbered sheet(s) attached hereto.

Title of the Invention: COMBINATIONS OF NANOPARTICLES COMPRISING SOLID STATE FORMS OF EPLERENONE

Correspondence Address: Customer Number 000321

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The application includes:

[x] Specification - Number of Pages 102

[x] Drawings - Number of Sheets 92

[ ] Small Entity Statement

[x] Return Receipt Postcard

[ ] Other _____

Method of Payment of Filing Fee:

[X] A check in the amount of $ 150.00 is enclosed to cover the Provisional Filing Fee.

[ ] The Commissioner is hereby authorized to charge filing fees and credit Deposit Account No. 19-1345.

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

[X] No
[ ] Yes, the name of the U.S. Government agency and the Government contract number are: _____

Respectfully submitted,

Scott A. Williams, Reg. No. 39,876

Date: 12/8/99

SAWskg

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FEE TRANSMITTAL

Application Number TBA
Filing Date December 8, 1999
First Named Inventor Subash Desai
Group Art Unit TBA
Examiner Name TBA
Attorney Docket Number SRL 6173 (C-3256)

METHOD OF PAYMENT

1. [ ] The Commissioner is hereby authorized to charge the indicated fees to Deposit Account No. 19-1345, in the name of Senniger, Powers, Leavitt & Roedel.

   [ ] The Commissioner is hereby authorized to charge any additional filing and claim fees under 37 CFR 1.16 and application processing fees under 37 CFR 1.17 to Deposit Account No. 19-1345, in the name of Senniger, Powers, Leavitt & Roedel.

2. [x] Check Enclosed. The Commissioner is hereby authorized to charge any under payment or credit any over payment to Deposit Account No. 19-1345, in the name of Senniger, Powers, Leavitt & Roedel.

FEE CALCULATION

1. [x] BASIC FILING FEE $150.00 (Type: Provisional)
   Entity Status: large

2. [ ] CLAIM FEE $_____

   Total Claims _____
   Independent Claims _____
   Multiple Dependent Claims _____

3. [ ] ADDITIONAL FEES $_____

   [ ] Surcharge - late filing fee or oath
   [ ] Surcharge - late provisional filing fee or cover sheet
   [ ] Extension for reply within _____ month
   [ ] Notice of Appeal
   [ ] Filing a Brief in Support of an appeal
   [ ] Request for Reexamination
   [ ] Petitions to the Commissioner
   [ ] Submission of Information Disclosure Statement
   [ ] Recording each patent assignment per property
   [ ] Other: _____

TOTAL AMOUNT OF PAYMENT $ 150.00

Scott A. Williams, Reg. No. 39,876 — 12/8/99 Date

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COMBINATIONS OF NANOPARTICLES COMPRISING SOLID STATE FORMS OF EPLERENONE

Field of the Invention

This invention is in the field of aldosterone receptor antagonist pharmaceutical agents and specifically relates to combinations of nanoparticles comprising crystalline and amorphous solid state forms of eplerenone, methods of preparing such combinations of eplerenone nanoparticles, pharmaceutical compositions comprising such combinations of eplerenone nanoparticles, and methods for the treatment and/or prophylaxis of aldosterone-mediated conditions and/or disorders, including conditions and disorders associated with hyperaldosteronism such as hypertension.

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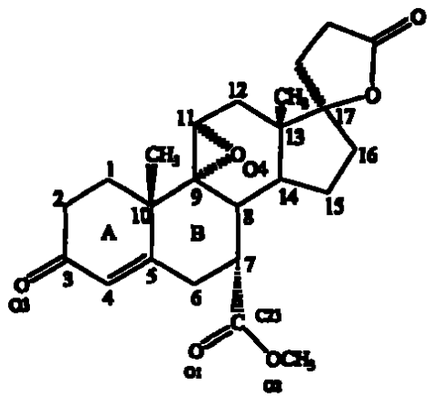
Background of the Invention

The compound methyl hydrogen 9,11-epoxy-17-hydroxy-3-oxopregn-4-ene-7,21-dicarboxylate, γ-lactone 1 (also referred to herein as "eplerenone") was first reported in Grob et al., U.S. Patent 4,559,332 which discloses a class of 9,11-epoxy steroid compounds and their salts. Eplerenone is an aldosterone receptor antagonist that can be administered in a therapeutically effective amount where use of an aldosterone receptor antagonist is indicated, such as in the treatment of pathological conditions associated with hyperaldosteronism including hypertension, heart failure including cardiac insufficiency, and cirrhosis of the liver.



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U.S. Patent 4,559,332 generally discloses the preparation of eplerenone and the preparation of pharmaceutical formulations comprising eplerenone.

Additional processes for the preparation of 9,11-epoxy steroid compounds and their salts, including eplerenone, are disclosed in Ng et al., WO97/21720 and Ng et al., WO98/25948.

Grob et al., "Steroidal Aldosterone Antagonists: Increased Selectivity of 9α,11-Epoxy Derivatives", Helvetica Chimica Acta, Vol. 80, pp. 566-585 (1997), discloses an X-ray crystal structure analysis of a solvate of eplerenone prepared by crystallizing eplerenone from a methylene chloride/diethyl ether solvent system.

De Gasparo et al., "Antialdosterones: Incidence and Prevention of Sexual Side Effects", J. Steroid Biochem., Vol. 32, No. 13, pp. 223-227 (1989), discloses the use of non-formulated eplerenone having a 20 micron particle size in a single dose study of eplerenone.

Spironolactone 2, a 20-spiroxane-steroid having activity as an aldosterone receptor antagonist, is commercially available for the treatment of hypertension. Spironolactone, however, has antiandrogenic activity that can result in gynecomastia and impotence in men. It also has weak progestational activity that can produce menstrual irregularities in women. Accordingly, there is interest in the development of additional active aldosterone receptor antagonists such as eplerenone that do not interact with other steroid receptor systems such as glucocorticoid, progestin and androgen steroid receptor systems and/or that provide for a broader range of treatment.

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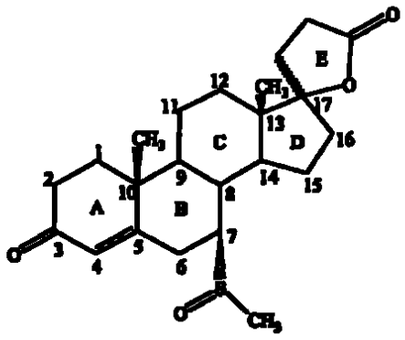

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Agafonov et al., "Polymorphism of Spironolactone", J. Pharm. Sciences, Vol. 80, No. 2, pp 181-185 (1991), discloses an acetonitrile solvate, an ethanol solvate, an ethyl acetate solvate, a methanol solvate and two non-solvated polymorphic crystalline forms of spironolactone. Brittan, Polymorphism in Pharmaceutical Solids, pp. 114-116, 207, 235, and 261 (Marcel Dekker, Inc. 1999), likewise discloses these solid state forms of spironolactone.

Summary of the Invention

A first aspect of the invention comprises novel combinations comprising particles of a first solid state form of eplerenone and particles of a second solid state form of eplerenone wherein said solid state forms of eplerenone are selected from the Form I crystalline form of eplerenone; the Form II crystalline form of eplerenone; a crystalline form of eplerenone comprising a solvent; and amorphous eplerenone; and wherein at least 90% of the particles have a particle size less than about 15 microns. These eplerenone particles possess activity as aldosterone receptor antagonists and have unique properties relative to each other.

A second aspect of the invention comprises comprises pharmaceutical compositions comprising particles of a first solid state form of eplerenone and particles of a second solid state form of eplerenone wherein at least 90% of the particles have a particle size less than about 15 microns.

A third aspect of the invention comprises a method for the prophylaxis and/or treatment of an aldosterone-mediated condition and/or disorder comprising administering to a subject a therapeutically-effective amount of particles of a first solid state form of eplerenone and particles of a second solid state form of eplerenone, either alone or in combination with other active ingredients, wherein at least 90% of the particles have a particle size less than about 15 microns.

A fourth aspect of the invention comprises novel particles comprising the amorphous form of eplerenone wherein at least 90% of the particles have a particle size less than about 15 microns.

A fifth aspect of the invention comprises methods for the preparation of novel particles comprising the amorphous form of eplerenone wherein at least 90% of the particles have a particle size less than about 15 microns.

Additional aspects of the invention are discussed throughout the specification of this application.

Brief Description of the Drawings

Fig. 1-A shows X-ray powder diffraction patterns of Form I eplerenone.

Fig. 1-B shows X-ray powder diffraction patterns of Form II eplerenone.

Fig. 1-C shows X-ray powder diffraction patterns of the methyl ethyl ketone solvate of eplerenone.

Figs. 1-D through 1-O show X-ray powder diffraction patterns for the following eplerenone solvates: n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, acetone solvate, toluene solvate, isopropanol solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, methyl acetate solvate, and propyl acetate solvate, respectively.

Fig. 2-A shows a differential scanning calorimetry (DSC) thermogram of non-milled Form II directly crystallized from methyl ethyl ketone.

Fig. 2-B shows a differential scanning calorimetry (DSC) thermogram of non-milled Form II prepared by desolvation of a solvate obtained by crystallization of a high purity eplerenone from methyl ethyl ketone.

Fig. 2-C shows a differential scanning calorimetry (DSC) thermogram of Form II prepared by crystallizing a solvate from a solution of high purity eplerenone in methyl ethyl ketone, desolvating the solvate to yield Form II, and milling the resulting Form II.

Fig. 2-D shows a differential scanning calorimetry (DSC) thermogram of non-milled Form I prepared by desolvation of a solvate obtained by digestion of low purity eplerenone from appropriate solvents.

Figs. 2-E through 2-T show DSC thermograms for the following eplerenone solvates: n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, chloroform solvate, acetone solvate, toluene solvate, isopropanol solvate, t-butyl acetate solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate

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solvate, methyl acetate solvate, propyl acetate solvate, n-butanol solvate, and n-octanol solvate, respectively.

Fig. 3-A shows the infrared spectra (diffuse reflectance, DRIFTS) of Form I eplerenone.

Fig. 3-B shows the infrared spectra (diffuse reflectance, DRIFTS) of Form II eplerenone.

Fig. 3-C shows the infrared spectra (diffuse reflectance, DRIFTS) of the methyl ethyl ketone solvate of eplerenone.

Fig. 3-D shows the infrared spectra (diffuse reflectance, DRIFTS) of epleranone in chloroform solution.

Figs. 3-E through 3-Q show infrared absorption spectra for the following eplerenone solvates: n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, acetone solvate, toluene solvate, isopropanol solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, propyl acetate solvate, methyl acetate solvate, propylene glycol solvate, and t-butyl acetate solvate respectively.

Fig. 4 shows ^{13}C NMR spectra for Form I of eplerenone.

Fig. 5 shows ^{13}C NMR spectra for Form II of eplerenone.

Figs. 6-A through 6-R show thermogravimetry analysis profiles for the following eplerenone solvates: methyl ethyl ketone solvate, n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, chloroform solvate, acetone solvate, toluene solvate, isopropanol solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, methyl acetate solvate, propyl acetate solvate, propylene glycol solvate, n-butanol solvate, n-octanol solvate, and t-butyl acetate solvate, respectively.

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Fig. 7 is a scanning electron micrograph of Form II eplerenone prepared by desolvation of the methyl ethyl ketone solvate.

Fig. 8 is a scanning electron micrograph of Form II eplerenone prepared by direct crystallization from ethyl acetate.

Fig. 9 shows an X-ray powder diffraction pattern of a crystalline form of 7-methyl hydrogen 4α,5α:9α,11α-diepoxy-17-hydroxy-3-oxo-17α-pregnane-7α,21-dicarboxylate, γ-lactone isolated from methyl ethyl ketone.

Fig. 10 shows an X-ray powder diffraction pattern of the crystalline form of 7-methyl hydrogen 11α,12α-epoxy-17-hydroxy-3-oxo-17α-pregn-4-ene-7α,21-dicarboxylate, γ-lactone isolated from isopropanol.

Fig. 11 shows an X-ray powder diffraction pattern of the crystalline form of 7-methyl hydrogen 17-hydroxy-3-oxo-17α-pregna-4,9(11)-diene-7α,21-dicarboxylate, γ-lactone isolated from n-butanol.

Fig. 12 illustrates the Gibbs free energy-temperature relationship for enantiotropically related polymorphs.

Fig. 13 shows the X-ray powder diffraction patterns for the wet cake (methyl ethyl ketone solvate) obtained from (a) 0%, (b) 1%, (c) 3%, and (d) 5% diepoxide-doped methyl ethyl ketone crystallizations.

Fig. 14 shows the X-ray powder diffraction patterns for the dried solids obtained from (a) 0%, (b) 1%, (c) 3%, and (d) 5% diepoxide-doped methyl ethyl ketone crystallizations.

Fig. 15 shows the X-ray powder diffraction patterns for the dried solids from the methyl ethyl ketone crystallization with 3% doping of diepoxide (a) without grinding of the

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solvate prior to drying, and (b) with grinding of the solvate prior to drying.

Fig. 16 shows the X-ray powder diffraction patterns for the wet cake (methyl ethyl ketone solvate) obtained from (a) 0%, (b) 1%, (c) 5%, and (d) 10% 11,12-epoxide-doped methyl ethyl ketone crystallizations.

Fig. 17 shows the X-ray powder diffraction patterns for the dried solids obtained from (a) 0%, (b) 1%, (c) 5%, and (d) 10% 11,12-epoxide-doped methyl ethyl ketone crystallizations.

Fig. 18 shows a cube plot of product purity, starting material purity, cooling rate and endpoint temperature based on the data reported in Table X-7A.

Fig. 19 shows a half normal plot prepared using the cube plot of Fig. 18 to determine those variables having a statistically significant effect on the purity of the final material.

Fig. 20 is an interaction graph based on the results reported in Table X-7A showing the interaction between starting material purity and cooling rate on final material purity.

Fig. 21 shows a cube plot of Form I weight fraction, starting material purity, cooling rate and endpoint temperature based on the data reported in Table X-7A.

Fig. 22 shows a half normal plot prepared using the cube plot of Fig. 21 to determine those variables having a statistically significant effect on the purity of the final material.

Fig. 23 is an interaction graph based on the results reported in Table X-7A showing the interaction between

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starting material purity and endpoint temperature on final material purity.

Fig. 24 shows an X-ray diffraction pattern of amorphous eplerenone.

Fig. 25 shows a DSC thermogram of amorphous eplerenone.

Fig. 26 shows the dissolution rates measured for four polymorph samples.

Detailed Description of the Preferred Embodiments

As with all pharmaceutical compounds and compositions, the chemical and physical properties of eplerenone are important in its commercial development. These properties include, but are not limited to: (1) packing properties such as molar volume, density and hygroscopicity, (2) thermodynamic properties such as melting temperature, vapor pressure and solubility, (3) kinetic properties such as dissolution rate and stability (including stability at ambient conditions, especially to moisture and under storage conditions), (4) surface properties such as surface area, wettability, interfacial tension and shape, (5) mechanical properties such as hardness, tensile strength, compactibility, handling, flow and blend; and (6) filtration properties. These properties can affect, for example, the processing and storage of pharmaceutical compositions comprising eplerenone. Solid state forms of eplerenone that provide an improvement in one or more of these properties relative to other solid state forms of eplerenone are desirable.

The present invention comprises such novel solid state forms of eplerenone. Specifically, these novel solid state forms include solvated crystalline forms, non-solvated and

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non-hydrated crystalline forms (designated "Form I" and "Form II"), and an amorphous form of eplerenone. Each solid state form of eplerenone described in the present application possesses one or more of the above-described advantageous chemical and/or physical properties relative to the other solid state forms described herein or otherwise disclosed in the literature.

In one embodiment of the invention, the solid state form comprises Form I eplerenone. Form I exhibits a faster dissolution rate (approximately 30% higher) at lower temperatures (i.e., temperatures below the enantiotropic transition temperature as later discussed) than, for example, Form II. Where dissolution of eplerenone in the gastrointestinal tract is the rate-controlling step for delivery of the eplerenone to the target cells, faster dissolution generally results in improved bioavailability. Form I, therefore, can provide an improved bioavailability profile relative to Form II. In addition, selection of a solid state form of eplerenone having a faster dissolution rate likewise provides greater flexibility in the selection of excipients for, and in the formulation of, immediate release pharmaceutical compositions relative to other solid state forms having a slower dissolution rate.

In another embodiment of the invention, the solid state form comprises Form II eplerenone. Form II possesses greater physical stability at lower temperatures (i.e., at temperatures below the enantiotropic transition temperature as later discussed) than, for example, Form I. Solid state forms of eplerenone such as Form II that do not require the use of special processing or storage conditions, and that avoid the need for frequent inventory replacement, are

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desirable. For example, selection of a solid state form of eplerenone that is physically stable during the manufacturing process (such as during milling of eplerenone to obtain a material with reduced particle size and increased surface area) can avoid the need for special processing conditions and the increased costs generally associated with such special processing conditions. Similarly, selection of a solid state form of eplerenone that is physically stable at different conditions of storage (especially considering the different possible storage conditions during the lifetime of the eplerenone product) can help avoid polymorphic or other degradative changes in the eplerenone that can lead to product loss or deterioration of product efficacy. Therefore, the selection of a solid state form of eplerenone such as Form II having greater physical stability provides a meaningful benefit over less stable eplerenone forms.

In another embodiment of the invention, the solid state form of eplerenone comprises a solvated crystalline form of eplerenone. Such solvated crystalline forms are useful as intermediates in the preparation of Form I and Form II eplerenone. An additional benefit from the use of a solvated crystalline form as an intermediate is the "intrinsic micronizing" of the crystal that results upon desolvation as discussed later in this application. Such "intrinsic micronizing" can reduce or eliminate milling requirements. Further, where additional milling is still required, it is easier to mill certain solvates before the desolvation step than to mill Form I or Form II after desolvation of the solvate.

Pharmaceutically acceptable solvated crystalline forms also can be used directly in pharmaceutical compositions.

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These solvated crystalline forms preferably do not comprise methylene chloride, isopropanol or ethyl ether; and, in yet another embodiment, do not comprise methylene chloride, isopropanol, ethyl ether, methyl ethyl ketone or ethanol; and, in yet another embodiment, do not comprise methylene chloride, isopropanol, ethyl ether, methyl ethyl ketone, ethanol, ethyl acetate or acetone. More preferably, the solvated crystalline forms are substantially exclusive of solvents that are not pharmaceutically-acceptable solvents. Because Form I and Form II typically are more physically stable than the crystalline solvates at room temperature and under atmospheric pressure, the solvated crystalline forms used in such compositions generally comprise a pharmaceutically acceptable higher boiling and/or hydrogen bonding solvent such as, but not limited to, butanol. It is believed that the solvated crystalline forms collectively can offer a range of different dissolution rates and, where dissolution of eplerenone in the gastrointestinal tract is the rate-controlling step for delivery of the eplerenone to the target cells, bioavailabilities relative to Form I and Form II.

In another embodiment of the invention, the solid state form of eplerenone comprises an amorphous form of eplerenone. Amorphous eplerenone is useful as an intermediate in the preparation of Form I and Form II eplerenone. In addition, it is believed that amorphous eplerenone possesses a different dissolution rate and, where dissolution of eplerenone in the gastrointestinal tract is the rate-controlling step for delivery of the eplerenone to the target cells, bioavailability relative to Form I and Form II.

In another embodiment, the invention comprises combinations of solid state eplerenone forms selected from

the group consisting of Form I eplerenone, Form II eplerenone, solvated crystalline eplerenone and amorphous eplerenone. Such combinations are useful, for example, in the preparation of pharmaceutical compositions having differing dissolution profiles, including controlled release compositions.

In addition, depending upon the intended use of the solid state form of eplerenone, processing considerations may favor the selection of a specific solid state form of eplerenone or a specific combination of such solid state forms. Pure phase Form II, for example, generally is more easily prepared than pure phase Form I. A mixture of Form I and Form II, however, generally is more easily prepared than phase pure Form II and allows for the use of a low purity eplerenone starting material. Use of a solvated crystalline form instead of Form I or Form II in a composition eliminates a processing step, desolvation, for those processes that otherwise would proceed by desolvation of the solvated crystalline form. Alternatively, the desolvation step can be eliminated, for example, if Form II is directly crystallized from an appropriate solvent without the intervening preparation and desolvation of the intermediate solvated crystalline form. These processes are described in greater detail later in the application.

It has been discovered that reduction of the particle size of the solid state form of eplerenone to a D_{90} particle size (later defined herein) between about 10 nm to about 15 microns can improve the bioavailability of unformulated eplerenone and pharmaceutical compositions comprising that solid state form of eplerenone. Accordingly, the D_{90} particle size of the unformulated eplerenone or the eplerenone used as

a starting material in the pharmaceutical composition generally is less than about 15 microns, preferably less than about 1 micron, more preferably less than about 800 nm, still more preferably less than about 600 nm, and still more preferably less than about 400 nm. In one embodiment, the D_{90} particle size is between about 10 nm to about 1 micron. In another embodiment, the D_{90} particle size is between about 100 nm to about 800 nm. In another embodiment, the D_{90} particle size is between about 200 nm to about 600 nm. In another embodiment, the D_{90} particle size is between about 400 nm to about 800 nm.

Solid state forms of eplerenone having a particle size less than about 15 microns can be prepared in accordance with applicable particle size reduction techniques known in the art. Such techniques include, but are not limited to those described in U.S. Patents 5,145,684, 5,318,767, 5,384,124 and 5,747,001. U.S. Patents 5,145,684, 5,318,767, 5,384,124 and 5,747,001 are expressly incorporated by reference as if fully set forth at length. In accordance with the method of U.S. Patent 5,145,684, for example, particles of suitable size are prepared by dispersing the eplerenone in a liquid dispersion medium and wet-grinding the mixture in the presence of grinding media to reduce the particles to the desired size. If necessary or advantageous, the particles can be reduced in size in the presence of a surface modifier.

Definitions

The term "amorphous" as applied to eplerenone refers to a solid state wherein the eplerenone molecules are present in a disordered arrangement and do not form a distinguishable crystal lattice or unit cell. When subjected to X-ray powder

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diffraction, amorphous eplerenone does not produce any characteristic crystalline peaks.

Where reference is made in this application to the "boiling point" of a substance or solution, the term "boiling point" means the boiling point of the substance or solution under the applicable process conditions.

The term "crystalline form" as applied to eplerenone refers to a solid state form wherein the eplerenone molecules are arranged to form a distinguishable crystal lattice (i) comprising distinguishable unit cells, and (ii) yielding diffraction peaks when subjected to X-ray radiation.

The term "crystallization" as used throughout this application can refer to crystallization and/or recrystallization depending upon the applicable circumstances relating to the preparation of the eplerenone starting material.

The term "digestion" means a process in which a slurry of solid eplerenone in a solvent or mixture of solvents is heated at the boiling point of the solvent or mixture of solvents under the applicable process conditions.

The term "direct crystallization" as used herein refers to the crystallization of eplerenone directly from a suitable solvent without the formation and desolvation of an intermediate solvated crystalline solid state form of eplerenone.

The term "particle size" as used herein refers to particle size as measured by conventional particle size measuring techniques well known in the art, such as laser light scattering, sedimentation field flow fractionation, photon correlation spectroscopy, or disk centrifugation. The term "D_{90} particle size" means the particle size of at least

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90% of the particles as measured by such conventional particle size measuring techniques.

The term "DSC" means differential scanning calorimetry.

The term "HPLC" means high pressure liquid chromatography.

The term "IR" means infra red.

The term "min" means minute.

The term "psi" means pounds per square inch absolute.

The term "purity" means the chemical purity of eplerenone according to conventional HPLC assay. As used herein, "low purity eplerenone" generally means eplerenone that contains an effective amount of a Form I growth promoter and/or a Form II growth inhibitor. As used herein, "high purity eplerenone" generally means eplerenone that does not contain, or contains less than an effective amount of, a Form I growth promoter and/or a Form II growth inhibitor.

The term "phase purity" means the solid state purity of eplerenone with regard to a particular crystalline or amorphous form of the eplerenone as determined by the infrared spectroscopy analytical methods described herein.

The term "XPRD" means X-ray powder diffraction.

The term "rpm" means revolutions per minute.

The term "TGA" means thermogravimetric analysis.

The term "T_m" means melting temperature.

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Characterization of Crystalline Form

1. Molecular Conformation

Single crystal X-ray analysis indicates that the eplerenone molecular conformation differs between Form I and Form II, particularly with respect to the orientation of the

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ester group at the 7-position of the steroid ring. The orientation of the ester group can be defined by the C8-C7-C23-02 torsion angle.

In the Form I crystal lattice, the eplerenone molecule adopts a conformation in which the methoxy group of the ester is approximately aligned with the C-H bond at the 7-position and the carbonyl group is approximately positioned over the center of the B-steroid ring. The C8-C7-C23-02 torsion angle is approximately -73.0° in this conformation. In this orientation, the carbonyl oxygen atom of the ester group (01) is in close contact with the oxygen atom of the 9,11-epoxide ring (04). The 01-04 distance is about 2.97 Å, which is just below the van der Waal's contact distance of 3.0 Å (assuming van der Waal's radii of 1.5Å for the oxygen).

In the Form II crystal lattice, the eplerenone molecule adopts a conformation in which the ester group is rotated approximately 150° relative to that of Form I and has a C8-C7-C23-02 torsion angle of approximately +76.9°. In this orientation, the methoxy group of the ester is directed toward the 4,5-alkene segment of the A-steroid ring. In this orientation, the distance between either oxygen atom of the ester group (01,02) and the oxygen atom of the 9,11-epoxide ring is increased relative to the distance determined for Form I. The 02-04 distance is approximately 3.04Å, falling just above the van der Waal's contact distance. The 01-04 distance is about 3.45Å.

The eplerenone molecule appears to adopt a conformation characteristic of Form II in the solvated crystalline forms analyzed by single crystal X-ray diffraction to date.

2. X-Ray Powder Diffraction

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The various crystalline forms of eplerenone were analyzed with either a Siemens D5000 powder diffractometer or an Inel Multipurpose Diffractometer. For the Siemens D500 powder diffractometer, the raw data was measured for 2q values from 2 to 50, with steps of 0.020 and step periods of two seconds. For the Inel Multipurpose Diffractometer, samples were placed in an aluminum sample holder and raw data was collected for 30 minutes at all two theta values simultaneously.

Tables 1A, 1B and 1C set out the significant parameters of the main peaks in terms of 2q values and intensities for the Form I (prepared by desolvation of the ethanol solvate obtained by digestion of low purity eplerenone), Form II (prepared by desolvation of the methyl ethyl ketone solvate obtained by recrystallization of high purity eplerenone), and methyl ethyl ketone solvate (prepared by room temperature slurry conversion of high purity eplerenone in methyl ethyl ketone) crystalline forms of eplerenone, respectively (X-ray radiation at a wavelength of 1.54056 Angstroms).

Minor shifts in peak positioning may be present in the diffraction patterns of Form I and Form II as a result of imperfections in the spacing of the crystal diffraction planes due to the route of manufacture of Form I and Form II (i.e. desolvation of a solvate). In addition, Form I is isolated from a solvate prepared by digestion of crude eplerenone. This method results in a lower overall chemical purity (approximately 90%) of the Form I. Finally, the solvated forms of eplerenone are expected to show some shifting in the positioning of the diffraction peaks due to the increased mobility of the solvent molecules within the solvent channels in the crystal lattice.

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TABLE 1A: FORM I DATA

Angle 2-theta	d-spacing Angstrom	Intensity	Intensity %
6.994	12.628	1186	7.2
8.291	10.655	2137	13
10.012	8.827	577	3.5
11.264	7.849	1854	11.3
12.04	7.344	7707	46.8
14.115	6.269	3121	19
14.438	6.13	15935	96.8
15.524	5.703	637	3.9
16.169	5.477	1349	8.2
16.699	5.305	1663	10.1
16.94	5.23	1692	10.3
17.147	5.167	2139	13
17.66	5.018	6883	41.8
17.91	4.949	16455	100
18.379	4.823	3106	18.9
18.656	4.752	1216	7.4
19.799	4.48	1499	9.1
20.235	4.385	383	2.3
21.707	4.091	1267	7.7
21.8	4.073	1260	7.7
21.959	4.044	1279	7.8
22.461	3.955	4264	25.9
23.191	3.832	1026	6.2
23.879	3.723	1000	6.1
24.599	3.616	1688	10.3
25.837	3.445	931	5.7
26.034	3.42	686	4.2
26.988	3.316	912	5.5
27.083	3.286	1322	8
27.762	3.209	1236	7.5
28.34	3.147	1845	11.2
28.861	3.091	957	5.8
29.868	2.9892	745	4.5
30.627	2.9166	992	6
31.108	2.8728	1205	7.3
33.216	2.6951	1267	7.6
33.718	2.656	802	4.9
34.434	2.6024	914	5.6

TABLE 1B: FORM II DATA

Angle 2-Theta	d-spacing Angstrom	Intensity Cps	Intensity %
7.992	11.054	11596	26.6
10.044	8.799	12048	27.6
11.206	7.889	4929	11.3
12.441	7.109	1747	4
12.752	6.938	4340	9.9
13.257	6.673	2444	5.6
14.705	6.019	43646	100
15.46	5.727	2670	6.1
15.727	5.63	7992	18.3
16.016	5.529	3519	8.1
17.671	5.015	8897	20.4
17.9	4.951	2873	6.6
18.352	4.83	612	1.4
18.703	4.74	689	1.6
19.524	4.543	1128	2.6
20.103	4.413	3763	8.6
20.63	4.302	1451	3.3
21.067	4.214	876	2
21.675	4.097	2760	6.3
22.232	3.995	1951	4.5
22.652	3.922	1657	3.8
23.624	3.763	827	1.9
24.279	3.663	1242	2.8
25.021	3.556	5144	11.8
25.485	3.492	1702	3.9
25.707	3.463	2493	5.7
26.251	3.392	1371	3.1
26.85	3.318	1970	4.5
27.319	3.262	1029	2.4
27.931	3.192	440	1
27.969	3.187	440	1
28.937	3.083	1128	2.6
29.703	3.005	1211	2.8
30.173	2.9594	1506	3.5
30.584	2.9206	1602	3.7
30.885	2.8928	1550	3.6
31.217	2.8628	1068	2.4
31.605	2.8285	1038	2.4
32.059	2.7895	1211	2.8
32.64	2.7412	684	1.6
32.747	2.7324	758	1.7
33.48	2.6759	506	1.2
34.194	2.6201	1085	2.5
34.545	2.5943	915	2.1

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TABLE 1C: METHYL ETHYL KETONE DATA

Angle 2-Theta	d-spacing Angstrom	Intensity Cps	Intensity %
7.584	11.648	5629	32.6
7.753	11.393	15929	92.3
10.151	8.707	2877	16.7
11.31	7.817	701	4.1
12.646	6.994	1027	5.9
13.193	6.705	15188	88
13.556	6.526	14225	82.4
14.074	6.287	1966	11.4
14.746	6.002	2759	16
15.165	5.837	801	4.6
15.548	5.694	1896	11
17.031	5.202	7960	46.2
17.26	5.127	17267	100
17.706	5.005	6873	39.8
18.555	4.778	545	3.2
18.871	4.699	1112	6.4
19.766	4.488	1704	9.9
20.158	4.401	1396	8.1
20.725	4.282	2644	15.3
21.787	4.076	1127	6.5
22.06	4.026	451	2.6
22.864	3.886	1542	8.9
23.412	3.796	14185	82.2
23.75	3.743	1154	6.7
24.288	3.662	3063	17.7
25.253	3.524	1318	7.6
25.603	3.49	1736	10.1
25.761	3.455	1225	7.1
26.178	3.402	1348	7.8
26.548	3.356	1098	6.4
27.357	3.257	1944	11.3
27.605	3.229	2116	12.3
27.9	3.195	858	5
28.378	3.142	583	3.4
28.749	3.103	763	4.4
29.3	3.048	1162	6.8
29.679	3.008	2606	15.1
30.402	2.9377	2184	12.6
30.739	2.9063	648	3.8

Graphical examples of the x-ray diffraction patterns for Form I, Form II, and the methyl ethyl ketone solvate

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crystalline forms of eplerenone are shown in Figs. 1-A, 1-B, and 1-C, respectively. Form I shows distinguishing peaks at 7.0 ± 0.2, 8.3 ± 0.2, and 12.0 ± 0.2 degrees two theta. Form II shows distinguishing peaks at 8.0 ± 0.2, 12.4 ± 0.2, 12.8 ± 0.2, and 13.3 ± 0.2 degrees two theta. The methyl ethyl ketone solvated crystalline form shows distinguishing peaks at 7.6 ± 0.2, 7.8 ± 0.2, and 13.6 ± 0.2 degrees two theta.

Examples of the x-ray diffraction patterns are shown in Figs. 1-D through 1-O for the following solvate crystalline forms of eplerenone: n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, acetone solvate, toluene solvate, isopropanol solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, methyl acetate solvate, and propyl acetate solvate, respectively.

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3. Melting/Decomposition Temperature

The temperatures of melting and/or decomposition of non-solvated eplerenone crystalline forms were determined using a TA Instruments 2920 differential scanning calorimeter. Each sample (1-2 mg) was placed in either a sealed or unsealed aluminum pan and heated at 10°C/minute. Melting/decomposition ranges were defined from the extrapolated onset to the maximum of the melting/decomposition endotherm.

The melting of the non-solvated eplerenone crystals forms (Form I and Form II) was associated with chemical decomposition and loss of trapped solvent from the crystal lattice. The melting/decomposition temperature also was affected by the manipulation of the solid prior to analysis. For example, non-milled Form II (approximate D_{90} particle size of about 180-450 microns) prepared by direct crystallization

from an appropriate solvent or from desolvation of a solvate obtained from crystallization of high purity eplerenone in an appropriate solvent or mixture of solvents generally had a melting range of about 237-242°C. Milled Form II (approximate D_{90} particle size of about 80-100 microns) (Form II prepared by crystallizing a solvate from a solution of high purity eplerenone in an appropriate solvent or mixture of solvents, desolvating the solvate to yield Form II, and milling the resulting Form II) generally had a lower and broader melting/decomposition range of about 223-234°C. Non-milled Form I (approximate D_{90} particle size of about 180-450 microns) prepared by desolvation of a solvate obtained by digestion of low purity eplerenone generally had a higher melting/decomposition range of about 247-251°C. Examples of the DSC thermograms of (a) non-milled Form II directly crystallized from methyl ethyl ketone, (b) non-milled Form II prepared by desolvation of a solvate obtained by crystallization of a high purity eplerenone from methyl ethyl ketone, (c) Form II prepared by milling a desolvated solvate obtained by crystallization of high purity eplerenone from methyl ethyl ketone, and (d) non-milled Form I prepared by desolvation of a solvate obtained by digestion of low purity eplerenone from methyl ethyl ketone are given in Figures 2-A, 2-B, 2-C and 2-D, respectively.

DSC thermograms of solvated forms of eplerenone were determined using a Perkin Elmer Pyris 1 differential scanning calorimeter. Each sample (1-10 mg) was placed in an unsealed aluminum pan and heated at 10°C/minute. One or more endothermal events at lower temperatures were associated with enthalpy changes that occurred as solvent was lost from the solvate crystal lattice. The highest temperature endotherm

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or endotherms were associated with the melting/decomposition of Form II or Form I eplerenone. Examples of the DSC thermograms are shown in Figs. 2-E through 2-T for the following solvated crystalline forms of eplerenone: n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, chloroform solvate, acetone solvate, toluene solvate, isopropanol solvate, t-butyl acetate solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, methyl acetate solvate, propyl acetate solvate, n-butanol solvate, and n-octanol solvate, respectively.

4. Infrared Absorption Spectroscopy

Infrared absorption spectra of the non-solvated forms of eplerenone (Form I and Form II) were obtained with a Nicolet DRIFT (diffuse reflectance infrared fourier transform) Magna System 550 spectrophotometer. A Spectra-Tech Collector system and a microsample cup were used. Samples (5%) were analyzed in potassium bromide and scanned from 400-4000 cm^{-1}. Infrared absorption spectra of eplerenone in dilute chloroform solution (3%) or in the solvated crystal forms were obtained with a Bio-rad FTS-45 spectrophotometer. Chloroform solution samples were analyzed using a solution cell of 0.2 mm path length with sodium chloride salt plates. Solvate FTIR spectra were collected using an IBM micro-MIR (multiple internal reflectance) accessory. Samples were scanned from 400-4000 cm^{-1}. Examples of the infrared absorption spectra of (a) Form I, (b) Form II, (c) the methyl ethyl ketone solvate, and (d) eplerenone in chloroform solution are shown in Figures 3-A, 3-B, 3-C and 3-D, respectively.

Table 2 discloses illustrative absorption bands for

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eplerenone in the Form I, Form II, and methyl ethyl ketone solvate crystal forms. Illustrative absorption bands for eplerenone in chloroform solution are also disclosed for comparison. Differences between Form I and either Form II or the methyl ethyl ketone solvate were observed, for example, in the carbonyl region of the spectrum. Form I has an ester carbonyl stretch of approximately 1739 cm^{-1} while both Form II and the methyl ethyl ketone solvate have the corresponding stretch at approximately 1724 and 1722 cm^{-1}, respectively. The ester carbonyl stretch occurs at approximately 1727 cm^{-1} in the eplerenone in chloroform solution. The change in stretching frequency of the ester carbonyl between Form I and Form II reflects the change in orientation of the ester group between the two crystal forms. In addition, the stretch of the ester of the conjugated ketone in the A-steroid ring shifts from approximately 1664-1667 cm^{-1} in either Form I or the methyl ethyl ketone solvate to approximately 1655 cm^{-1} in Form II. The corresponding carbonyl stretch occurs at approximately 1665 cm^{-1} in dilute solution.

Another difference between Form I and Form II was seen in the C-H bending region. Form I has an absorption at approximately 1399 cm^{-1} which is not observed in Form II, the methyl ethyl ketone solvate, or the eplerenone in chloroform solution. The 1399 cm^{-1} stretch occurs in the region of CH_2 scissoring for the C2 and C21 methylene groups adjacent to carbonyl groups.

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Table 2

Absorption Region	Form I (cm^{-1})	Form II (cm^{-1})	Methyl Ethyl Ketone Solvate (cm^{-1})	Eplerenone in Chloroform (cm^{-1})
ν C=O(lactone)	1773	1775	1767	1768
ν C=O(ester)	1739	1724	1722	1727
ν C=O(3keto)	1664	1655	1667	1665
ν C=C (3,4-olefin)	1619	1619	1622	1623
δ_{as}CH3,δCH2, δCH2(α to carbonyl)	1460, 1444, 1426	1467, 1438, 1422, 1399	1467, 1438, 1422	1464, 1438, 1422
δ_{s}CH3	1380	1381	~1380	1378

Examples of infrared absorption spectra are shown in Figs. 3-E through 3-S for the following solvated crystalline forms of eplerenone: n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, acetone solvate, toluene solvate, isopropanol solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, propyl acetate solvate, methyl acetate solvate, propylene glycol solvate, and t-butyl acetate solvate respectively.

5. Nuclear Magnetic Resonance

^{13}C NMR spectra were obtained at a field of 31.94 MHz. Examples of the ^{13}C NMR spectra of Form I and Form II eplerenone are shown in Figs. 4 and 5, respectively. The Form I eplerenone analyzed to obtain the data reflected in Fig. 4 was not phase pure and included a small amount of Form II eplerenone. Form I is most clearly distinguished by the carbon resonances at around 64.8 ppm, 24.7 ppm and 19.2 ppm.

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Form II is most clearly distinguished by the carbon resonances at around 67.1 ppm and 16.0 ppm.

6. Thermogravimetry

Thermogravimetric analysis was performed using a TA Instruments TGA 2950 thermogravimetric analyzer. Samples were placed in an unsealed aluminum pan under nitrogen purge. Starting temperature was 25°C with the temperature increased at a rate of about 10°C/minute.

Examples of thermogravimetry analysis profiles are shown in Figs. 6-A through 6-R for the following solvated crystalline forms of eplerenone: methyl ethyl ketone solvate, n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, chloroform solvate, acetone solvate, toluene solvate, isopropanol solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, methyl acetate solvate, propyl acetate solvate, propylene glycol solvate, n-butanol solvate, n-octanol solvate, and t-butyl acetate solvate, respectively.

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7. Microscopy

Hot-stage microscopy was performed on single crystals of the methyl ethyl ketone solvate using a Linkam THMS 600 Hot Stage with Zeiss Universal Polarized Light Microscope. Under polarized light at room temperature the solvate crystal was birefringent and translucent indicating that the crystal lattice was highly ordered. As the temperature increased to about 60°C, noticeable defects began to emerge along the long crystal dimension. A scanning electron micrograph of Form II eplerenone obtained by desolvation of the methyl ethyl ketone solvate is shown in Fig. 7 and reveals surface defects,

pores, cracks and fractures within the crystal lattice. A scanning electron micrograph of Form II eplerenone obtained by direct crystallization from ethyl acetate is shown in Fig. 8 and does not exhibit similar surface defects, pores, cracks and fractures within the crystal lattice.

8. Unit Cell Parameters

Tables 3A, 3B and 3C below summarize the unit cell parameters determined for Form I, Form II, and several solvated crystalline forms.

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TABLE 3A

Parameter	Form I	Form II	Methyl ethyl ketone Solvate
Crystal system	Ortho-rhombic	Monoclinic	Orthorhombic
Space group	$P2_12_12_1$	$P2_1$	$P2_12_12_1$
a	21.22 Å	8.78 Å	23.53 Å
b	15.40 Å	11.14 Å	8.16 Å
c	6.34 Å	11.06 Å	13.08 Å
α	90°	90°	90°
β	90°	93.52°	90°
γ	90°	90°	90°
z	4	2	4
Volume (Å)	2071.3	1081.8	2511.4
ρ (calculated)	1.329 g/cm³	1.275 g/cm³	1.287 g/cm³
R	0.0667	0.062	0.088

TABLE 3B

Parameter	Acetone Solvate	Toluene Solvate	Butyl Acetate Solvate[1]
Crystal system	Ortho-rhombic	Ortho-rhombic	Ortho-rhombic
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
a	23.31 Å	23.64 Å	23.07 Å
b	13.13 Å	13.46 Å	13.10 Å
c	8.28 Å	8.16 Å	8.24 Å
α	90°	90°	90°
β	90°	90°	90°
γ	90°	90°	90°
z	4	4	4
Volume (Å)	2533.7	2596.6	2490.0
ρ (calculated)	1.239 g/cm³	1.296 g/cm³	1.334 g/cm³
R	0.058	0.089	0.093

[1]The solvate molecules were not completely refined due to disorder of the solvent molecules in the channels.

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TABLE 3C

Parameter	Isobutyl Acetate Solvate[1]	Isopropanol Solvate[1]	Ethanol Solvate[1]
Crystal system	Ortho-rhombic	Ortho-rhombic	Ortho-rhombic
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
a	23.19 Å	23.15 Å	23.51 Å
b	12.95 Å	12.73 Å	13.11 Å
c	8.25 Å	8.25 Å	8.27 Å
α	90°	90°	90°
β	90°	90°	90°
γ	90°	90°	90°
z	4	4	4
Volume (Å)	2476.4	2433.2	2548.6
ρ (calculated)	1.337 g/cm³	1.296 g/cm³	1.234 g/cm³
R	0.098	0.152	0.067

[1]The solvate molecules were not refined completely due to disorder of the solvent molecules in the channels.

Additional information on selected solvated crystalline forms of eplerenone is reported in Table 4 below. The unit cell data reported in Table 3A above for the methyl ethyl ketone solvate also are representative of the unit cell parameters for many of these additional eplerenone crystalline solvates. Most of the eplerenone crystalline solvates tested are substantially isostructural to each other. While there may be some minor shifting in the X-ray powder diffraction peaks from one solvated crystalline form to the next due to the size of the incorporated solvent molecule, the overall diffraction patterns are substantially the same and the unit cell parameters and molecular positions are substantially identical for most of the solvates tested.

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TABLE 4

Solvent	Stoichiometry (Solvent: Eplerenone)	Isostructural to Methyl Ethyl ketone Solvate?	Desolvation Temperature[1] (°C)
Methyl Ethyl Ketone	1:1	N/A	89
2-Pentanone	---	---	---
Acetic Acid	1:2	Yes	203
Acetone	1:1	Yes	117
Butyl Acetate	1:2	Yes	108
Chloroform	---	Yes	125
Ethanol	1:1	Yes	166
Isobutanol	---	---	---
Isobutyl Acetate	1:2	Yes	112
Isopropanol	1:1	Yes	121
Methyl Acetate	1:1	Yes	103
Ethyl Propionate	1:1	Yes	122
n-Butanol	1:1	Yes	103
n-Octanol	---	Yes	116
n-Propanol	1:1	Yes	129
Propyl Acetate	1:1	Yes	130
Propylene Glycol	---	Yes	188
t-Butanol	---	---	---
Tetrahydrofuran	1:1	Yes	136
Toluene	1:1	Yes	83
t-Butyl Acetate	---	Yes	109

[1]Defined as the extrapolated desolvation temperature from the final solvent weight loss step as determined by thermogravimetric analysis at a heating rate of 10°C/minute under nitrogen purge. Desolvation temperatures, however, can be affected by the method of manufacture of the solvate. Different methods can produce different numbers of nucleation sites capable of initiating desolvation in the solvate at lower temperatures.

The unit cell of the solvate is composed of four eplerenone molecules. The stoichiometry of the eplerenone molecules and solvent molecules in the unit cell is also reported in Table 4 above for a number of solvates. The unit cell of Form I is composed of four eplerenone molecules. The unit cell of Form II is composed of two eplerenone molecules. The solvate unit cells are converted during desolvation into

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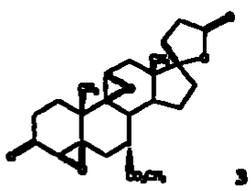
Form I and/or Form II unit cells when the eplerenone molecules undergo translation and rotation to fill the spaces left by the solvent molecules. Table 4 also reports the desolvation temperatures for a number of different solvates.

9. Crystal Properties of Impurity Molecules

Selected impurities in eplerenone can induce the formation of Form I during the desolvation of the solvate. In particular, the effect of the following two impurity molecules was evaluated: 7-methyl hydrogen 4α,5α:9α,11α-diepoxy-17-hydroxy-3-oxo-17α-pregnane-7α,21-dicarboxylate, γ-lactone 3 (the "diepoxide"); and 7-methyl hydrogen 11α,12α-epoxy-17-hydroxy-3-oxo-17α-pregn-4-ene-7α,21-dicarboxylate, γ-lactone 4 (the "11,12-epoxide").



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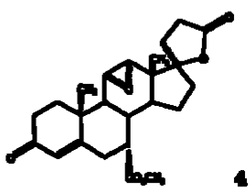

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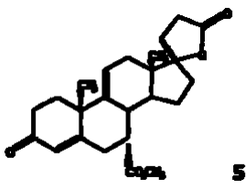
The effect of these impurity molecules on the eplerenone crystalline form resulting from desolvation is described in greater detail in the examples of this application.

Given the similarity in single crystal structure of 7-methyl hydrogen 17-hydroxy-3-oxo-17α-pregna-4,9(11)-diene-7α,21-dicarboxylate, γ-lactone 5 (the "9,11-olefin") and Form I, it is hypothesized that the 9,11-olefin also can induce the formation of Form I during the desolvation of the solvate.



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A single crystal form was isolated for each impurity compound. Representative X-ray powder diffraction patterns for the crystal forms isolated for the diepoxide, 11,12-epoxide and 9,11-olefin are given in Figs. 9, 10 and 11, respectively. The X-ray powder diffraction pattern of each impurity molecule is similar to the X-ray powder diffraction pattern of Form I, suggesting that Form I and the three impurity compounds have similar single crystal structures.

Single crystals of each impurity compound also were isolated and subjected to X-ray structure determination to verify that these three compounds adopt single crystal structures similar to that of Form I. Single crystals of the diepoxide were isolated from methyl ethyl ketone. Single crystals of the 11,12-epoxide were isolated from isopropanol. Single crystals of the 9,11-olefin were isolated from n-butanol. Crystal structure data determined for the crystalline form of each impurity compound are given in Table 5. The resulting crystal system and cell parameters were substantially the same for the Form I, diepoxide, 11,12-epoxide, and 9,11-olefin crystalline forms.

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TABLE 5

Parameter	Form I	Diepoxide	11,12 Epoxide	9,11 olefin
Crystal system	Ortho-rhombic	Ortho-rhombic	Ortho-rhombic	Ortho-rhombic
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
a	21.22 Å	21.328 Å	20.90 Å	20.90 Å
b	15.40 Å	16.16 Å	15.55 Å	15.74 Å
c	6.34 Å	6.15 Å	6.38 Å	6.29 Å
α	90°	90°	90°	90°
β	90°	90°	90°	90°
γ	90°	90°	90°	90°
z	4	4	4	4
Volume (Å)	2071.3	2119.0	2073.2	2069.3
ρ (calculated)	1.329 g/cm³	1.349 g/cm³	1.328 g/cm³	1.279 g/cm³
R	0.0667	0.0762	0.0865	0.0764

The four compounds reported in Table 5 crystallize into the same space group and have similar cell parameters (i.e., they are isostructural). It is hypothesized that the diepoxide, 11,12-epoxide and 9,11-olefin adopt a Form I conformation. The relative ease of isolation of a Form I packing (directly from solution) for each impurity compound, indicates that the Form I lattice is a stable packing mode for this series of structurally similar compounds.

Preparation of Eplerenone

The eplerenone starting material used to prepare the novel crystalline forms of the present invention can be prepared using the methods set forth in Ng et al., WO97/21720; and Ng et al., WO98/25948, particularly scheme 1 set forth in WO97/21720 and WO98/25948.

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Preparation of Crystalline Forms

1. Preparation of Solvated Crystalline Form

The solvated crystalline forms of eplerenone can be prepared by crystallization of eplerenone from a suitable solvent or a mixture of suitable solvents. A suitable solvent or mixture of suitable solvents generally comprises an organic solvent or a mixture of organic solvents that solubilizes the eplerenone together with any impurities at an elevated temperature, but upon cooling, preferentially crystallizes the solvate. The solubility of eplerenone in such solvents or mixtures of solvents generally is about 5 to about 200 mg/mL at room temperature. The solvent or mixtures of solvents preferably are selected from those solvents previously used in the process to prepare the eplerenone starting material, particularly those solvents that would be pharmaceutically acceptable if contained in the final pharmaceutical composition comprising the eplerenone crystalline form. For example, a solvent system comprising methylene chloride that yields a solvate comprising methylene chloride generally is not desirable.

Each solvent used preferably is a pharmaceutically acceptable solvent, particularly a Class 2 or Class 3 solvent as defined in "Impurities: Guideline For Residual Solvents", International Conference On Harmonisation Of Technical Requirements For Registration Of Pharmaceuticals For Human Use (Recommended for Adoption at Step 4 of the ICH Process on July 17, 1997 by the ICH Steering Committee). Still more preferably, the solvent or mixture of solvents is selected from the group consisting of methyl ethyl ketone, 1-propanol,

2-pentanone, acetic acid, acetone, butyl acetate, chloroform, ethanol, isobutanol, isobutyl acetate, methyl acetate, ethyl propionate, n-butanol, n-octanol, isopropanol, propyl acetate, propylene glycol, t-butanol, tetrahydrofuran, toluene, methanol and t-butyl acetate. Still more preferably, the solvent is selected from the group consisting of methyl ethyl ketone and ethanol.

In another embodiment of this process, the solvent or mixture of solvents is selected from the group consisting of 1-propanol, 2-pentanone, acetic acid, acetone, butyl acetate, chloroform, isobutanol, isobutyl acetate, methyl acetate, ethyl propionate, n-butanol, n-octanol, propyl acetate, propylene glycol, t-butanol, tetrahydrofuran, toluene, methanol and t-butyl acetate.

In another embodiment of this process, the solvent or mixture of solvents is selected from the group consisting of 1-propanol, 2-pentanone, acetic acid, acetone, butyl acetate, chloroform, isobutanol, isobutyl acetate, methyl acetate, ethyl propionate, n-butanol, n-octanol, n-propanol, propyl acetate, propylene glycol, t-butanol, tetrahydrofuran, toluene, methanol and t-butyl acetate.

To prepare the solvated crystalline form of eplerenone, an amount of the eplerenone starting material is solubilized in a volume of the solvent and cooled until crystals form. The solvent temperature at which the eplerenone is added to the solvent generally will be selected based upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, this solvent temperature typically is at least about 25°C, preferably from about 30°C to the boiling point of the solvent, and more preferably from about 25°C below the

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boiling point of the solvent to the boiling point of the solvent.

Alternatively, hot solvent may be added to the eplerenone and the mixture can be cooled until crystals form. The solvent temperature at the time it is added to the eplerenone generally will be selected based upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, the solvent temperature typically is at least 25°C, preferably from about 50°C to the boiling point of the solvent, and more preferably from about 15°C below the boiling point of the solvent to the boiling point of the solvent.

The amount of the eplerenone starting material mixed with a given volume of solvent likewise will depend upon the solubility curve of the solvent or mixture of solvents. Typically, the amount of eplerenone added to the solvent will not completely solubilize in that volume of solvent at room temperature. For most of the solvents described herein, for example, the amount of eplerenone starting material mixed with a given volume of solvent usually is at least about 1.5 to about 4.0 times, preferably about 2.0 to about 3.5 times, and more preferably about 2.5 times, the amount of eplerenone that will solubilize in that volume of solvent at room temperature.

After the eplerenone starting material has completely solubilized in the solvent, the solution typically is cooled slowly to crystallize the solvated crystalline form of eplerenone. For most of the solvents described herein, for example, the solution is cooled at a rate slower than about 20°C/minute, preferably at a rate of about 10°C/minute or slower, more preferably at a rate of about 5°C/minute or

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slower, and still more preferably at a rate of about 1°C/minute or slower.

The endpoint temperature at which the solvated crystalline form is harvested will depend upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, the endpoint temperature typically is less than about 25°C, preferably less than about 5°C, and more preferably less than about -5°C. Decreasing the endpoint temperature generally favors the formation of the solvated crystalline form.

Alternatively, other techniques may be used to prepare the solvate. Examples of such techniques include, but are not limited to, (i) dissolving the eplerenone starting material in one solvent and adding a co-solvent to aid in the crystallization of the solvate crystalline form, (ii) vapor diffusion growth of the solvate, (iii) isolation of the solvate by evaporation, such as rotary evaporation, and (iv) slurry converstion.

The crystals of the solvated crystalline form prepared as described above can be separated from the solvent by any suitable conventional means such as by filtration or centrifugation. Increased agitation of the solvent system during crystallization generally results in smaller crystal particle sizes.

2. Preparation of Form II From Solvate

Form II eplerenone can be prepared directly from the solvated crystalline form by desolvation. Desolvation can be accomplished by any suitable desolvation means such as, but not limited to, heating the solvate, reducing the ambient pressure surrounding the solvate, or combinations thereof.

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If the solvate is heated to remove the solvent, such as in an oven, the temperature of the solvate during this process typically does not exceed the enantiotropic transition temperature for Form I and Form II. This temperature preferably does not exceed about 150°C.

The desolvation pressure and time of desolvation are not narrowly critical. The desolvation pressure preferably is about one atmosphere or less. As the desolvation pressure is reduced, however, the temperature at which the desolvation can be carried out and/or the time of desolvation likewise is reduced. Particularly for solvates having higher desolvation temperatures, drying under vacuum will permit the use of lower drying temperatures. The time of desolvation need only be sufficient to allow for the desolvation, and thus the formation of Form II, to reach completion.

To ensure the preparation of a product that comprises substantially all Form II, the eplerenone starting material typically is a high purity eplerenone, preferably substantially pure eplerenone. The eplerenone starting material used to prepare Form II eplerenone generally is at least 90% pure, preferably at least 95% pure, and more preferably at least 99% pure. As discussed in greater detail elsewhere in this application, certain impurities in the eplerenone starting material can adversely affect the yield and Form II content of the product obtained from the process.

The crystallized eplerenone product prepared in this manner from a high purity eplerenone starting material generally comprises at least 10% Form II, preferably at least 50% Form II, more preferably at least 75% Form II, still more preferably at least 90% Form II, still more preferably at

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least about 95% Form II, and still more preferably substantially phase pure Form II.

3. Preparation of Form I From Solvate

A product comprising Form I can be prepared in substantially the same manner as set forth above for the preparation of Form II by (i) using a low purity eplerenone starting material instead of a high purity eplerenone starting material, (ii) seeding the solvent system with phase pure Form I crystals, or (iii) a combination of (i) and (ii).

A. Use Of Impurities As Growth Promoters and Inhibitors

The presence and amount of selected impurities in the eplerenone starting material, rather than the total amount of all impurities in the eplerenone starting material, affect the potential for Form I crystal formation during the desolvation of the solvate. The selected impurity generally is a Form I growth promoter or Form II growth inhibitor. It may be contained in the eplerenone starting material, contained in the solvent or mixture of solvents before the eplerenone starting material is added, and/or added to the solvent or mixture of solvents after the eplerenone starting material is added. Bonafede et al., "Selective Nucleation and Growth of an Organic Polymorph by Ledge-Directed Epitaxy on a Molecular Crystal Substate", J. Amer. Chem. Soc., Vol. 117, No. 30 (August 2, 1995) discusses the use of growth promoters and growth inhibitors in polymorph systems and is incorporated by reference herein. For the present invention, the impurity generally comprises a compound having a single crystal structure substantially identical to the single crystal structure of Form I. The impurity preferably is a compound

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having an X-ray powder diffraction pattern substantially identical to the X-ray powder diffraction pattern of Form I, and more preferably is selected from the group consisting of the diepoxide, the 11,12-epoxide, the 9,11-olefin and combinations thereof.

The amount of impurity needed to prepare Form I crystals typically can depend, in part, upon the solvent or mixture of solvents and the solubility of the impurity relative to eplerenone. In the crystallization of Form I from a methyl ethyl ketone solvent, for example, the weight ratio of diepoxide to low purity eplerenone starting material typically is at least about 1:100, preferably at least about 3:100, more preferably between about 3:100 and about 1:5, and still more preferably between about 3:100 and about 1:10. The 11,12-epoxide has a higher solubility in methyl ethyl ketone than the diepoxide and generally requires a larger amount of the 11,12-epoxide generally is necessary to prepare Form I crystals. Where the impurity comprises the 11,12-epoxide, the weight ratio of the diepoxide to the low purity eplerenone starting material typically is at least about 1:5, more preferably at least about 3:25, and still more preferably between about 3:25 and about 1:5. Where both the diexpoxide and the 11,12-epoxide impurities are used in the preparation of the Form I crystals, the weight ratio of each impurity to the eplerenone starting material may be lower than the corresponding ratio when only that impurity is used in the preparation of the Form I crystals.

A mixture of Form I and Form II is generally obtained when a solvate comprising the selected impurity is desolvated. The weight fraction of Form I in the product resulting from the initial desolvation of the solvate

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typically is less than about 50%. Further treatment of this product by crystallization or digestion, as discussed below, generally will increase the weight fraction of Form II in the product.

B. Seeding

Form I crystals also can be prepared by seeding the solvent system with phase pure Form I crystals (or a Form I growth promoter and/or Form II growth inhibitor as previously discussed above) prior to crystallization of the eplerenone. The eplerenone starting material can be either a low purity eplerenone or a high purity eplerenone. When the resulting solvate prepared from either starting material is desolvated, the weight fraction of Form I in the product typically is at least about 70% and may be as great as about 100%.

The weight ratio of Form I seed crystals added to the solvent system to the eplerenone starting material added to the solvent system generally is at least about 0.75:100, preferably between about 0.75:100 to about 1:20, and more preferably between about 1:100 to about 1:50. The Form I seed crystals can be prepared by any of the methods discussed in this application for the preparation of Form I crystals, particularly the preparation of Form I crystals by digestion as discussed below.

The Form I seed crystals may be added at one time, in multiple additions or substantially continually over a period of time. The addition of the Form I seed crystals, however, generally is completed before the eplerenone begins to crystallize from solution, i.e., the seeding is completed before the cloud point (the lower end of the metastable zone) is reached. Seeding typically is performed when the solution

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temperature ranges from about 0.5°C above the cloud point to about 10°C above the cloud point, preferably within about 2°C to about 3°C above the cloud point. As the temperature above the cloud point at which the seeds are added increases, the amount of seeding needed for crystallization of Form I crystals generally increases.

The seeding preferably occurs not only above the cloud point, but within the metastable zone. Both the cloud point and the metastable zone are dependent on the eplerenone solubility and concentration in the solvent or mixture of solvents. For a 12 volume dilution of methyl ethyl ketone, for example, the high end of the metastable zone generally is between about 70°C to about 73°C and the lower end of the metastable zone (i.e., the cloud point) is between about 57°C and 63°C. For a concentration of 8 volumes of methyl ethyl ketone, the metastable zone is even narrower because the solution is supersaturated. At this concentration, the cloud point of the solution occurs at about 75°C to about 76°C. Because the boiling point of methyl ethyl ketone is about 80° C under ambient conditions, seeding for this solution typically occurs between about 76.5°C and the boiling point.

An illustrative non-limiting example of seeding with Form I is set forth below in Example 7.

The crystallized eplerenone product obtained using a Form I growth promoter or Form II growth inhibitor, and/or Form I seeding generally comprises at least 2% Form I, preferably at least 5% Form I, more preferably at least 7% Form I, and still more preferably at least about 10% Form I.

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The remaining crystallized eplerenone product generally is Form II.

C. Form I Prepared By Grinding Eplerenone

In yet another alternative, it has been discovered that a small amount of Form I can be prepared by suitable grinding eplerenone. Concentrations of Form I in ground eplerenone as high as about 3% have been observed.

4. Preparation of Form II From Solvate Prepared From Low Purity Eplerenone

As discussed above, crystallization of low purity eplerenone to form a solvate followed by desolvation of the solvate generally yields a product comprising both Form I and Form II. A product having a greater Form II content can be prepared from low purity eplerenone in substantially the same manner as set forth above for the preparation of Form I by seeding the solvent system with phase pure Form II crystals, or by using a Form II growth promoter and/or Form I growth inhibitor. The seeding protocol and the weight ratio of the amount of Form II seed crystals added to the solvent system to the amount of the eplerenone starting material added to the solvent system generally are similar to those ratios previously discussed above for the preparation of Form I eplerenone by seeding with phase pure Form I crystals.

The crystallized eplerenone product prepared in this manner generally comprises at least 10% Form II, preferably at least 50% Form II, more preferably at least 75% Form II, more preferably at least 90% Form II, still more preferably at least about 95% Form II, and still more preferably substantially phase pure Form II.

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The seeding protocols described in this section and in the prior section relating to the preparation of Form I eplerenone also may allow for improved control of the particle size of the crystallized eplerenone.

5. Crystallization of Form II Directly From Solution

Form II eplerenone also can be prepared by the direct crystallization of eplerenone from a suitable solvent or mixture of solvents without the formation of an intermediate solvate and the accompanying need for desolvation. Typically, (i) the solvent has a molecular size that is incompatible with the available channel space in the solvate crystal lattice, (ii) the eplerenone and any impurities are soluble in the solvent at elevated temperatures, and (iii) upon cooling, results in the crystallization of the non-solvated Form II eplerenone. The solubility of eplerenone in the solvent or mixture of solvents generally is about 5 to about 200 mg/mL at room temperature. The solvent or mixture of solvents preferably comprises one or more solvents selected from the group consisting of methanol, ethyl acetate, isopropyl acetate, acetonitrile, nitrobenzene, water and ethyl benzene.

To crystallize Form II eplerenone directly from solution, an amount of the eplerenone starting material is solubilized in a volume of the solvent and cooled until crystals form. The solvent temperature at which the eplerenone is added to the solvent generally will be selected based upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, this solvent temperature typically is at least about 25°C, preferably from about 30°C to the boiling point of the

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solvent, and more preferably from about 25°C below the boiling point of the solvent to the boiling point of the solvent.

Alternatively, hot solvent may be added to the eplerenone and the mixture can be cooled until crystals form. The solvent temperature at the time it is added to the eplerenone generally will be selected based upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, the solvent temperature typically is at least 25°C, preferably from about 50°C to the boiling point of the solvent, and more preferably from about 15°C below the boiling point of the solvent to the boiling point of the solvent.

The amount of the eplerenone starting material mixed with a given volume of solvent likewise will depend upon the solubility curve of the solvent or mixture of solvents. Typically, the amount of eplerenone added to the solvent will not completely solubilize in that volume of solvent at room temperature. For most of the solvents described herein, for example, the amount of eplerenone starting material mixed with a given volume of solvent usually is at least about 1.5 to about 4.0 times, preferably about 2.0 to about 3.5 times, and more preferably about 2.5 times, the amount of eplerenone that will solubilize in that volume of solvent at room temperature.

To ensure the preparation of a product that comprises substantially phase pure Form II, the eplerenone starting material generally is a high purity eplerenone. The eplerenone starting material preferably is at least 65% pure, more preferably at least 90% pure, still more preferably at least 98% pure, and still more preferably at least 99% pure.

After the eplerenone starting material has completely solubilized in the solvent, the solution typically is cooled slowly to crystallize the solvated crystalline form of eplerenone. For most of the solvents described herein, for example, the solution is cooled at a rate slower than about 1.0°C/minute, preferably at a rate of about 0.2°C/minute or slower, and more preferably at a rate between about 5°C/minute and about 0.1°C/minute.

The endpoint temperature at which the Form II crystals are harvested will depend upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, the endpoint temperature typically is less than about 25°C, preferably less than about 5°C, and more preferably less than about -5°C.

Alternatively, other techniques may be used to prepare the Form II crystals. Examples of such techniques include, but are not limited to, (i) dissolving the eplerenone starting material in one solvent and adding a co-solvent to aid in the crystallization of Form II eplerenone, (ii) vapor diffusion growth of Form II eplerenone, (iii) isolation of Form II eplerenone by evaporation, such as rotary evaporation, and (iv) slurry conversion.

The crystals of the solvated crystalline form prepared as described above can be separated from the solvent by any suitable conventional means such as by filtration or centrifugation.

In addition, Form II eplerenone also can be prepared by digesting (as described below) a slurry of high purity eplerenone in methyl ethyl ketone and filtering the digested eplerenone at the boiling point of the slurry.

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6. Preparation of Form I Directly From Solution

It is hypothesized that if the crystallization is performed above the enantiotropic transition temperature (T_t) for Form I and Form II, particularly if Form I growth promoters or Form II growth inhibitors are present or the solvent is seeded with phase pure Form I crystals, Form I should crystallize directly from solution since Form I is more stable at these higher temperatures. The solvent system used preferably comprises a high boiling solvent such as nitrobenzene. Suitable Form I growth promoters would include, but would not be limited to, the diepoxide and the 11,12-olefin.

7. Digestion of Eplerenone With A Solvent

The solvated crystalline forms, Form I and Form II of eplerenone also can be prepared by digestion of an eplerenone starting material in a suitable solvent or mixture of solvents. In the digestion process, a slurry of eplerenone is heated at the boiling point of the solvent or mixture of solvents. For example, an amount of eplerenone starting material is combined with a volume of solvent or mixture of solvents, heated to reflux, and the distillate is removed while an additional amount of the solvent is added simultaneously with the removal of the distillate. Alternatively, the distillate can be condensed and recycled without the addition of more solvent during the digestion process. Typically, once the original volume of solvent has been removed or condensed and recycled, the slurry is cooled and solvated crystals form. The solvated crystals can be separated from the solvent by any suitable conventional means such as by filtration or centrifugation. Desolvation of the

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solvate as previously described yields either Form I or Form II eplerenone depending upon the presence or absence of the selected impurities in the solvated crystals.

A suitable solvent or mixture of solvents generally comprises one or more of the solvents previously disclosed herein. The solvent may be selected, for example, from the group consisting of methyl ethyl ketone and ethanol.

The amount of eplerenone starting material added to the solvent used in the digestion process generally is sufficient to maintain a slurry (i.e., the eplerenone in the solvent or mixture of solvents is not completely solubilized) at the boiling point of the solvent or mixture of solvents. Illustrative values include, but are not limited to, about one gram of eplerenone per four mL methyl ethyl ketone and about one gram of eplerenone per eight mL ethanol.

The solution generally is cooled slowly once solvent turnover is complete to crystallize the solvated crystalline form of eplerenone. For the solvents tested, for example, the solution is cooled at a rate slower than about 20°C/minute, preferably about 10°C/minute or slower, more preferably about 5°C/minute or slower, and still more preferably about 1°C/minute or slower.

The endpoint temperature at which the solvated crystalline form is harvested will depend upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, the endpoint temperature typically is less than about 25°C, preferably less than about 5°C, and more preferably less than about -5°C.

If a product comprising primarily or exclusively Form II is desired, a high purity eplerenone starting material

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typically is digested. The high purity eplerenone starting material preferably is at least 98% pure, more preferably at least 99% pure, and still more preferably at least 99.5% pure. The digested eplerenone product prepared in this manner generally comprises at least 10% Form II, preferably at least 50% Form II, more preferably at least 75% Form II, more preferably at least 90% Form II, still more preferably at least about 95% Form II, and still more preferably substantially phase pure Form II.

If a product comprising primarily or exclusively Form I is desired, a low purity eplerenone starting material typically is digested. The low purity eplerenone starting material generally contains only as much Form I growth promoter and/or Form II growth inhibitor as is needed to yield Form I. Preferably, the low purity eplerenone starting material is at least 65% pure, more preferably at least 75% pure, and still more preferably at least 80% pure. The digested eplerenone product prepared in this manner generally comprises at least 10% Form I, preferably at least 50% Form I, more preferably at least 75% Form I, more preferably at least 90% Form I, still more preferably at least about 95% Form I, and still more preferably substantially phase pure Form I.

8. Preparation of Amorphous Eplerenone

Amorphous eplerenone can be prepared in small quantities by suitable comminution of solid eplerenone, such as by crushing, grinding and/or micronizing. Phase pure amorphous eplerenone can be prepared, for example, by lyophilizing a solution of eplerenone, particularly an aqueous solution of

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eplerenone. These processes are illustrated in Examples 13 and 14 below.

Additional Processing Considerations

1. Thermodynamic Stability Considerations

Form II is more thermodynamically stable than Form I at ambient temperatures. As described in Example 5 below, when an organic slurry containing equivalent amounts of Form I and Form II was allowed to stand overnight at room temperature and the residual solids were then collected and analyzed by X-ray powder diffraction, the analytical results indicated that the eplerenone had completely converted to Form II. The differential scanning calorimetry ("DSC") data (discussed previously) indicate that Form I is more thermodynamically stable than Form II at higher temperatures since it has the higher melting/decomposition temperature. Taken together, the slurry conversion and DSC data indicate that Form I and Form II are related enantiotropically, i.e., a change in the stability relationship between the two polymorphs occurs around a transition temperature (T_t), with Form II being the more stable at lower temperatures. Fig. 12 shows the Gibbs free energy-temperature relationship generally observed for enantiotropically related polymorphs such as Form I and Form II, wherein I and II refer to the crystal forms, T_t refers to the transition temperature, T_m refers to the melting points of Form I and Form II, and L represents the liquid or melt state.

Accordingly, processing temperatures preferably are maintained below the transition temperature during the preparation of compositions comprising Form II. For example, drying temperatures employed for desolvation typically are

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less than about 150°C, preferably less than about 125°C, more preferably less than about 115°C, more preferably less than about 110°C, and still more preferably between about 80°C to about 110°C. In addition, cooling (such as by use of liquid nitrogen) may be necessary during particle size reduction process steps to maintain the temperature of the Form II crystals below the transition temperature.

2. Intrinsic Micronizing Considerations

The method used to prepare crystalline eplerenone may affect the properties of the resulting crystal. For example, Form II prepared by desolvation of a solvate exhibits a higher incidence of surface defects, pores, cracks and fractures within the crystal lattice than Form II prepared by direct crystallization from solution. This "intrinsic micronizing" of the desolvated crystal results in an increase in both the available surface area of the crystal and the dissolution rate of the crystal. Dissolution times, therefore, can be shortened by selection of Form II crystals prepared by desolvation, lengthened by selection of Form II crystals prepared by direct crystallization, or otherwise adjusted by selection of an appropriate combination of Form II crystals prepared by desolvation and Form II crystals prepared by direct crystallization.

This "intrinsic micronizing" also may effectively reduce or eliminate the need for crystal particle size reduction during processing steps where Form II crystals prepared by desolvation are used in the preparation of the pharmaceutical composition. One disadvantage to using such Form II crystals, however, is the need for a desolvation step that is

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not required for Form II crystals prepared by direct crystallization.

Product-By-Process Solid State Forms

The present invention also comprises each of the solid state eplerenone forms prepared in accordance with the processes disclosed in this application. In particular, the invention comprises solvated eplerenone, Form I eplerenone, Form II eplerenone, and combinations thereof, prepared as set forth in this application.

Combinations of Solid State Forms

Also embraced within this invention are combinations comprising a first solid state form of eplerenone and a second solid state form of eplerenone, wherein the first and second solid state forms of eplerenone are selected from Form I, Form II, solvated eplerenone and amorphous eplerenone. In genaral, the weight ratio of said first solid state form to said second solid state form preferably is at least about 1:9, more preferably is at least about 1:1, more preferably at least about 2:1, more preferably at least about 5:1, and still more preferably at least about 9:1.

In one embodiment, for example, the first solid state form is Form I and the second solid state form is Form II.

In another embodiment, for example, the first solid state form is Form II and the second solid state form is Form I.

Pharmaceutical Compositions

Also embraced within this invention is a class of pharmaceutical compositions comprising a first solid state

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form of eplerenone and a second solid state form of eplerenone in association with one or more pharmaceutically-acceptable carriers and/or diluents and/or adjuvants (collectively referred to herein as "carrier" materials) and, if desired, other active ingredients. The first solid state form of eplerenone and the second solid state form of eplerenone are selected from Form I, Form II, solvated eplerenone and amorphous eplerenone. In general, the weight ratio of said first solid state form to said second solid state form preferably is at least about 1:9, more preferably is at least about 1:1, more preferably at least about 2:1, more preferably at least about 5:1, and still more preferably at least about 9:1.

In one embodiment of the invention, the entire amount of eplerenone contained in the composition is present as phase pure Form II.

In another embodiment of the invention, the entire amount of eplerenone contained in the composition is present as phase pure Form I.

In still another embodiment of the invention, the entire amount of eplerenone contained in the composition is present as phase pure solvated crystalline eplerenone.

In still another embodiment of the invention, the entire amount of eplerenone contained in the composition is present as amorphous eplerenone.

In still another embodiment of the invention, the composition comprises both Form I and Form II. The ratio of the amount of Form II to Form I in the composition generally is between about 1:20 to about 20:1. In other embodiments, for example, this ratio is between about 10:1 to about 1:10;

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about 5:1 to about 1:5; about 2:1 to about 1:2; or is about 1:1.

In still another embodiment of the invention, the composition comprises a first solid state form of eplerenone and a second solid state form of eplerenone, wherein the first and second solid state forms of eplerenone are selected from Form I, Form II, solvated eplerenone and amorphous eplerenone. In general, the weight ratio of said first solid state form to said second solid state form preferably is at least about 1:9, preferably about 1:1, more preferably at least about 2:1, more preferably at least about 5:1, and still more preferably at least about 9:1.

In each of the above combination and pharmaceutical composition embodiments, the D_{90} particle size of the eplerenone used as a starting material in the pharmaceutical composition generally is between about 10 nm to about 15 microns, preferably less than about 1 micron, more preferably less than about 800 nm, still more preferably less than about 600 nm, and still more preferably less than about 400 nm. In one embodiment, the D_{90} particle size is between about 10 nm to about 1 micron. In another embodiment, the D_{90} particle size is between about 100 nm to about 800 nm. In another embodiment, the D_{90} particle size is between about 200 nm to about 600 nm.

These solid state forms of eplerenone of the present invention may be administered by any suitable route, preferably in the form of a pharmaceutical composition adapted to such a route, and in a dose effective for the treatment intended. The solid state forms of eplerenone and compositions thereof may, for example, be administered orally, intravascularly, intraperitoneally, subcutaneously,

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intramuscularly or topically.

The amount of eplerenone that is administered and the dosage regimen for treating a condition or disorder with the solid state forms of eplerenone and/or compositions of this invention depends on a variety of factors, including the age, weight, sex and medical condition of the subject, the severity of the disease, the route and frequency of administration, and the particular compound employed, and thus may vary widely. The pharmaceutical compositions may contain eplerenone in the range of about 0.1 to 2000 mg, preferably in the range of about 0.5 to 500 mg and most preferably between about 1 and 200 mg. A daily dose of about 0.01 to 100 mg/kg body weight, preferably between about 0.5 and about 20 mg/kg body weight and most preferably between about 0.1 to 10 mg/kg body weight, may be appropriate. The daily dose can be administered in one to four doses per day.

For oral administration, the pharmaceutical composition may be in the form of, for example, a tablet, capsule, or suspension. The pharmaceutical composition is preferably made in the form of a dosage unit containing a particular amount of eplerenone. Examples of such dosage units are tablets or capsules. The eplerenone may also be administered by injection of a suspension wherein, for example, saline, dextrose or water may be used as a suitable carrier.

Other formulations may be in the form of a topical ointment or cream, or a suppository, containing eplerenone in a total amount of, for example, 0.075 to 30% w/w, preferably 0.2 to 20% w/w and most preferably 0.4 to 15% w/w. When formulated in an ointment, the eplerenone may be employed with either paraffinic or a water-miscible ointment base. Alternatively, the eplerenone may be formulated in a cream

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with an oil-in-water cream base. If desired, the aqueous phase of the cream base may include, for example at least 30% w/w of a polyhydric alcohol such as propylene glycol, butane-1,3-diol, mannitol, sorbitol, glycerol, polyethylene glycol and mixtures thereof. The topical formulation may desirably include a compound which enhances absorption or penetration of the eplerenone through the skin or other affected areas. Examples of such dermal penetration enhancers include dimethylsulfoxide and related analogs.

The novel solid state forms of eplerenone also can be administered by a transdermal device. Preferably, topical administration will be accomplished using a patch either of the reservoir and porous membrane type or of a solid matrix variety. In either case, the eplerenone is delivered continuously from the reservoir or microcapsules through a membrane into the eplerenone permeable adhesive, which is in contact with the skin or mucosa of the recipient. If the eplerenone is absorbed through the skin, a controlled and predetermined flow of the eplerenone is administered to the recipient. In the case of microcapsules, the encapsulating agent may also function as the membrane.

The oily phase of the emulsions of this invention may be constituted from known ingredients in a known manner. While the phase may comprise merely an emulsifier, it may comprise a mixture of at least one emulsifier with a fat or an oil or with both a fat and an oil. Preferably, a hydrophilic emulsifier is included together with a lipophilic emulsifier which acts as a stabilizer. It is also preferred to include both an oil and a fat. Together, the emulsifier(s) with or without stabilizer(s) make-up the so-called emulsifying wax, and the wax together with the oil and fat make up the so-

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called emulsifying ointment base which forms the oily dispersed phase of the cream formulations. Emulsifiers and emulsion stabilizers suitable for use in the formulation of the present invention include Tween 60, Span 80, cetostearyl alcohol, myristyl alcohol, glyceryl monostearate, and sodium lauryl sulfate, among others.

The choice of suitable oils or fats for the formulation is based on achieving the desired cosmetic properties, since the solubility of the eplerenone in most oils likely to be used in pharmaceutical emulsion formulations is very low. Thus, the cream should preferably be a non-greasy, non-staining and washable product with suitable consistency to avoid leakage from tubes or other containers. Straight or branched chain, mono- or dibasic alkyl esters such as di-isoadipate, isocetyl stearate, propylene glycol diester of coconut fatty acids, isopropyl myristate, decyl oleate, isopropyl palmitate, butyl stearate, 2-ethylhexyl palmitate or a blend of branched chain esters may be used. These may be used alone or in combination depending on the properties required. Alternatively, high melting point lipids such as white soft paraffin and/or liquid paraffin or other mineral oils can be used.

Formulations suitable for topical administration to the eye also include eye drops wherein the eplerenone is suspended in suitable carrier, especially an aqueous carrier for the eplerenone. The eplerenone is preferably present in such formulations in a concentration of 0.5 to 20%, advantageously 0.5 to 10% and particularly about 1.5% w/w.

For therapeutic purposes, the novel solid state forms of eplerenone ordinarily are combined with one or more adjuvants appropriate to the indicated route of administration. If

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administered per os, the compound may be admixed with lactose, sucrose, starch powder, cellulose esters of alkanoic acids, cellulose alkyl esters, talc, stearic acid, magnesium stearate, magnesium oxide, sodium and calcium salts of phosphoric and sulfuric acids, gelatin, acacia gum, sodium alginate, polyvinylpyrrolidone, and/or polyvinyl alcohol, and then tableted or encapsulated for convenient administration. Such capsules or tablets may contain an immediate release formulation, or may contain a controlled-release formulation such as a dispersion of the eplerenone in hydroxypropylmethyl cellulose. Formulations for parenteral administration may be in the form of aqueous or non-aqueous isotonic sterile injection suspensions. These suspensions may be prepared from sterile powders or granules having one or more of the carriers or diluents mentioned for use in the formulations for oral administration. Other adjuvants and modes of administration are well and widely known in the pharmaceutical art.

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Methods of Treatment or Prophylaxis

The present invention also comprises methods for the treatment and/or prophylaxis of an aldosterone-mediated condition and/or disorder such as hypertension in a subject, the methods comprising treating the subject having or susceptible to such condition or disorder with a therapeutically-effective amount of Form I, Form II, solvated crystalline form, or an amorphous form of eplerenone, or combinations thereof. These solid state forms of eplerenone would be useful for the treatment and/or prophylaxis of a condition and/or disorder in a subject where administration of an aldosterone-antagonist is indicated including, but not

limited to, the treatment of hyperaldosteronism such as hypertension, heart failure including cardiac insufficiency, cirrhosis of the liver, excess collagen/fibrosis, benign prostate hypertrophy and depression.

Besides being useful for human treatment, these solid state forms of eplerenone are also useful for veterinary treatment of companion animals, exotic animals and farm animals, including mammals, rodents, and the like. More preferred animals include horses, dogs, and cats.

These solid state forms of eplerenone also may be used (i) in combination therapies partially or completely in place of other conventional aldosterone receptor antagonists, or (ii) in combination therapies with other active drugs. The phrase "combination therapy" is intended to embrace the administration of each agent in a sequential manner in a regimen that will provide beneficial effects of the drug combination, and is intended as well to embrace co-administration of these agents in a substantially simultaneous manner, such as in a single capsule or injection having a fixed ratio of these active agents or in multiple, separate capsules or injections for each agent. Non-limiting examples of combination therapy include the treatment of cardiovascular diseases using a combination of an aldosterone receptor antagonist and an angiotensin II receptor antagonist as described in WO96/24373; the treatment of congestive heart failure using a combination of an aldosterone receptor antagonist and an angiotensin II antagonist as described in WO96/40257; and the treatment of heart failure using a combination of an aldosterone receptor antagonist, an ACE inhibitor and a diuretic as described in WO96/24372.

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Working Examples

The following examples contain detailed descriptions of the methods of preparation of the various solid state forms of eplerenone described in this application. These detailed descriptions fall within the scope, and serve to exemplify the invention. These detailed descriptions are presented for illustrative purposes only and are not intended as a restriction on the scope of the invention. All parts are by weight and temperatures are in degrees Centigrade unless otherwise indicated. The eplerenone starting material used in each of the following examples was prepared in accordahce with scheme 1 set forth in Ng et al., WO98/25948.

Example 1: Preparation of (a) methyl ethyl ketone solvate from high purity eplerenone starting material and (b) Form II crystalline eplerenone from resulting solvate

A. Preparation of Methyl Ethyl Ketone Solvate:

High purity eplerenone (437 mg; greater than 99% purity with less than 0.2% diepoxide and 11,12 epoxide present) was dissolved in 10 mL of methyl ethyl ketone by heating to boiling on a hot plate with magnetic stirring at 900 rpm. The resulting solution was allowed to cool to room temperature with continuous magnetic stirring. Once at room temperature, the solution was transferred to a 1°C bath with maintenance of the stirring for one hour. After one hour, the solid methyl ethyl ketone solvate was collected by vacuum filtration.

B. Preparation of Form II crystalline eplerenone:

The solid methyl ethyl ketone solvate prepared in Step A above was dried in an oven at 100°C for four hours at ambient

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pressure. The dried solid was determined to be pure Form II by DSC and XPRD analysis.

Example 2: Preparation of additional solvates from high purity eplerenone starting material

Additional solvated crystalline forms were prepared by replacing methyl ethyl ketone with one of the following solvents: n-propanol, 2-pentanone, acetic acid, acetone, butyl acetate, chloroform, ethanol, isobutanol, isobutyl acetate, isopropanol, methyl acetate, ethyl propionate, n-butanol, n-octanol, propyl acetate, propylene glycol, t-butanol, tetrahydrofuran, and toluene and carrying out the crystallization substantially as described above in Step A of Example 1.

Example 3: Preparation of Methyl Ethyl Ketone Solvate by Vapor Diffusion Growth

Eplerenone (400 mg; greater than 99.9% purity) was dissolved in 20 mL of methyl ethyl ketone by warming on a hot plate to form a stock solution. An 8 mL amount of the stock solution was transferred to a first 20 mL scintillation vial and diluted to 10 mL with methyl ethyl ketone (80%). A 10 mL amount of the stock solution was transferred to a second 20 mL scintillation vial and diluted to 10 mL with methyl ethyl ketone (40%). The final 2 mL of the stock solution was diluted to 10 mL with methyl ethyl ketone (20%). The four vials containing the dilutions were transferred to a dessicator jar containing a small amount of hexane as an anti-solvent. The dessicator jar was sealed and the hexane vapor allowed to diffuse into the methyl ethyl ketone

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solutions. Methyl ethyl ketone solvate crystals grew in the 80% dilution sample by the next day.

Example 4: Preparation of Methyl Ethyl Ketone Solvate by Rotary Evaporation

About 400 mg of eplerenone (greater than 99.9% purity) is weighed into a 250 mL round bottom flask. Solvent (150 mL) is added to the flask and, if necessary, the solution is heated gently until the solid is dissolved. The resulting clear solution is placed on a Buchi rotary evaporator with a bath temperature of about 85°C and the solvent is removed under vacuum. Solvent removal is stopped when approximately 10 mL of solvent remain in the round bottom flask. The resulting solids are analyzed by appropriate method (XPRD, DSC, TGA, microscopy, etc.) for determination of form.

Example 5: Slurry Conversion

Approximately 150 mg of Form II eplerenone and 150 mg of Form I eplerenone were added to 5 mL of ethyl acetate. The resulting slurry was allowed to stir at 300 rpm (magnetic stirring) overnight. The next day a sample of the solid was collected by filtration. Analysis of the sample by XPRD indicated that the sample was entirely composed of Form II eplerenone.

Example 6: Preparation of (a) solvate from low purity eplerenone starting material and (b) Form I crystalline eplerenone from resulting solvate

Samples containing varying amounts of the impurity 7-methyl hydrogen 4α,5α:9α,11α-diepoxy-17-hydroxy-3-oxo-17α-pregnane-7α,21-dicarboxylate, γ-lactone (the "diepoxide") or

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the impurity 7-methyl hydrogen 11α,12α-epoxy-17-hydroxy-3-oxo-17α-pregn-4-ene-7α,21-dicarboxylate, γ-lactone (the "11,12-epoxide") were prepared by adding the desired amount of the impurity to a 7 mL scintillation vial together with an amount of eplerenone sufficient to provide a total sample mass of 100 mg. The weight percent of the diepoxide or 11,12-epoxide in each sample is given in Tables X-6A and X-6B, respectively. A micro-flea magnetic stirrer was added to each scintillation vial along with 1 mL of methyl ethyl ketone. The vials were loosely capped and the solid dissolved by heating to reflux on a hot plate with magnetic stirring. Once the solids were dissolved, the solutions were allowed to cool to room temperature on the hot plate. Magnetic stirring was maintained during the cooling period. After the solutions reached room temperature, the solids were collected by vacuum filtration and immediately analyzed by X-ray powder diffraction (XPRD). The solids were then placed in a 100°C oven and dried for one hour at ambient pressure. The dried solids were analyzed by XPRD for Form I content by monitoring the area of the Form I diffraction peak at about 12.1 degrees two theta. All XPRD diffraction patterns were recorded using an Inel Multipurpose Diffractometer.

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TABLE X-6A

Weight Percent Diepoxide	Weight Eplerenone (mg)	Weight Diepoxide (mg)
0%	100.44	--
1%	99.08	1.24
2%	98.09	2.24
3%	97.08	3.04
5%	95.09	5.04

TABLE X-6B

Weight Percent 11,12-Epoxide	Weight Eplerenone (mg)	Weight 11,12-Epoxide (mg)
0%	101.38	0
1%	99.23	1.10
5%	94.97	5.36
10%	90.13	10.86

A. Diepoxide Results

Fig. 13 shows the X-ray powder diffraction patterns for the wet cake (methyl ethyl ketone solvate) obtained from the (a) 0%, (b) 1%, (c) 3%, and (d) 5% diepoxide-doped methyl

ethyl ketone crystallizations. The peak intensities have been normalized for ease of comparison. No peaks characteristic of Form I or the diepoxide are present in the diffraction patterns. The patterns are characteristic of the methyl ethyl ketone solvate of eplerenone.

Fig. 14 shows the X-ray powder diffraction patterns for the dried solids obtained from the (a) 0%, (b) 1%, (c) 3%, and (d) 5% diepoxide-doped methyl ethyl ketone crystallizations. The peak intensities have been normalized for ease of comparison. No Form I was detected for the dried samples corresponding to the methyl ethyl ketone crystallizations performed at doping levels of 0 and 1%. Form I was detected in the dried samples corresponding to the methyl ethyl ketone crystallizations performed at doping levels of 3 and 5%. The area for the Form I diffraction peak at about 12.1 degrees two theta and the estimated Form I content for each sample are given in Table X-6C below.

TABLE X-6C

Weight Percent of Diepoxide in Starting Eplerenone Mixture	Weight Percent of Diepoxide in Resulting Crystals (HPLC)	Form I Peak Area 12° Two Theta Peak	Estimated Weight Percent of Form I
0%	---	None Detected	None Detected
1%	0.29%	None Detected	None Detected
3%	0.58%	1168	10%
5%	1.05%	4175	30%

The results reported in Table X-6C confirm that the presence of the diepoxide affects the formation of Form I during the desolvation. These results indicate that the

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diepoxide is effective in inducing the formation of Form I eplerenone when it is incorporated into and/or adsorbed onto the methyl ethyl ketone solvate crystals.

The 3% diepoxide doping experiment was repeated to analyze the impact of the route of preparation on the amount of Form I formed during the desolvation. In this experiment, the methyl ethyl ketone solvate obtained from the doped crystallization was divided into two portions. The first portion was left untreated while the second portion was lightly ground in a mortar and pestle to induce a higher level of crystal defects. The two portions were both dried at 100 °C for one hour at ambient pressure. The dried solids were analyzed by XPRD. The XPRD patterns are given in Fig. 15 for the dried solids from the methyl ethyl ketone crystallization with 3% doping of diepoxide (a) without grinding of the solvate prior to drying, and (b) with grinding of the solvate prior to drying. The XPRD patterns indicated a greater amount of Form I in the ground sample relative to the unground sample. These results suggest that the conditions under which the methyl ethyl ketone solvate is isolated and handled can affect the crystal form that results from the desolvation.

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B. 11,12-Epoxide Results

Fig. 16 shows the X-ray powder diffraction patterns for the wet cake (methyl ethyl ketone solvate) obtained from the (a) 0%, (b) 1%, (c) 5%, and (d) 10% 11,12-epoxide-doped methyl ethyl ketone crystallizations. The peak intensities have been normalized for ease of comparison. No peaks characteristic of Form I or the 11,12-epoxide are present in the diffraction

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patterns. The patterns are characteristic of the methyl ethyl ketone solvate of eplerenone.

Fig. 17 shows the X-ray powder diffraction patterns for the dried solids obtained from the (a) 0%, (b) 1%, (c) 5%, and (d) 10% 11,12-epoxide-doped methyl ethyl ketone crystallizations. The peak intensities have been normalized for ease of comparison. No Form I was detected for the dried samples corresponding to the methyl ethyl ketone crystallizations performed at doping levels of 0, 1% and 5%. Form I was detected in the dried samples corresponding to the methyl ethyl ketone crystallization performed at a doping level of 10%. The area for the Form I diffraction peak at 12.1 degrees two theta and estimated Form I content for each sample are given in Table X-6D.

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TABLE X-6D

Weight Percent 11,12-Epoxide in Starting Eplerenone Mixture	Weight Percent 11,12-Epoxide in Resulting Crystals (HPLC)	Form I Peak Area 12° Two Theta Peak	Estimated Weight Percent of Form I
0%	Not Available	None Detected	None Detected
1%	Not Available	None Detected	None Detected
5%	Not Available	None Detected	None Detected
10%	Not Available	1541	10-15%

The results reported in Table X-6D confirm that the presence of the 11,12-epoxide impacts the formation of Form I during the desolvation. The percentage of impurity in the methyl ethyl ketone crystallization required to induce the formation of Form I eplerenone appears to be greater for the 11,12-epoxide than for the diepoxide.

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Example 7: Effect of Crystallization and Drying on Final Crystal Form

The following four experiments analyzing the effect of crystallization and drying on the final crystal form were conducted: (i) methyl ethyl ketone crystallization of eplerenone (2^3+3 statistical design of experiment), (ii) crystallization of poor quality mother liquor residue, (iii) crystallization of high purity eplerenone with form I seeding, and (iv) crystallization of low purity eplerenone with form II seeding. Variables in the design of the experiments included cooling rate, starting material purity level, and end point temperature of crystallization. For purposes of this Example, high purity eplerenone was defined as ultra-pure milled eplerenone (HPLC analysis showed this material to be 100.8% pure) and low purity eplerenone was defined as 89% pure eplerenone. To prepare the low purity eplerenone, stripped-down mother liquors from the process for the preparation of eplerenone were analyzed and blended to yield a material that was 61.1% eplerenone, 12.8% diepoxide and 7.6% 11,12-epoxide. This material was then blended with a sufficient amount of high purity eplerenone to yield the 89% eplerenone.

A. Methyl Ethyl Ketone Crystallization

In the methyl ethyl ketone crystallization experiment, all runs were performed using 60 g of high purity eplerenone. High endpoint was defined as 45°C and low endpoint was defined as 5°C. High cooling rate was defined as 3°C/minute cooling and low cooling rate was defined as 0.1°C/minute cooling. Center points were 1.5°C/minute cooling, 94.5% pure eplerenone, and a 25°C endpoint.

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After a background reading was taken with the FTIR, 250 mL of methyl ethyl ketone was charged to a 1L Mettler RC-1, MP10 reactor and stirred at 100 rpm. After several scans, eplerenone was charged to the reactor followed by an additional 470 mL of methyl ethyl ketone. Agitation was increased to 500 rpm to suspend solids and the batch temperature was increased to 80°C. The batch temperature was held at 80°C to ensure dissolution of the eplerenone. Black or white specks generally were visible in the resulting transparent solution. The batch temperature was then ramp cooled at the desired rate to the desired endpoint, where it was maintained for one hour before being pulled into a transfer flask and filtered. The vacuum was reactor, transfer flask and cake were then washed with 120 mL methyl ethyl ketone. Once the wash was pulled through the cake, the stopped. About 10 grams of each wet cake were dried in a vacuum oven under nominal conditions of 75°C with a light nitrogen bleed. For the "high, high, high" and "low, low, low" experiments described below, fluid bed drying was operated under high and low conditions. High fluid bed drying was defined as 100°C with a blower setting of "4" while low fluid bed drying was defined as 40°C with a blower setting of "1".

B. Crystallization of Poor Quality Mother Liquor Residue

In the crystallization of poor quality mother liquor residue experiment, 60 g of the 61.1% pure material and 720 mL methyl ethyl ketonewere charged directly to a 1L Mettler RC-1, MP10 reactor. The 61.1% pure material was not blended with high purity eplerenone prior to being charged to the reactor. The resulting mixture was heated to 80°C and was an opaque

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slurry at that temperature. The crystallization continued and the mixture was filtered at 45°C under fast cooling conditions.

C. Form I Seeding

In the Form I seeding experiment, 60 g of pure (100.8%) eplerenone and 720 mL of methyl ethyl ketone were charged to a 1L Mettler RC-1, MP10 reactor. The mixture was heated to 80°C and then cooled to 25°C at a rate of 1.5° C/minute. When the solution had cooled to 62°C, it was seeded with 3 g of phase pure Form I crystals to initiate crystallization. The Form I seed crystals were prepared by the digestion process described in Example 9 below.

D. Form II Seeding

In the Form II seeding experiment, 66.6 g of 89.3% eplerenone (prepared by mixing 48.3 g of 100% eplerenone with 18.3 g of 61.1% eplerenone) and 720 mL of methyl ethyl ketone were charged to a 1L Mettler RC-1, MP10 reactor. The mixture was heated to 80°C and then cooled to 25°C at a rate of 1.5° C/minute. When the solution had cooled to 63°C, it was seeded with 3 g of phase pure Form II crystals to initiate crystallization. The Form II seed crystals were prepared by the crystallization and desolvation process described in Example 1 above.

Results from the experiments are reported in Table X-7A. In the n+1 crystallization experiment, Form I was detected only in the experiments employing low purity eplerenone where the product contained the diepoxide. Elevated levels of the diepoxide in the final product were also observed with higher cooling rates.

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The crystallization of poor quality mother liquor residue experiment yielded poor quality material that appeared to be a mixture of the diepoxide and Form I when analyzed by X-ray powder diffraction.

The Form I seeding experiment (where high purity eplerenone was seeded with Form I) yielded a product that was 77% Form I based on X-ray powder diffraction analysis, but entirely Form I based on DSC. The X-ray powder diffraction model, however, had not been tested for linearity beyond about 15% Form I. This experiment was the only one of the four experiments of this Example where Form I was created in the absence of the diepoxide.

The Form II seeding experiment (where low purity eplerenone was seeded with Form II) yielded a product that was entirely Form II.

The data obtained for the high fluid bed drying of eplerenone appeared to correspond to the data obtained for the vacuum oven drying. The low fluid bed dryings yielded results that differed from those of the vacuum oven dryings.

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TABLE X-7A

Cooling Rate[1]	Cooling Endpoint[2]	Impurity Level[3]	Nucleation Temperature (°C)	Weight Percent 11,12-Epoxide[4]	Weight Percent Diepoxide[4]	Assay For Desolvated Crystal	Percent Yield	Weight Percent Form I (XPRD)
+	+	-	57.0	ND	ND	100.3	66.1	ND
+	-	-	54.9	ND	ND	100.3	98.1	ND
-	+	-	60.9	ND	ND	100.3		ND
-	-	-	63.4	ND	ND	100.5	79.3	ND
+	+	++	N/A	4.8	36.6	43.3	27	100[5]
+	+	+	52.2	0.49	0.88	98.3	62	29
+	-	+	53.3	0.56	1.0	98.1	87	9
0	0	0	59.0	0.18	0.36	99.4	75	5
-	+	+	63.3	0.20	0.44	99.4	36	31
-	-	+	61.4	0.18	0.40	99.5	87	ND
0	0	0	60.6	0.18	0.36	99.5	79.2	ND
0	0	0	55.9	0.38	0.80	98.6	80.5	<3%
0	0	100.8% eplerenone seeded with Form I		0.03	ND	100.4	82.2	77/100[6]
0	0	89.3% eplerenone seeded with Form II		0.33	0.50	97.5	80.2	ND

[1]Cooling Rate: (+) = 3°C/min.; (0) = 1.5 °C/min.; and (-) = 0.1 °C/min.

[2]Cooling Endpoint: (+) = 45°C; (0) = 25 °C; and (-) = 5°C.

[3]Impurity Level: : (+) = 89.3% purity eplerenone starting material; (++) = 61.1% purity eplerenone starting material; (0) = 100.8% purity eplerenone starting material; and (-) = 94.5% purity eplerenone starting material.

[4]Weight percent after drying solvate in a vacuum oven at 75°C.

[5]Appears to be mixture of Form I and diepoxide when analysed by XPRD.

[6]Appears to be 77% Form I when analysed by XPRD and 100% Form I when analysed by DSC.

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A. Material Purity

A cube plot of product purity, starting material purity, cooling rate and endpoint temperature based on the data reported in Table X-7A is shown in Fig. 18. The cube plot suggests that the use of a higher purity material at the start of crystallization will yield a higher purity product. The endpoint temperature of crystallization does not appear to greatly affect the product purity. The cooling rate, however, appears to have an effect with slightly less pure product resulting from a faster cooling rate. In fact, the level of diepoxide generally was higher with faster cooling rates.

Fig. 19 shows a half normal plot that was prepared using the results of cube plot to determine which variables, if any, had a statistically significant effect on the product purity. Starting material purity had the greatest statistically significant effect on product purity, although cooling rate and the interaction between cooling rate and starting material purity were also seen as statistically significant effects.

Fig. 20 is an interaction graph based on these results and showing the interaction between starting material purity and cooling rate on product purity. With the high purity eplerenone (100.8% eplerenone starting material) the cooling rate appears to have little or no effect on final purity. With the low purity eplerenone (89.3% eplerenone starting material), however, the product purity decreases as cooling rate increases. This result suggests that more impurities crystallize out in eplerenone crystallizations conducted at higher cooling rates.

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B. Form I Content

A cube plot of Form I weight fraction, starting material product purity, cooling rate and endpoint temperature based on the data reported in Table X-7A is shown in Fig. 21. The cube plot suggests that the use of a higher purity eplerenone at the start of crystallization will yield a lower amount of Form I. The endpoint temperature of crystallization also appears to have an effect on the form of the final product. The cooling rate does not appear to greatly affect the formation of Form I although some Form I may result from faster cooling at the low endpoint temperature in the presence of impurities.

Fig. 22 shows a half normal plot that was prepared using the results of the cube plot to determine which variables, if any, had a statistically significant effect on the amount of Form I in the final material. Starting material purity, endpoint temperature of the crystallization and the interaction between the two variables were seen as statistically significant effects.

Fig. 23 is an interaction graph based on these results and showing the interaction between starting material purity and endpoint temperature on final Form I content. With the high purity eplerenone (100.8% eplerenone starting material), endpoint temperature appears to have little effect on Form I content. No Form I resulted in either case with pure eplerenone. With low purity eplerenone (89.3% eplerenone starting material), however, Form I was present in both cases, with significantly more Form I at higher endpoint temperatures.

Table X-7B reports the weight fraction of Form I measured in materials dried using either a fluid bed (LAB-LINE/P.R.L.

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Hi-Speed Fluid Bed Dryer, Lab-Line Instruments, Inc.) or a vacuum oven (Baxter Scientific Products Vacuum Drying Oven, Model DP-32). Similar Form I content was observed for comparable materials dried in either the high fluid bed or the vacuum oven. A difference was observed, however, for comparable materials dried in the low fluid bed relative to the vacuum oven.

TABLE X-7B

Cooling Rate	End Point	Impurity Level	Drying Type	Weight Percent Form I
High	High	High	Vacuum Oven	29%
High	High	High	High Fluid Bed	25%
High	High	High	Low Fluid Bed	4.7%
Low	Low	Low	Vacuum Oven	ND
Low	Low	Low	High Fluid Bed	ND
Low	Low	Low	Low Fluid Bed	5.5%

Example 8: Crystallization of a Mixture of Form I and Form II From Methyl Ethyl Ketone To Prepare a Solvate, and (b) Desolvation of the Solvate to Prepare Form II

Form I eplerenone (10 g) was combined with 80 mL of methyl ethyl ketone. The mixture was heated to reflux (79°C) and stirred at this temperature for about 30 minutes. The resulting slurry was then cooled with a stepwise, holdpoint protocol by maintaining the slurry at 65°C, 50°C, 35°C and 25°C for about 90 minutes at each temperature. The slurry was filtered and rinsed with about 20 mL methyl ethyl ketone. The isolated solid was initially dried on the filter and then in a vacuum oven at 40-50°C. The drying was completed in the vacuum oven

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at 90-100°C. The desolvated solid was obtained with an 82% recovery. XPRD, MIR and DSC confirmed that the solid had a Form II crystalline structure.

Example 9: Digestion of Low Purity Eplerenone Starting Material With a Solvent to Prepare Form I

A. Digestion With Ethanol Solvent:

Low purity eplerenone (24.6 g; 64% by weight assay via HPLC) was combined with 126 mL of ethanol 3A. The slurry was heated to reflux and the distillate removed. An additional 126 mL of ethanol 3A was simultaneously added as 126 ml of solvent was removed via atmospheric distillation. Upon completion of the solvent turnover, the mixture was cooled to 25 °C and stirred for one hour. The solid was filtered and rinsed with ethanol 3A. The solid was air-dried to give the ethanol solvate. The solvate was further dried in a vacuum oven at 90-100°C for six hours to obtain 14.9 g of Form I eplerenone.

B. Digestion With Methyl Ethyl Ketone Solvent

In an alternative digestion process, 1 gram of low purity eplerenone (about 65% pure) was digested in 4 mL of methyl ethyl ketone for two hours. After the two hours, the mixture was allowed to cool to room temperature. Once cooled, the solid was collected by vacuum filtration and determined to be the methyl ethyl ketone solvate by XPRD analysis. The solid was dried at 100°C for 30 to 60 minutes. The dried solids were determined to be pure Form I by XPRD.

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Example 10: Digestion of High Purity Eplerenone Starting Material With a Solvent to Prepare Form II

A. Digestion With Ethanol Solvent:

High purity eplerenone (1 gram) was digested in 8 mL of ethanol for approximately two hours. The solution was then allowed to cool to room temperature and the solids were collected by vacuum filtration. Analysis of the solids by XPRD immediately after filtration indicated that the solids were a solvate (presumably the ethanol solvate). The solids were subsequently dried at 100°C at atmospheric pressure for 30 minutes. The dried solid was analyzed by XPRD and determined to be predominately Form II (no Form I detected).

B. Digestion with Methyl Ethyl Ketone Solvent:

High purity eplerenone (1 gram) was digested in 4 mL of methyl ethyl ketone for two hours. After the two hours, the solution was allowed to cool to room temperature and the solids collected by vacuum filtration. The solid was immediately analyzed by XPRD and determined to be a solvate of eplerenone (presumably the methyl ethyl ketone solvate). The solvate was subsequently dried at 100°C at ambient pressure for 30 to 60 minutes. The dried solids were analyzed by XPRD and determined to be primarily Form II with no diffraction peaks for Form I present.

Example 11: Crystallization of Form II Directly From Solution

Procedure A: Eplerenone (2.5 g) was dissolved in ethyl acetate by heating to 75°C. Once the eplerenone dissolved,

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the solution was held at 75°C for 30 minutes to ensure complete dissolution. The solution was then cooled at 1 °C/min to 13°C. Once at 13°C, the slurry was allowed to stir for two hours at 750 rpm with an overhead stirrer. The crystals were collected by vacuum filtration and dried in a vacuum oven at 40°C for one hour. The XPRD pattern and DSC thermogram of the solid were characteristic of Form II eplerenone. Thermal gravimetric analysis (TGA) of the solid indicated no weight loss from the solid up to 200°C.

Procedure B: In an alternative procedure, 2 g of eplerenone was dissolved in 350 mL of 15/85% acetonitrile/water by heating on a hot plate with magnetic stirring. Once the eplerenone was dissolved, the solution was allowed to cool to room temperature overnight with magnetic stirring. The resulting solid was collected by vacuum filtration. The crystals were birefringent and had a triangular, plate-like crystal habit. The solid had an XPRD and DSC characteristic of Form II eplerenone. TGA indicated no weight loss up to 200°C.

Procedure C: In an alternative procedure, 640 mg of eplerenone was placed in a 50 mL flask with 20 mL of ethyl benzene. The resulting slurry was heated to 116°C and became a clear solution. The clear solution was cooled to 25°C over 30 minutes. Nucleation began at 84°C during the cooling period. The resulting solids were filtered from the solution and air-dried to give 530 mg of solids (83% recovery). Hot-stage microscopy and XPRD confirmed that the solids were Form II crystals.

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Procedure D: In an alternative procedure, 1.55 g of eplerenone was added to 2.0 mL of nitrobenzene and heated to 200°C. The resulting slurry was stirred overnight at 200°C. The solution was allowed to cool to room temperature (natural air convection) the following day and the solid was isolated. The solid was determined to be Form II eplerenone by XPRD and polarized light microscopy.

Procedure E: In an alternative procedure, 5.0 g of eplerenone (purity greater than 99%) was added to 82 g of methanol (104 mL). Under stirring action (210 rpm), the solution was heated to 60°C and held at that temperature for 20 minutes to ensure complete dissolution. The solution was then cooled to -5°C at a rate of 0.16°C/minute under stirring. The crystals were collected by filtration and dried in a vacuum oven at 40°C for 20 hours. The dried solids were determined to be pure Form II eplerenone by DSC and XPRD analysis.

Procedure F: In an alternative procedure, 6.0 g of eplerenone (ethanol solvate containing 9% ethanol and having a corrected purity of 95.2%) was added to 82 g of methanol (104 mL). Under stirring action (210 rpm), the solution was heated to 60°C and held at that temperature for 20 minutes to ensure complete dissolution. The solution was then cooled to 50°C at a rate of 0.14°C/minute and then held at that temperature for about 2.5 hours. The solution was then cooled to -5°C at a rate of 0.13°C/minute under stirring. The crystals were collected by filtration and dried in a vacuum

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oven at 40°C for 16 hours. The dried solids were determined to be pure Form II eplerenone by DSC and XPRD analysis.

Example 12: Crystallization of Form I Directly From Solution

150.5 mg of the diepoxide and 2.85 g of eplerenone were added to 1.5 mL of nitrobenzene. The mixture was magnetically stirred at 200°C for several hours. The slurry was then allowed to cool to room temperature by natural air convection. The sample was dried and analyzed by polarized light microscopy and XPRD. The XPRD indicated that the sample was a mixture of Form I and Form II. The crystals were translucent by microscopy, indicating that desolvation (and conversion to either Form I or Form II) did not occur.

Example 13: Preparation of Amorphous Eplerenone By Comminution

Approximately one-half of a steel Wig-L-Bug container was filled with about 60 g of eplerenone (greater than 99.9% purity). A steel ball and cap were placed on the sample container and agitated for 30 seconds by the Wig-L-Bug apparatus. The eplerenone was scraped off the surface of the Wig-L-Bug container and the container agitated for an additional 30 seconds. The resulting solid was analyzed by XPRD and DSC and determined to be a mixture of amorphous eplerenone and Form II crystalline eplerenone.

Example 14: Preparation of Amorphous By Lyophilization

Approximately 100 mg of crude eplerenone was weighed into a beaker containing 400 mL of water. The solution was heated slightly for five minutes, and then sonicated and heated with stirring for an additional five minutes.

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Approximately 350 mL of the eplerenone solution was filtered into a 1000 mL round bottom flask containing 50 mL of HPLC water. The solution was flashed frozen in a dry ice/acetone bath over a time period of one to two minutes. The flask was attached to a Labconco Freezone 4.5 freeze dryer and dried overnight. The solids in the flask were transferred to a small brown bottle. A small aliquot was observed under polarized light microscopy at 10X, 1.25X optivar in cargille oil (1.404) and observed to be at least 95% amorphous eplerenone. Figures 24 and 25 show the XPRD pattern and DSC thermogram obtained for the amorphous eplerenone. The peak observed at 39 degrees two theta in Figure 24 is attributable to the aluminum sample container.

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Example 15: Measurement of Form II Solubility

The aqueous solubility of Form II eplerenone was measured at pH 7 (100 mM phosphate buffer) at 5, 25, and 40°C. Approximately 30 mg of Form II eplerenone was mixed with approximately 10 mL of buffer to form a slurry of eplerenone at both 5 and 25 °C. Approximately 40 mg of Form II eplerenone was mixed with approximately 10 mL of buffer to form a slurry of eplerenone at 40 °C. Samples were prepared in duplicate for each condition. The slurries were allowed to equilibrate in appropriate temperature water shaker baths and the solutions were analyzed for eplerenone content by ultraviolet visible analysis (245 nm) at the 1, 5, 12, 19, 27, and 36 day time intervals. Data from each condition was appropriately averaged to determine the solubility of eplerenone at each condition and is reported in Table X-15. The residual solids from each time point were analyzed by DSC and TGA at the end of the 36 day equilibration and were

determined to be Form II.

TABLE X-15

Temperature (°C)	Form II Solubility
5	0.24 mg/mL
25	0.29 mg/mL
40	0.39 mg/mL

Example 16: Measurement of Intrinsic Dissolution Rates

Intrinsic dissolution rates were measured for the following four eplerenone polymorph samples: (i) Form II eplerenone prepared by direct crystallization from acetonitrile using water as an anti-solvent in the same manner as Example 12, Procedure B, (ii) Form I eplerenone prepared by digestion in ethanol in the same manner as Example 10, Procedure A, (iii) a mixture of Form I (5 weight percent) and Form II (95 weight percent), and (iv) Form II eplerenone that was micronized to provide the following particle size distribution: 10% of the particles under 9 microns, 50% of the particles under 22 microns, and 90% of the particles under 41 microns.

Eplerenone (150 mg) was weighed and placed into a VanKel intrinsic dissolution cavity. The powder was compressed at 1200 psi using a Carver press. The sample was then mounted onto the intrinsic dissolution apparatus. The dissolution media used was 1% SDS in HPLC grade water. All experiments were conducted at 37°C for two hours. Before the start of the experiment, 500 mL of the dissolution medium was equilibrated at 37°C for 30 minutes in the dissolution bathing chamber. An initial sample was taken from each dissolution vessel which served as time zero for the experiment. The eplerenone

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tablets were then lowered into the dissolution medium. Samples were drawn at determined intervals for the determination of rate of dissolution. Care was taken to avoid air bubbles forming at the surface of the tablet. The samples were analyzed by UV absorbance detection at 243 nm. Intrinsic dissolution rates were calculated from the slope of the linear portion of the concentration versus time profiles corrected for volume and normalized for the surface area of the dissolution tablet (0.5 cm^2).

Fig. 26 reports the intrinsic dissolution rates measured for the four samples. These studies appear to indicate that Form I eplerenone has faster intrinsic dissolution rate than Form II eplerenone. X-Ray powder diffraction measurements comparing compressed against uncompressed eplerenone confirmed that the polymorphs did not interconvert upon compression or during the course of the dissolution studies.

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Example 17: Eplerenone Polymorph Composition

Tablets containing 25 mg, 50 mg, 100 mg and 200 mg doses of Form II eplerenone are prepared and have the following composition:

Ingredient	Weight % of Tablet
Form II Eplerenone	29.41
Form I Eplerenone	Not Detected
Lactose Monohydrate (#310, NF)	42.00
Microcrystalline Cellulose (NF, Avicel PH101)	18.09
Croscarmellose Sodium (NF, Ac-Di-Sol)	5.00
Hydroxypropyl Methylcellulose (#2910, USP, Pharmacoat 603)	3.00
Sodium Lauryl Sulfate (NF)	1.00
Talc (USP)	1.00
Magnesium Stearate (NF)	0.5
Total	100.00

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Example 18: Eplerenone Polymorph Composition

Capsules (hard gelatin capsule, #0) are prepared containing a 100 mg dose of eplerenone and have the following composition:

Ingredient	Amount (mg)
Form II Eplerenone	90.0
Form I Eplerenone	10.0
Lactose, Hydrous, NF	231.4
Microcrystalline Cellulose, NF	45.4
Talc, USP	10.0
Croscarmellose Sodium, NF	8.0
Sodium Lauryl Sulfate, NF	2.0
Colloidal Silicon Dioxide, NF	2.0
Magnesium Stearate, NF	1.2
Total Capsule Fill Weight	400.0

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Example 19: Eplerenone Polymorph Composition

Capsules (hard gelatin capsule, size #0) are prepared containing a 200 mg dose of eplerenone and have the following composition:

Ingredient	Amount (mg)
Form II Eplerenone	190.0
Form I Eplerenone	10.0
Lactose, Hydrous, NF	147.8
Microcrystalline Cellulose, NF	29.0
Talc, USP	10.0
Croscarmellose Sodium, NF	8.0
Sodium Lauryl Sulfate, NF	2.0
Colloidal Silicon Dioxide, NF	2.0
Magnesium Stearate, NF	1.2
Total Capsule Fill Weight	400.0

Example 20: Preparation of Milled Eplerenone

Dried methyl ethyl ketone solvate is first delumped by passing the solvate through a 20 mesh screen on a Fitzmill. The delumped solid is then pin milled using an Alpine Hosakawa stud disk pin mill operating under liquid nitrogen cooling at a feed rate of approximately 250 kilograms/hour. Pin milling produces milled eplerenone with a D_{90} size of approximately 65-100 microns.

Example 21: Effect of Eplerenone Particle Size (Dog Study)

The effect of the particle size of Form II eplerenone on eplerenone plasma concentrations and relative bioavailability was studied in a dog model. Four heathy female beagle dogs

weighing between 8 to 12 kg were intragastrically administered one immediate release capsule containing the formulation described in Table X-21A below followed by about 10 mL of water.

Table X-21A

INGREDIENT	WEIGHT % OF TABLET	Amount (mg)
Eplerenone	50.00	200.00
Lactose, Fast-Flo, Hydrous	36.95	147.80
Microcrystalline Cellulose (Avicel PH102)	7.25	29.00
Sodium Lauryl Sulfate	0.50	2.00
Croscarmellose Sodium	2.00	8.00
Talc	2.50	10.00
Colloidal Silicon Dioxide	0.50	2.00
Magnesium Stearate	0.30	1.20
Total	100.00	400.00
Capsules, Size #0, White Opaque	1 Capsule	

The dogs were fasted for 15 to 20 hours prior to administration of the capsule and were not fed again until at least 4 hours after dose administration. Blood samples (approximately 3 mL) were collected by venipuncture in chilled tubes containing heparin at 0, 0.5, 1, 2, 3, 4, 6, 8 and 24 hours after dose administration. The blood samples were immediately placed on ice. Separation of plasma from the blood samples was complete after about 15 minutes of centrifugation. The resulting plasma samples were frozen at about -20°C and stored until analyzed. Analysis was performed using an LC/MS/MS procedure.

This experiment was conducted using the same four dogs for three formulations that were identical except for the

particle size of the eplerenone starting material. The three formulations tested used eplerenone starting materials having a D_{90} particle size (that is, at least 90% of the particles were) less than about 212 microns, less than about 86 microns, and less than about 36 microns, respectively. A minimum of a five day wash-out period was allowed between administration of each of the formulations. Reducing the D_{90} particle size of the eplerenone starting material from about 212 microns to above 86 microns increased relative bioavailability by almost 100%. The mean results are reported in Tables X-21B and X-21C below.

Table X-21B

Time (hours)	Blood Serum Eplerenone Concentration (μg/mL)		
	D_{90} = 212 microns	D_{90} = 86 microns	D_{90} = 36 microns
0	0	0	0
0.5	1.83	3.65	1.99
1.0	2.40	6.18	5.86
2.0	3.77	6.89	6.77
3.0	2.85	5.70	6.60
4.0	2.61	4.39	5.56
6.0	1.63	3.11	3.31
8.0	1.10	1.90	2.09
24.0	0.0252	0.032	0.0706

Table X-21C

Pharmacokinetic Parameter	Pharmacokinetic Parameter Value		
	D_{90} = 212 microns	D_{90} = 86 microns	D_{90} = 36 microns
C_{max} (μg/mL)	3.98	7.02	7.39
T_{max} (hours)	1.50	1.75	2.25
AUC ((μg/mL)hr)	26.6	49.2	53.1
Relative Bioavailability (%)	53.25	100	107.9

Example 22: Effect of Eplerenone Particle Size (Human Study)

The effect of the particle size of a Form II eplerenone starting material used in a pharmaceutical composition on eplerenone plasma concentrations and relative bioavailability was studied in a human model. Subjects received single doses of Form II eplerenone medication on Days 1, 8, 15, 22 and 29 according to a randomization schedule. All medication was administered with 180 mL of water at 0800 hours. Blood samples for eplerenone pharmacokinetic analyses were collected at -0.5 (predose), 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 16, 24, 36 and 48 hours postdose. Urine samples were collected and pooled between the hours of 0-24 and 24-48 hour postdose.

Plasma and urinary concentrations of eplerenone were determined using a validated high performance liquid chromatography method with MS/MS Detection. Pharmacokinetic data obtained is reported in Table X-23A. The particle size distributions of the eplerenone used in the preparation of the medications was determined in the dry powder state using laser light scattering.

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Table X-23A

Pharmacokinetic Parameter	Pharmacokinetic Parameter Value		
	100 mg Capsule A (D_{90}=40 microns)	100 mg Tablet (D_{90}=82 microns)	100 mg Capsule B (D_{90}=96 microns)
C_{max} (ng/mL)	1747	1704	1669
T_{max} (hours)	1.8	1.8	1.3
AUC ((ng/mL)hr)	11349	11945	11981

The specific composition of each medication is reported in Table X-23B below.

Table X-23B

INGREDIENT	WEIGHT % OF TABLET/CAPSULE		
	Capsule A	Tablet A	Capsule B
Form II Eplerenone	25	30	25
Lactose Monohydrate	–	42	57.86 (fast flo lactose)
Lactose, Hydrous NF	57.8	–	–
Microcrystalline Cellulose (Avicel PH101)	11.4	17.5 (7.5% intragranular plus 10% extragranular)	11.34 (Avicel PH 102)
Croscarmellose Sodium (Ac-Di-Sol)	2	5	2
Hydroxypropyl Methylcellulose (Pharmacoat 603)	–	3	–
Sodium Lauryl Sulfate	0.5	1	0.5
Talc	2.5	1	2.5
Magnesium Stearate	0.3	0.5	0.3
Colloidal Silicon Dioxide	0.5	–	0.5
Total	100	100	100

All mentioned references are expressly incorporated by reference herein as if fully set forth at length. In addition, U.S. Provisional Application Serial No. 60/111,646 filed December 9, 1998, and the corresponding nonprovisional application, U.S. Application Serial No. 09/_____ filed December 8, 1999, are expressly incorporated by reference herein as if fully set forth at length.

When introducing elements of the present invention or the preferred embodiment(s) thereof, the articles "a", "an", "the" and "said" are intended to mean that there are one or more of the elements. The terms "comprising", "including" and "having" are intended to be inclusive and mean that there may be additional elements other than the listed elements.

Although this invention has been described with respect to specific embodiments, the details of these embodiments are not to be construed as limitations.

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WHAT IS CLAIMED IS:

1. A combination comprising particles comprising a first solid state form of eplerenone and particles comprising a second solid state form of eplerenone,

wherein said solid state forms of eplerenone are selected from:

the Form I crystalline form of eplerenone;

the Form II crystalline form of eplerenone;

a crystalline form of eplerenone comprising a solvent; and

amorphous eplerenone; and

wherein at least 90% of said particles have a particle size less than about 15 microns.

2. The combination of Claim 1 wherein said solid state forms of eplerenone are selected from:

the Form I crystalline form of eplerenone;

the Form II crystalline form of eplerenone;

a crystalline form of eplerenone comprising a solvent, wherein said form does not comprise methylene chloride, ethyl ether or isopropanol; and

amorphous eplerenone.

3. The combination of Claim 1 wherein said solid state forms of eplerenone are selected from:

the Form I crystalline form of eplerenone;

the Form II crystalline form of eplerenone;

a crystalline form of eplerenone comprising a solvent, wherein said form does not comprise methylene chloride, ethyl ether, isopropanol, methyl ethyl ketone or ethanol; and

amorphous eplerenone.

4. The combination of Claim 1 wherein the weight ratio of said first solid state form to said second solid state form is at least about 1:1.

5. The combination of Claim 4 wherein the weight ratio is at least about 2:1.

6. The combination of Claim 4 wherein the weight ratio is at least about 5:1.

7. The combination of Claim 4 wherein the weight ratio is at least about 9:1.

8. The combination of Claim 1 wherein the first solid state form is the Form II crystalline form of eplerenone, and the second solid state form is the Form I crystalline form of eplerenone.

9. The combination of Claim 8 wherein the weight ratio of said first solid state form to said second solid state form is at least about 1:9.

10. The combination of Claim 8 wherein the weight ratio is at least about 1:1.

11. The combination of Claim 8 wherein the weight ratio is at least about 2:1.

12. The combination of Claim 8 wherein the weight ratio

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is at least about 5:1.

13. The combination of Claim 8 wherein the weight ratio is at least about 9:1.

14. The combination of Claim 1 wherein at least 90% of said particles have a particle size less than about 1 micron.

15. The combination of Claim 14 wherein at least 90% of said particles have a particle size less than about 800 nm.

16. The combination of Claim 14 wherein at least 90% of said particles have a particle size between about 10 nm to about 1 micron.

17. The combination of Claim 14 wherein at least 90% of said particles have a particle size between about 100 nm to about 800 nm.

18. The combination of Claim 14 wherein at least 90% of said particles have a particle size between about 200 nm to about 800 nm.

19. The combination of Claim 14 wherein at least 90% of said particles have a particle size between about 400 nm to about 800 nm.

20. A pharmaceutical composition comprising particles comprising a first solid state form of eplerenone and particles comprising a second solid state form of eplerenone in association with at least one pharmaceutically-acceptable

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carrier, adjuvant or diluent;

wherein said solid state forms of eplerenone are selected from:

the Form I crystalline form of eplerenone;

the Form II crystalline form of eplerenone;

a crystalline form of eplerenone comprising a solvent; and

amorphous eplerenone; and

wherein said first solid state form and said second solid state form in combination comprise a therapeutically-effective amount of eplerenone; and

wherein at least 90% of said particles have a particle size less than about 15 microns.

21. The pharmaceutical composition of Claim 20 wherein said solid state forms of eplerenone are selected from:

the Form I crystalline form of eplerenone;

the Form II crystalline form of eplerenone;

a crystalline form of eplerenone comprising a solvent, wherein said form does not comprise methylene chloride, ethyl ether or isopropanol; and

amorphous eplerenone.

22. The pharmaceutical composition of Claim 20 wherein said solid state forms of eplerenone are selected from:

the Form I crystalline form of eplerenone;

the Form II crystalline form of eplerenone;

a crystalline form of eplerenone comprising a solvent, wherein said form does not comprise methylene chloride, ethyl ether, isopropanol, methyl ethyl ketone or ethanol; and

amorphous eplerenone.

23. The pharmaceutical composition of Claim 20 wherein the weight ratio of said first solid state form to said second solid state form is at least about 1:9.

24. The pharmaceutical composition of Claim 23 wherein the weight ratio is at least about 1:1.

25. The pharmaceutical composition of Claim 23 wherein the weight ratio is at least about 9:1.

26. The pharmaceutical composition of Claim 20 wherein the first solid state form is the Form II crystalline form of eplerenone, and the second solid state form is Form I crystalline form of eplerenone.

27. The pharmaceutical composition of Claim 26 wherein the weight ratio of said first solid state form to said second solid state form is at least about 1:9.

28. The pharmaceutical composition of Claim 27 wherein the weight ratio is at least about 1:1.

29. The pharmaceutical composition of Claim 27 wherein the weight ratio is at least about 2:1.

30. The pharmaceutical composition of Claim 27 wherein the weight ratio is at least about 5:1.

31. The pharmaceutical composition of Claim 27 wherein the weight ratio is at least about 9:1.

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32. The pharmaceutical composition of Claim 20 wherein at least 90% of said particles have a particle size less than about 1 micron.

33. The pharmaceutical composition of Claim 32 wherein at least 90% of said particles have a particle size less than about 800 nm.

34. The pharmaceutical composition of Claim 32 wherein at least 90% of said particles have a particle size between about 10 nm to about 1 micron.

35. The pharmaceutical composition of Claim 32 wherein at least 90% of said particles have a particle size between about 100 nm to about 800 nm.

36. The pharmaceutical composition of Claim 32 wherein at least 90% of said particles have a particle size between about 200 nm to about 800 nm.

37. The pharmaceutical composition of Claim 32 wherein at least 90% of said particles have a particle size between about 400 nm to about 800 nm.

38. A method of treating or preventing an aldosterone-mediated condition or disorder in a subject, said method comprising administering to a subject having or susceptible to said condition or disorder a combination comprising particles comprising a first solid state form of eplerenone and particles comprising a second solid state form of

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eplerenone,

wherein said solid state forms of eplerenone are selected from:

the Form I crystalline form of eplerenone;

the Form II crystalline form of eplerenone;

a crystalline form of eplerenone comprising a solvent; and

amorphous eplerenone; and

wherein said first solid state form and said second solid state form in combination comprise a therapeutically-effective amount of eplerenone; and

wherein at least 90% of said particles have a particle size less than about 15 microns.

39. A method of Claim 38 comprising administering to a subject having or susceptible to said condition or disorder a combination comprising particles comprising a first solid state form of eplerenone and particles comprising a second solid state form of eplerenone in association with at least one pharmaceutically-acceptable carrier, adjuvant or diluent;

wherein said solid state forms of eplerenone are selected from:

the Form I crystalline form of eplerenone;

the Form II crystalline form of eplerenone;

a crystalline form of eplerenone comprising a solvent; and

amorphous eplerenone; and

wherein said first solid state form and a second solid state form in combination comprise a therapeutically-effective amount of eplerenone; and

wherein at least 90% of said particles have a particle

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size less than about 15 microns.

40. The method of Claim 39 for use in treatment of hyperaldosteronism.

41. The method of Claim 39 for use in treatment of hypertension.

42. The method of Claim 39 for use in treatment of heart failure.

43. The method of Claim 39 for use in treatment of cirrhosis of the liver.

44. The method of Claim 39 for use in treatment of a condition selected from the group consisting of excess collagen/fibrosis, benign prostate hypertrophy and depression.

45. Particles comprising an amorphous form of eplerenone having an X-ray powder diffraction pattern substantially lacking any characteristic peaks, wherein at least 90% of said particles have a particle size less than about 15 microns.

46. The particles of Claim 45 wherein at least 90% of said particles have a particle size less than about 1 micron.

47. The particles of Claim 45 wherein at least 90% of said particles have a particle size less than about 800 nm.

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48. The particles of Claim 45 wherein at least 90% of said particles have a particle size between about 10 nm to about 1 micron.

49. The particles of Claim 45 wherein at least 90% of said particles have a particle size between about 100 nm to about 800 nm.

50. The particles of Claim 45 wherein at least 90% of said particles have a particle size between about 200 nm to about 800 nm.

51. The particles of Claim 45 wherein at least 90% of said particles have a particle size between about 400 nm to about 800 nm.

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ABSTRACT OF THE DISCLOSURE

Novel combinations of nanoparticles of crystalline and amorphous solid state forms of eplerenone are described. These forms of eplerenone each have unique chemical and physical properties relative to one another and are characterized by their x-ray powder diffraction patterns, infrared spectra, nuclear magnetic resonance spectra, differential scanning calorimetric thermograms, and other physical characterizations.

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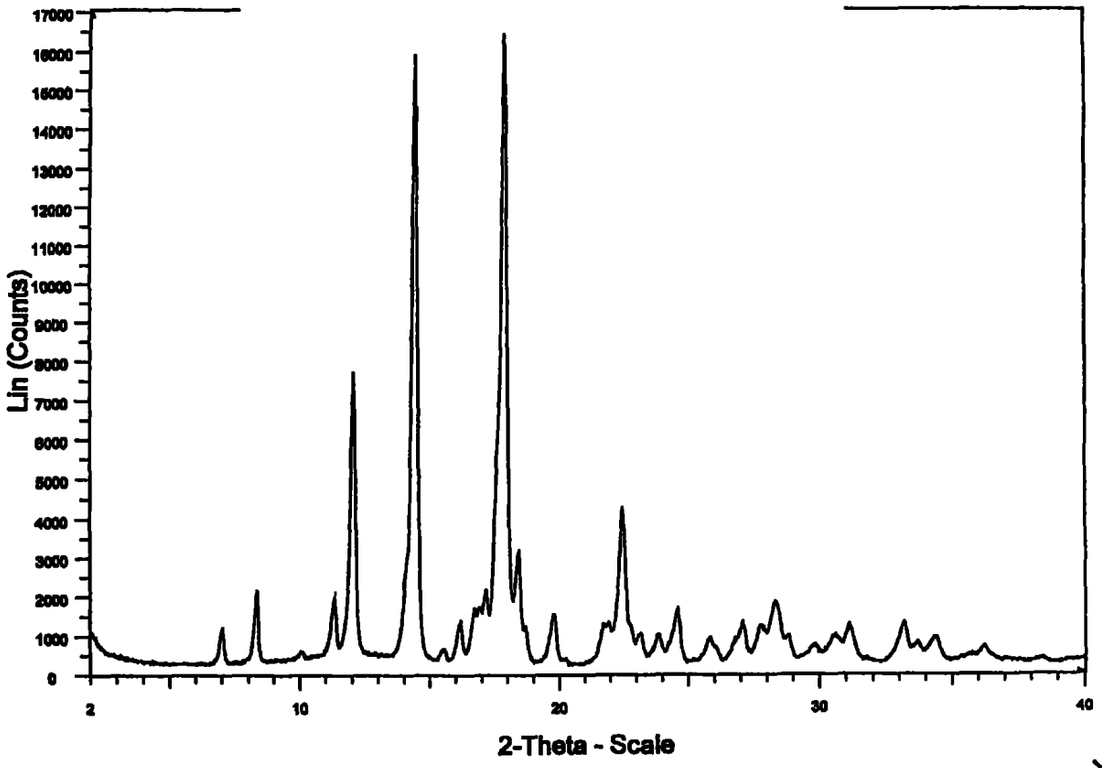
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16000
15000
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12000
11000
10000
9000
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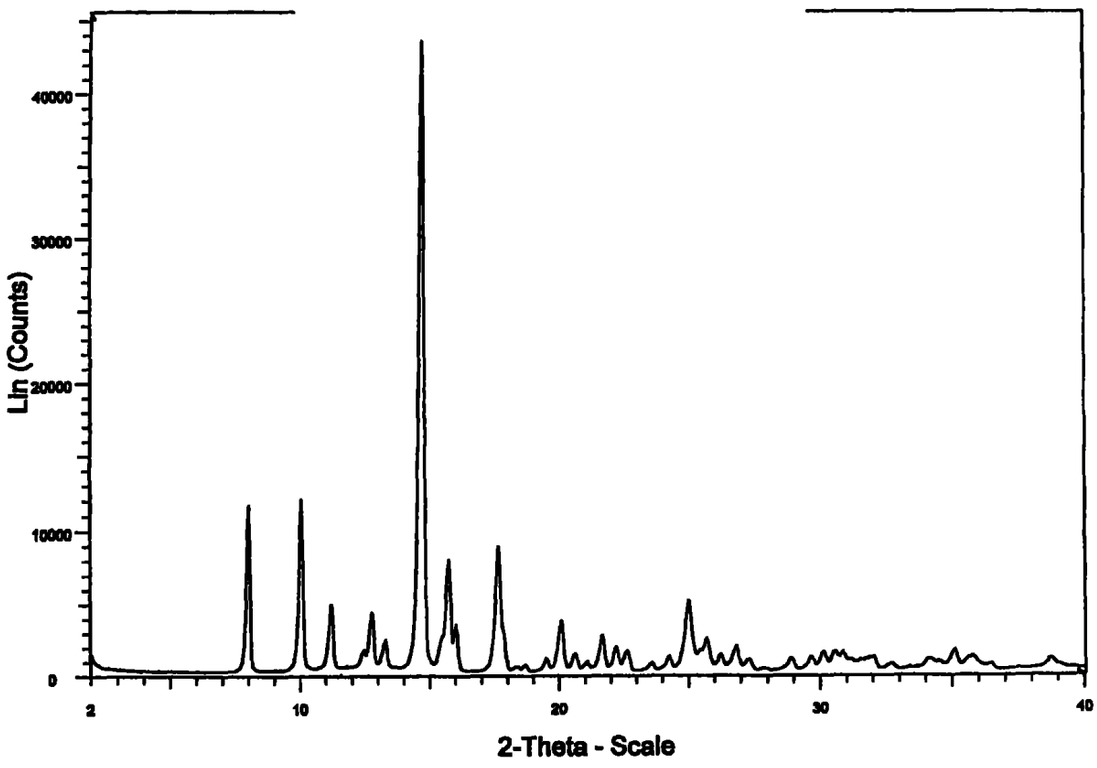
20
30
40
2-Theta - Scale


Lin (Counts)
40000
30000
20000
10000
0
2
10
20
30
40
2-Theta - Scale

386

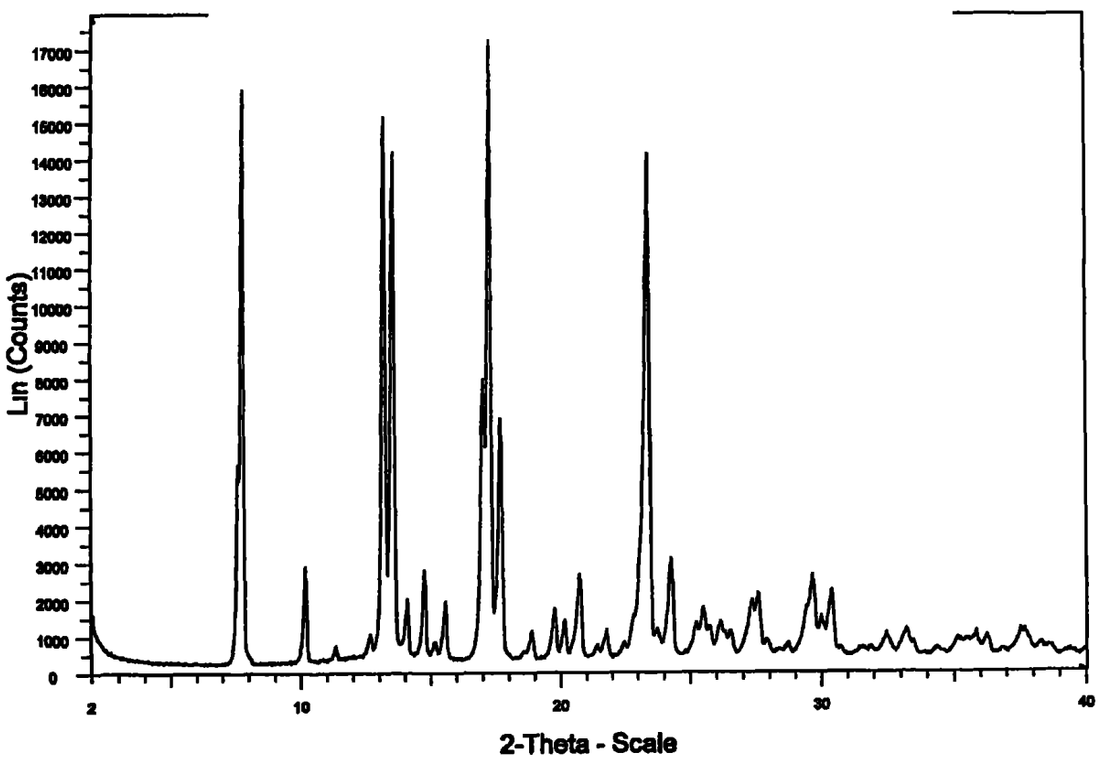

Lin (Counts)
17000
16000
15000
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
2
10
20
30
40
2-Theta - Scale

Figure 1

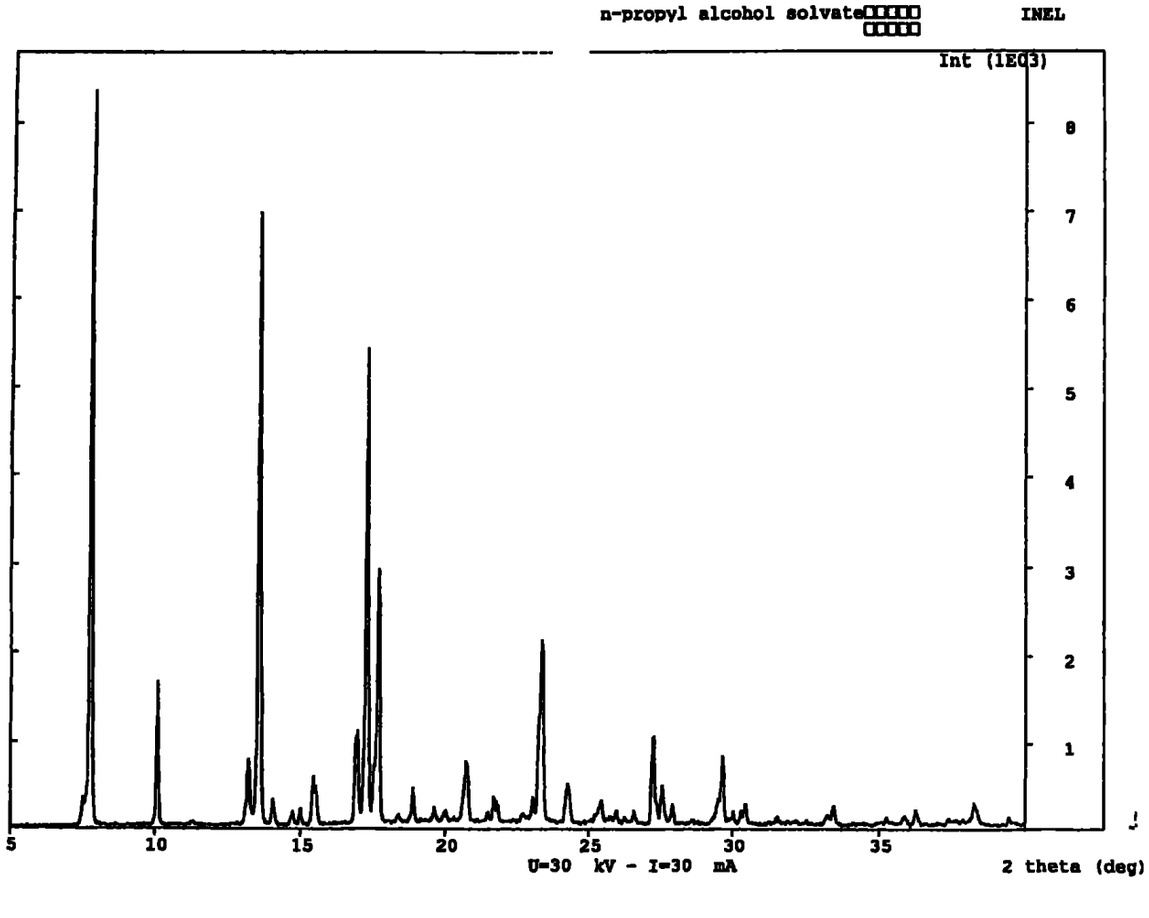

n-propyl alcohol solvate
INEL
Int (1E03)
8
7
6
5
4
3
2
1
5
10
15
20
25
30
35
U=30 kV - I=30 mA
2 theta (deg)

Figure 2

THF solvate INEL

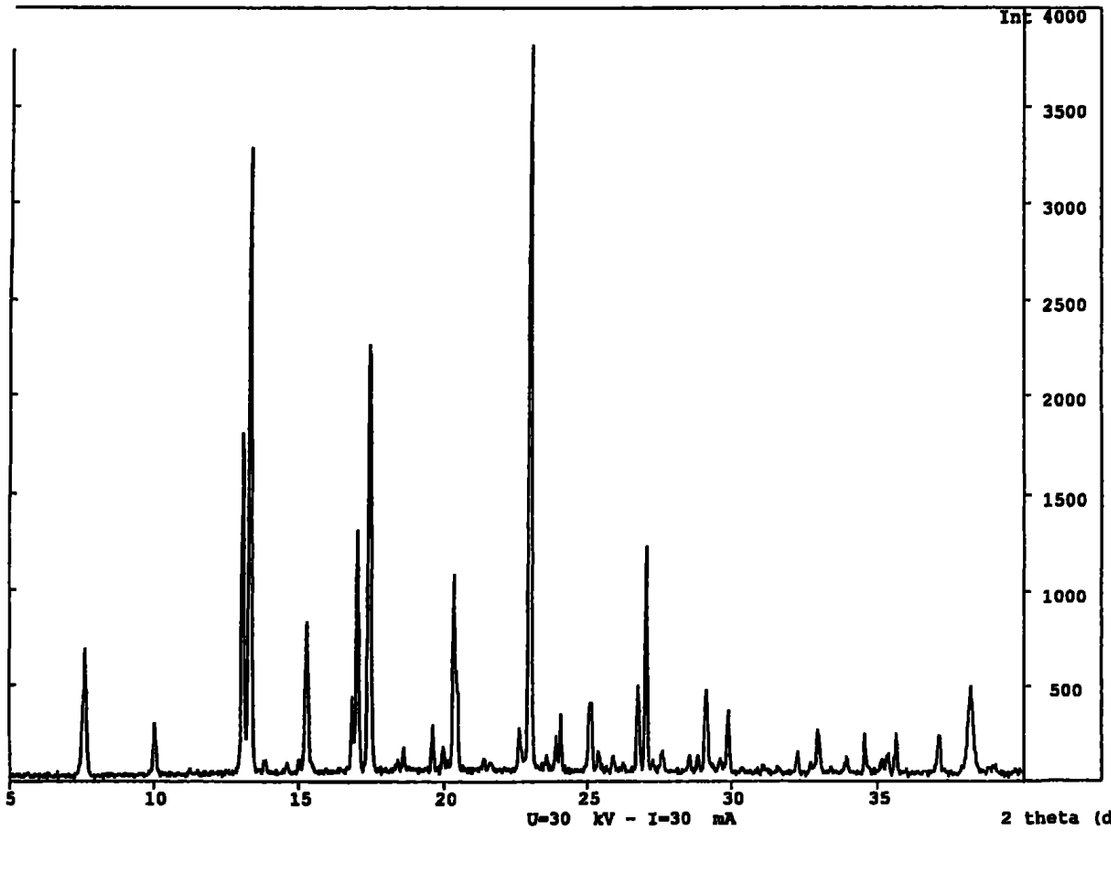
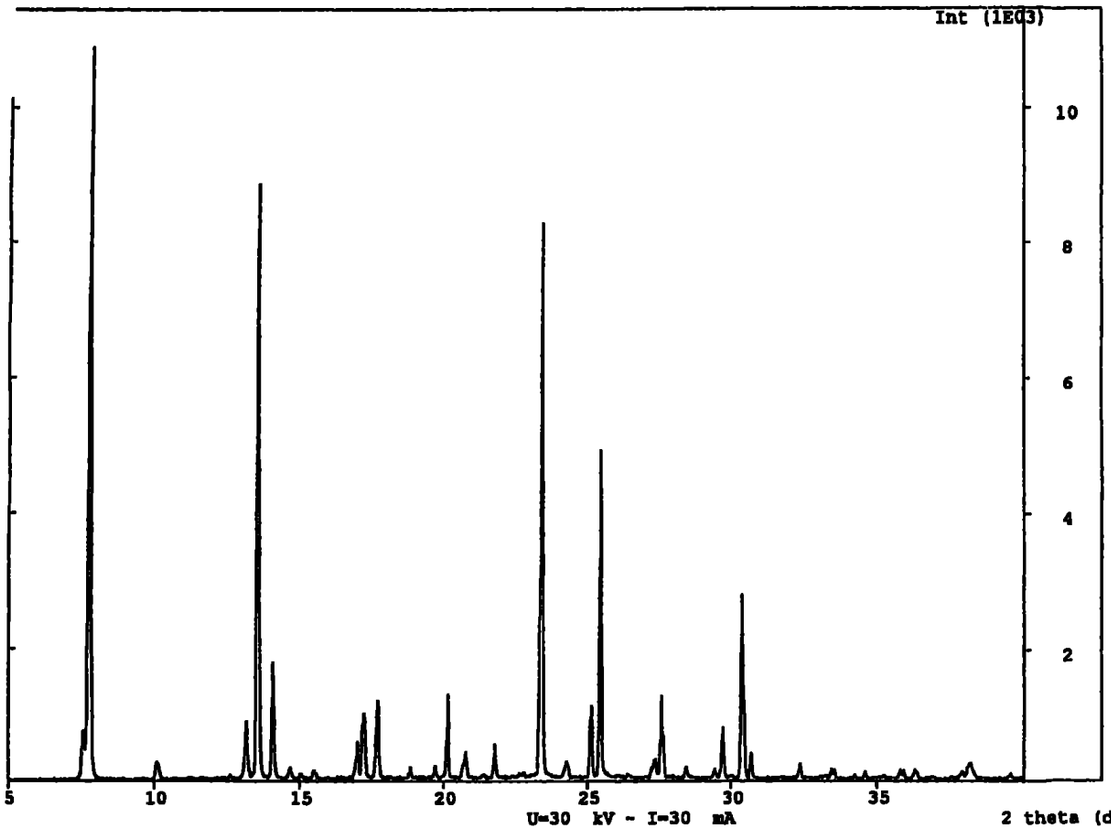
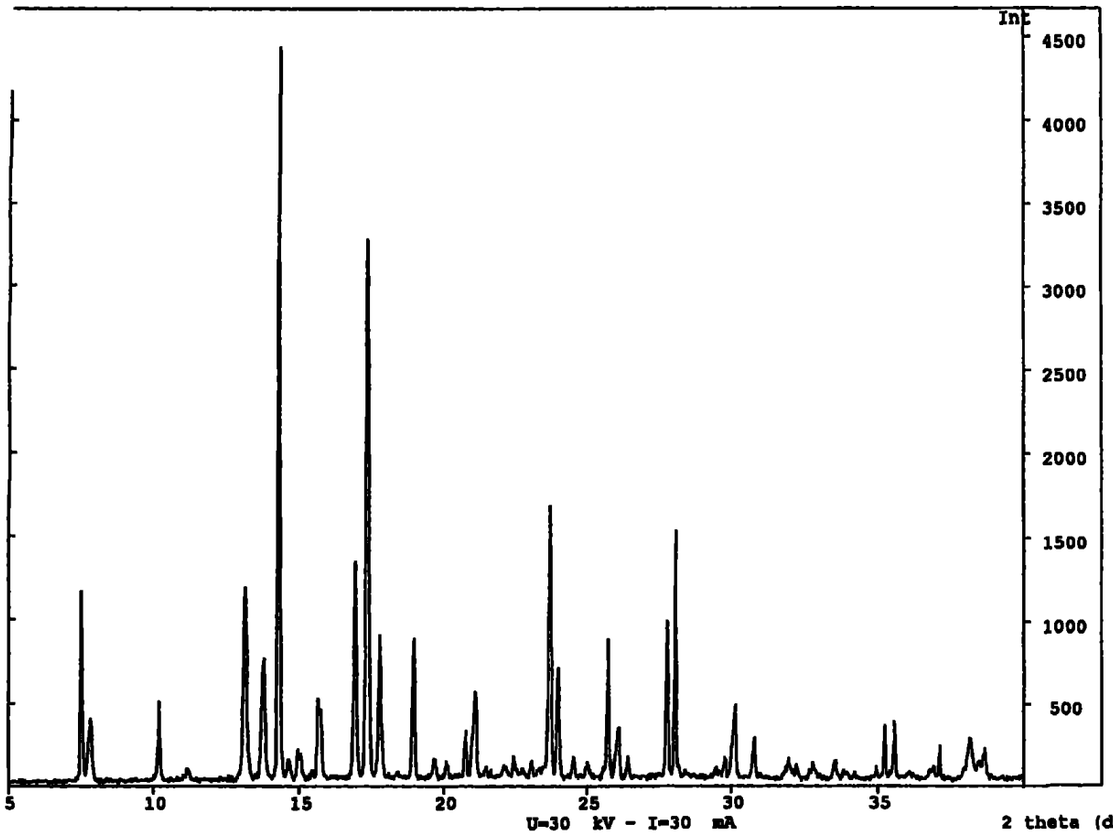


Figure 39

Ethyl Propionate solvate INEL



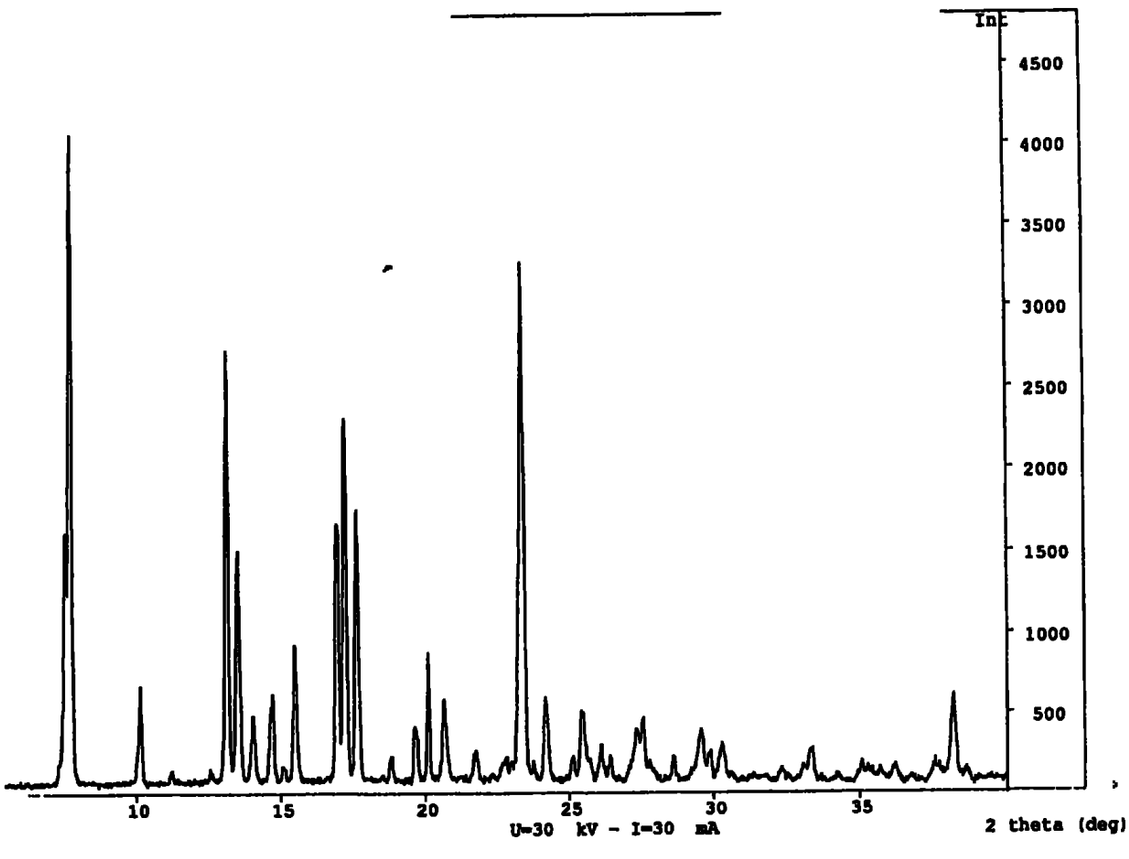
Acetic Acid solvate [redacted] INEL



391

66802 Figure 469709

acetone solvate



toluene solvate

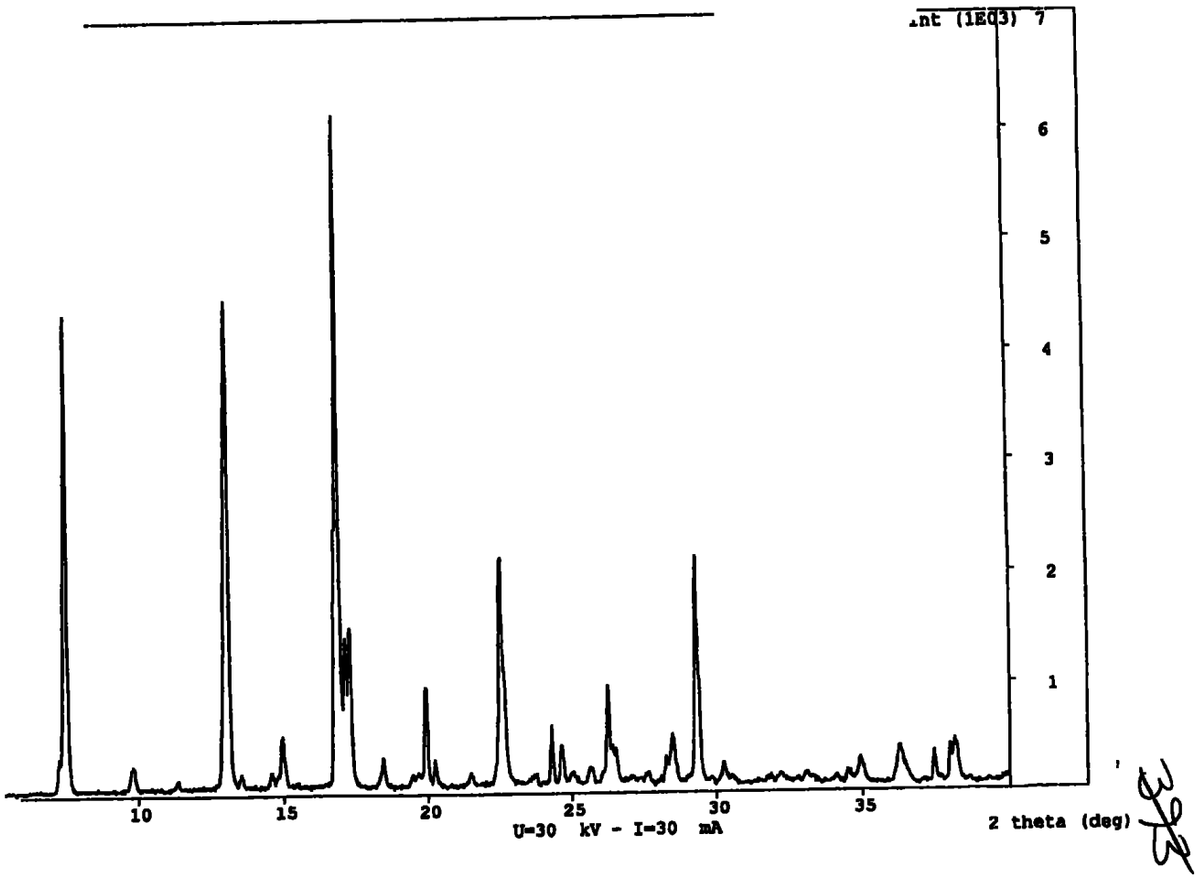
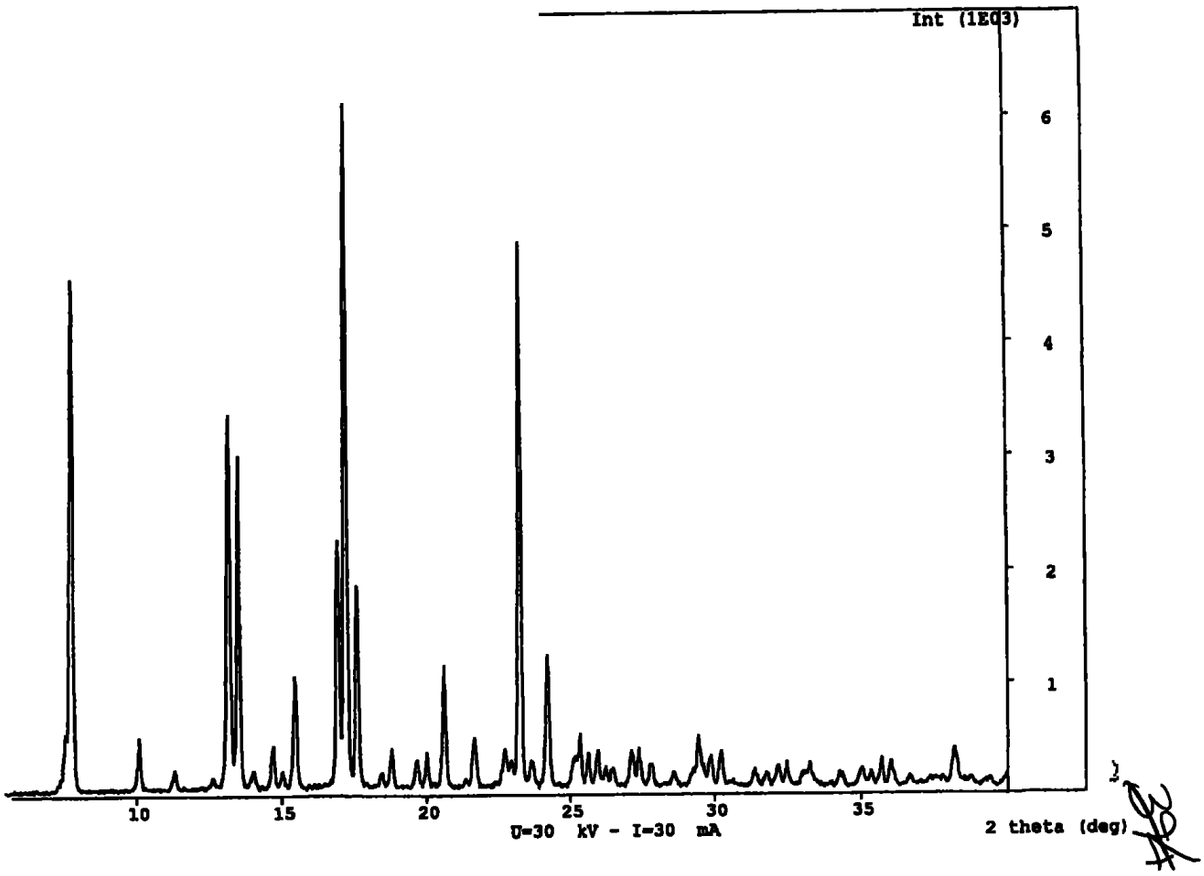
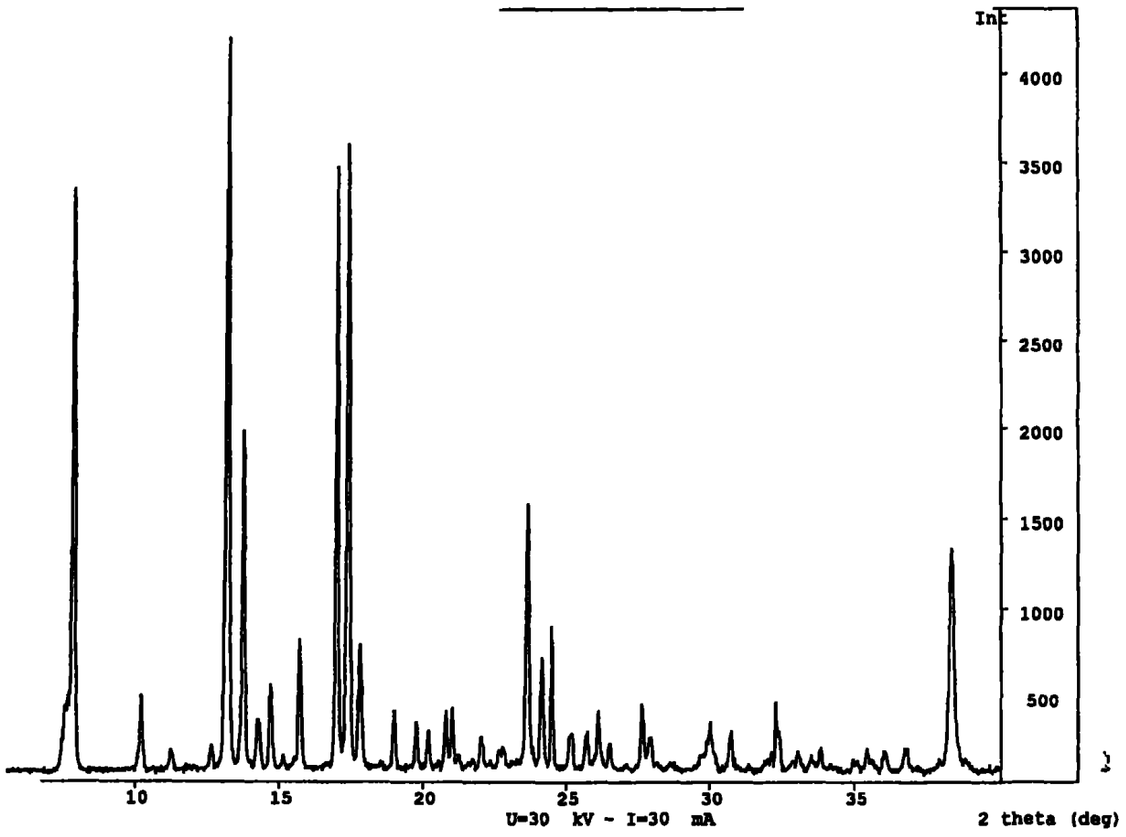


Figure 1J

isopropanol solvate

INEL



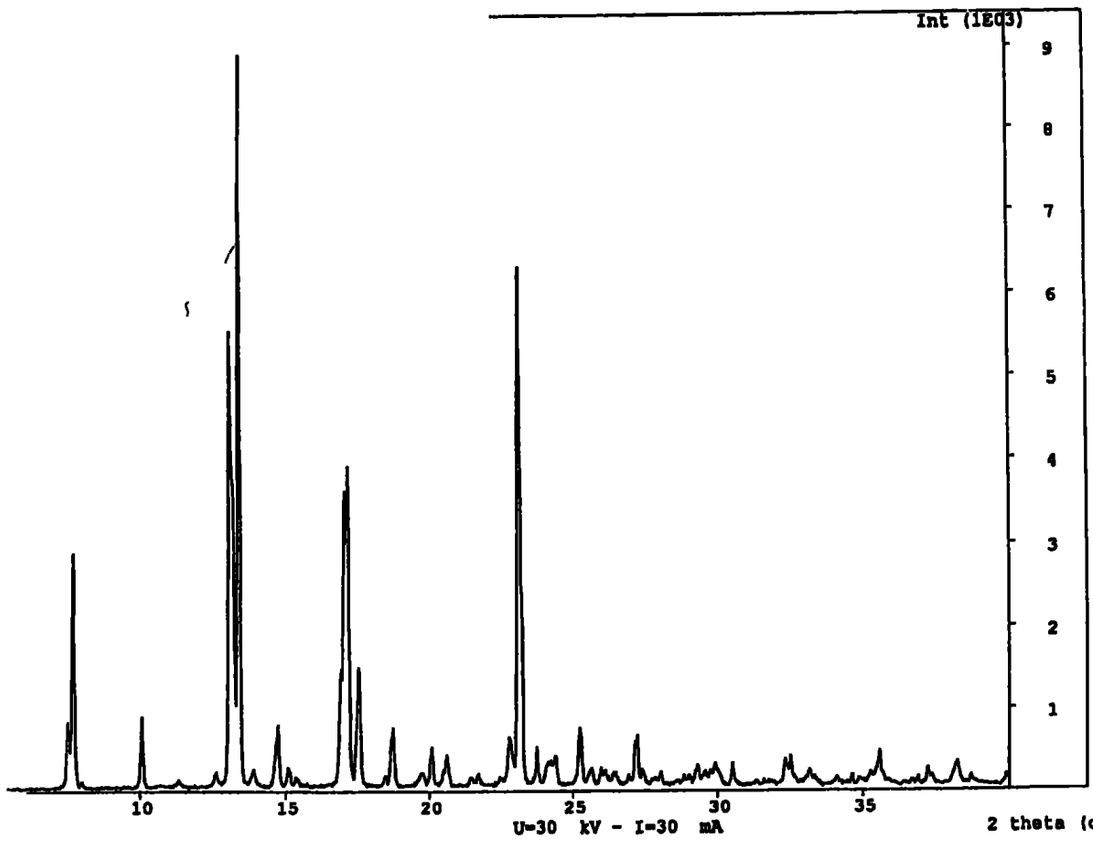

ethanol solvate
INEL
Int
4000
3500
3000
2500
2000
1500
1000
500
10
15
20
25
30
35
U=30 kV - I=30 mA
2 theta (deg)

395

6681021 2569709

Figure 11

isobutyl acetate solvate



396

Figure 1-M

butyl acetate solvate

INEL

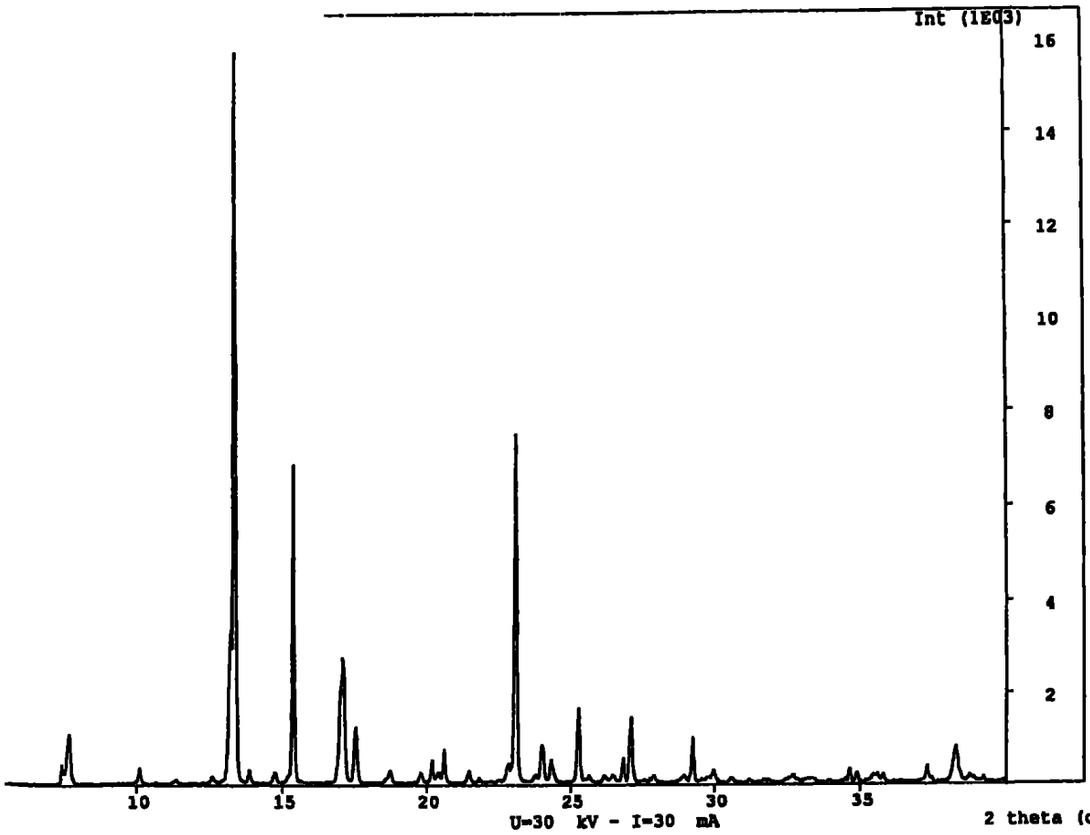
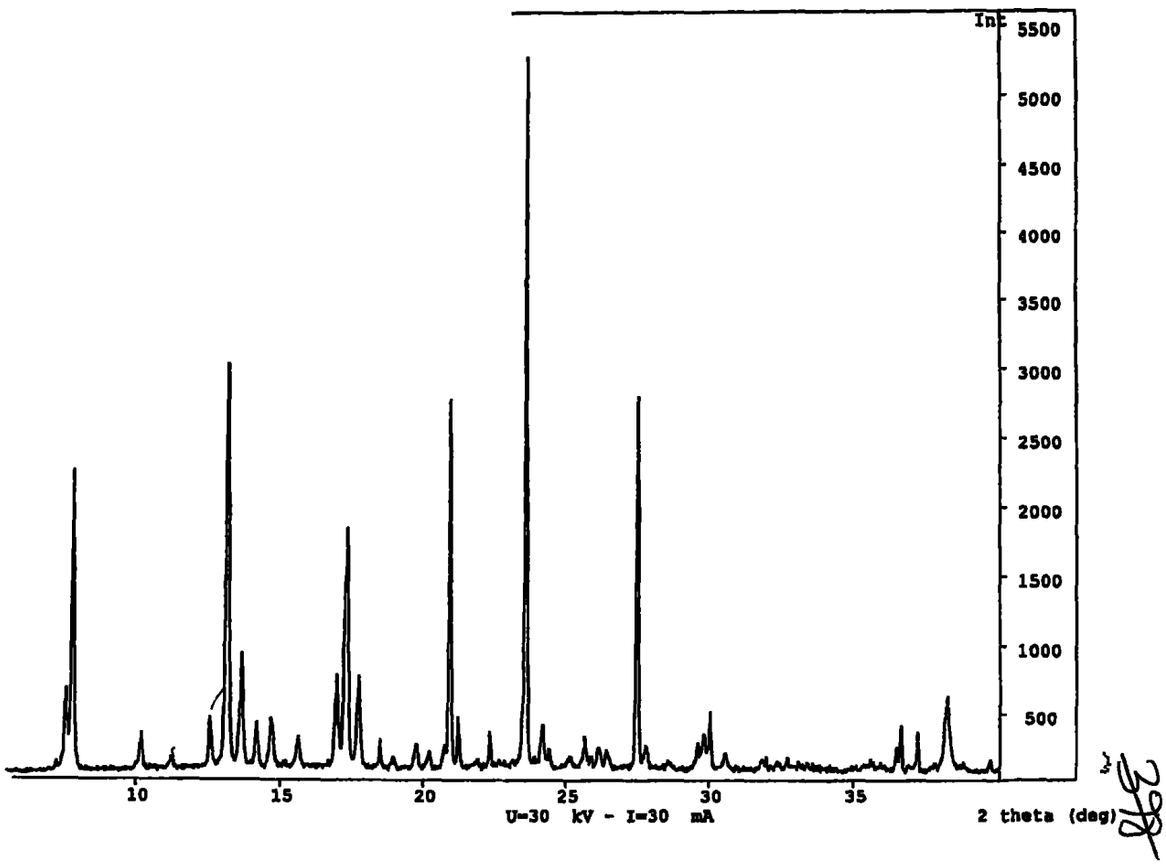


Figure 1-N

methyl acetate solvate

INEL

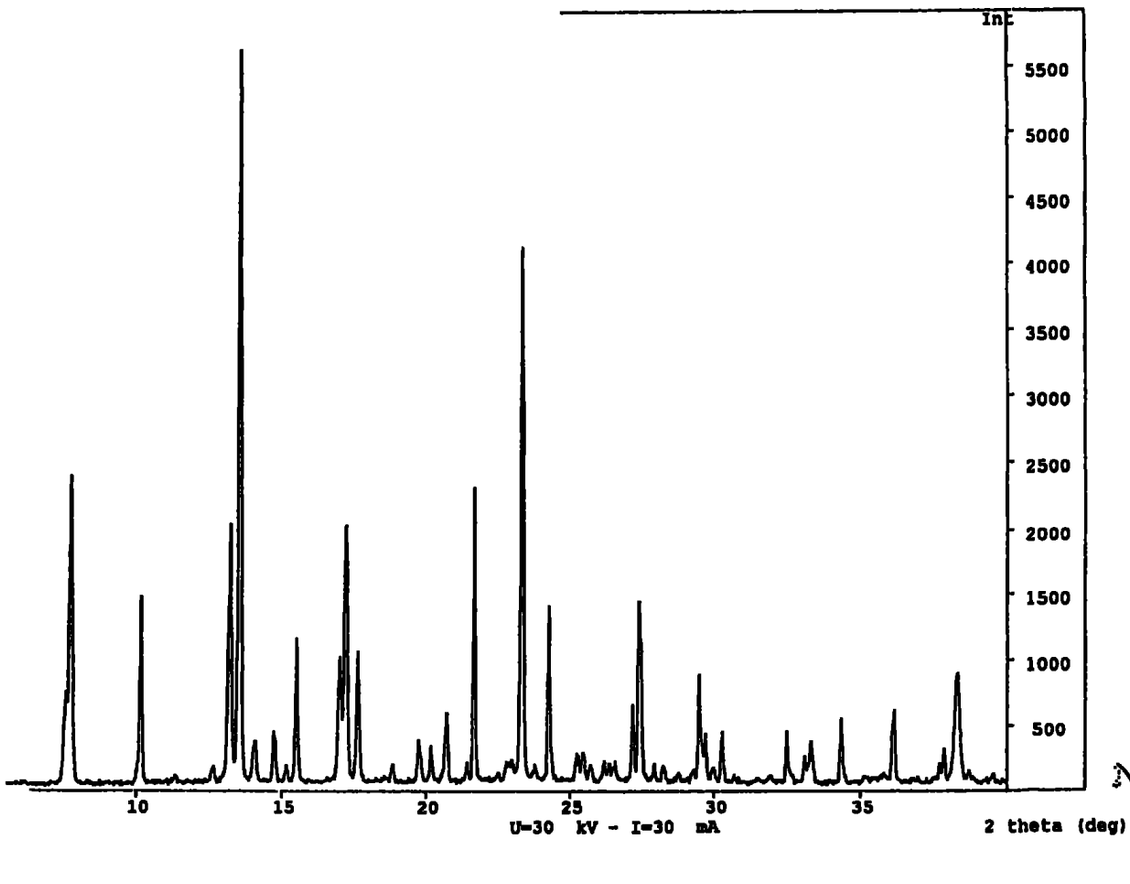


668021 6569709

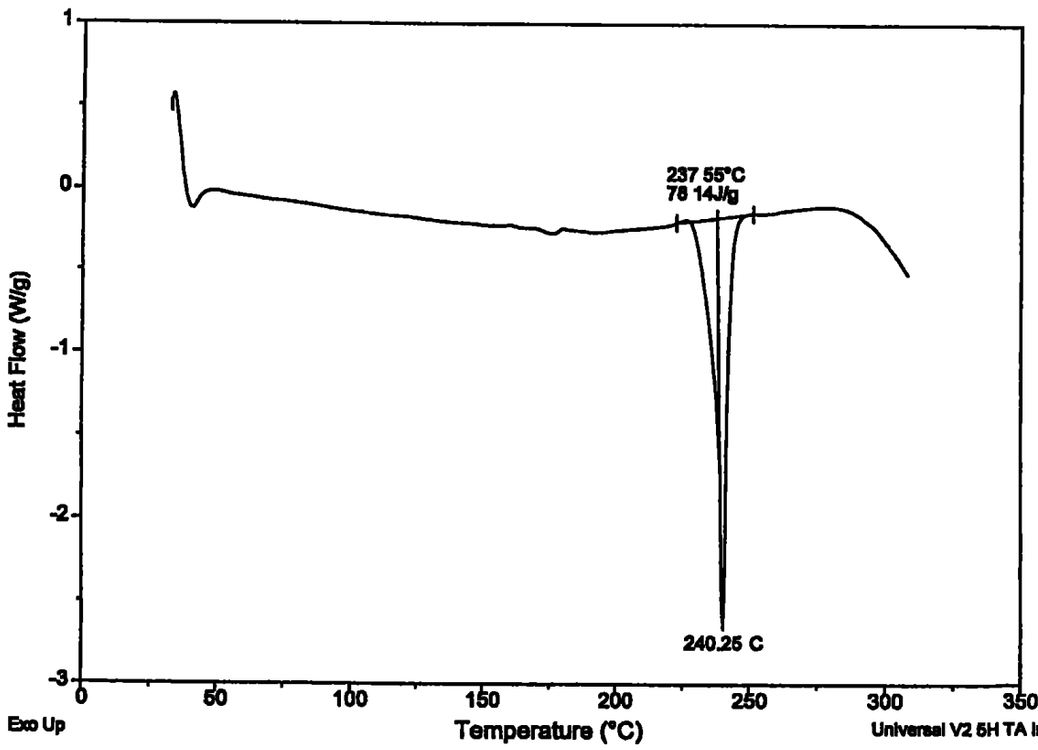
Figure 1-O

propyl acetate solvate

INEL



Size 0 6360 mg
Method 10 DEG C/MIN AMB TO 300
Comment SEALED PAN



B480

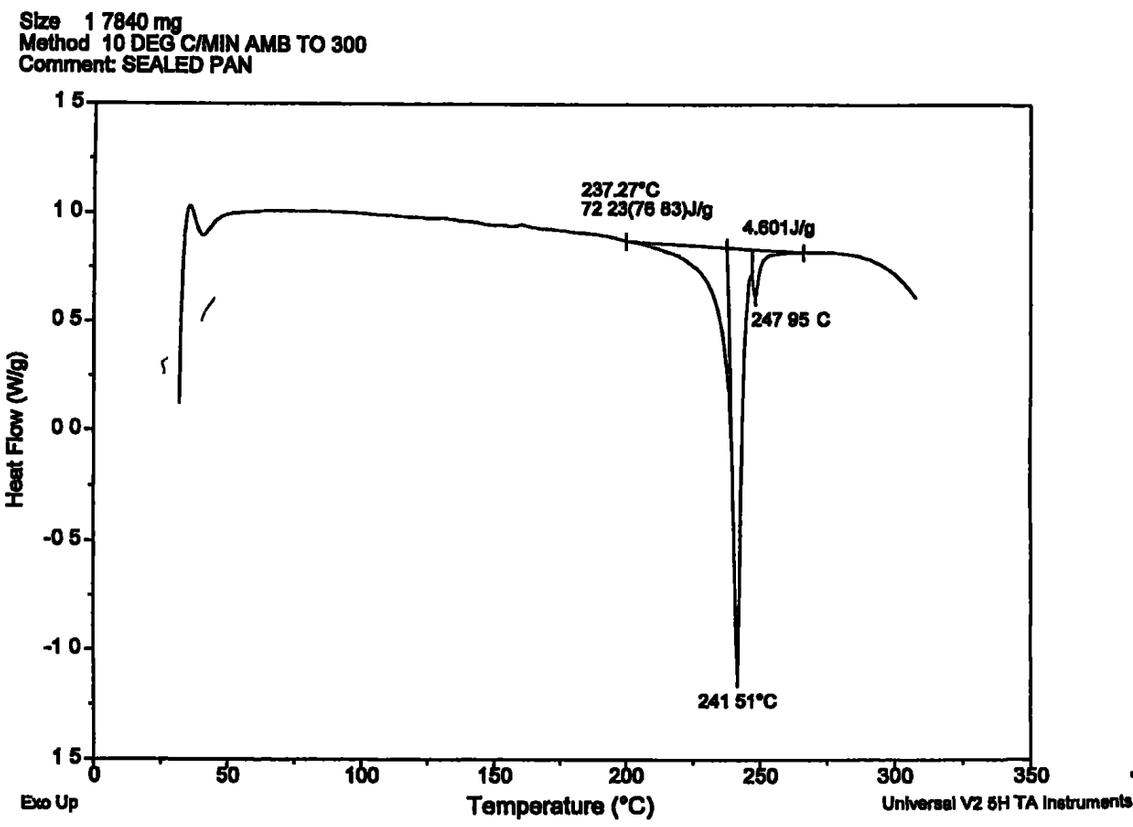
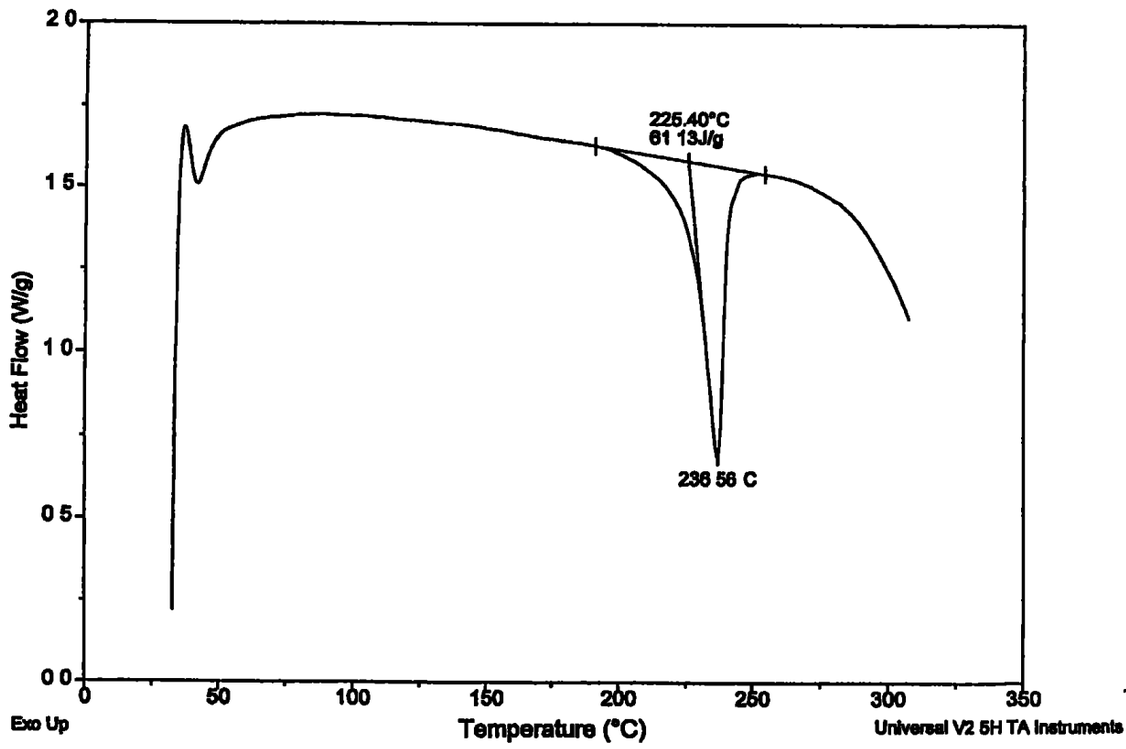

Size 1 7840 mg
Method 10 DEG C/MIN AMB TO 300
Comment: SEALED PAN
237.27°C
72 23(76 83)J/g
4.601J/g
247 95 C
241 51°C
Heat Flow (W/g)
Exo Up
Temperature (°C)
Universal V2 5H TA Instruments

Figure 2-C


Size. 1 4230 mg
Method 10 DEG C/MIN AMB TO 300
Comment: SEALED PAN
225.40°C
61 13J/g
236 56 C
Heat Flow (W/g)
Exo Up
Temperature (°C)
Universal V2 5H TA Instruments

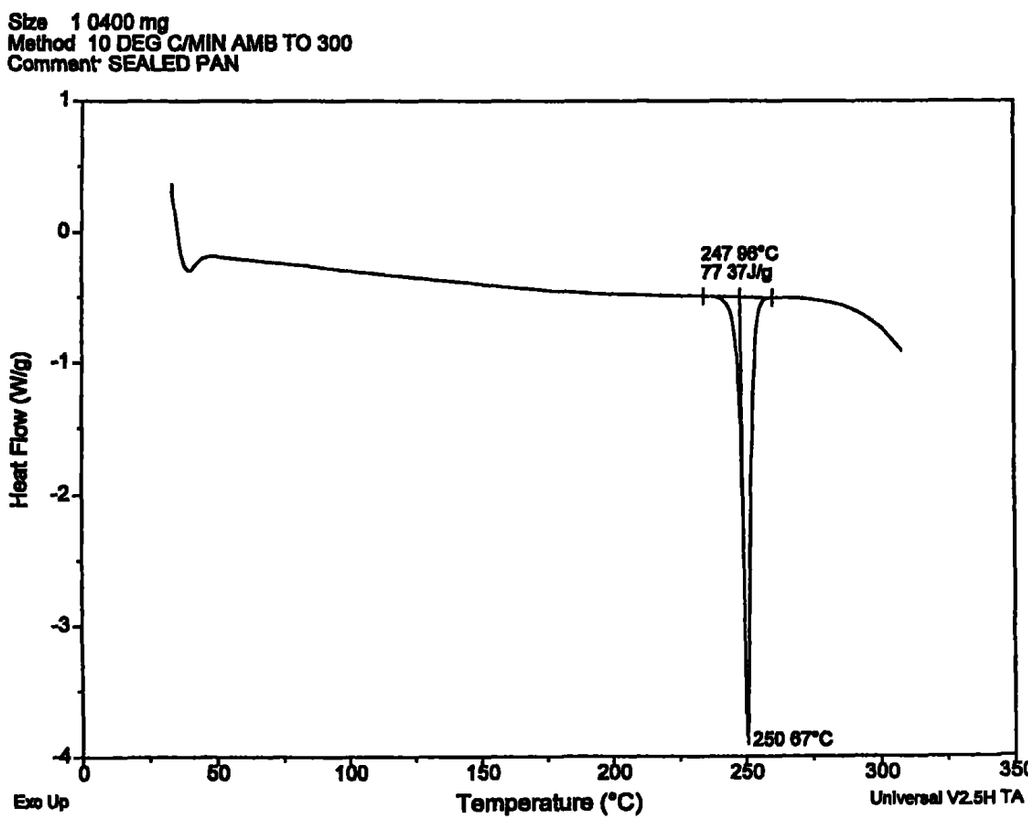

Size 1 0400 mg
Method 10 DEG C/MIN AMB TO 300
Comment SEALED PAN
247 96°C
77 37J/g
250 67°C
Heat Flow (W/g)
Exo Up
Temperature (°C)
Universal V2.5H TA Instruments
1
0
-1
-2
-3
-4
0
50
100
150
200
250
300
350

Figure 2-2

399

Sample Weight: 15.300 mg
Comment:
N-Propyl Alcohol solvate 1
unwashed pan

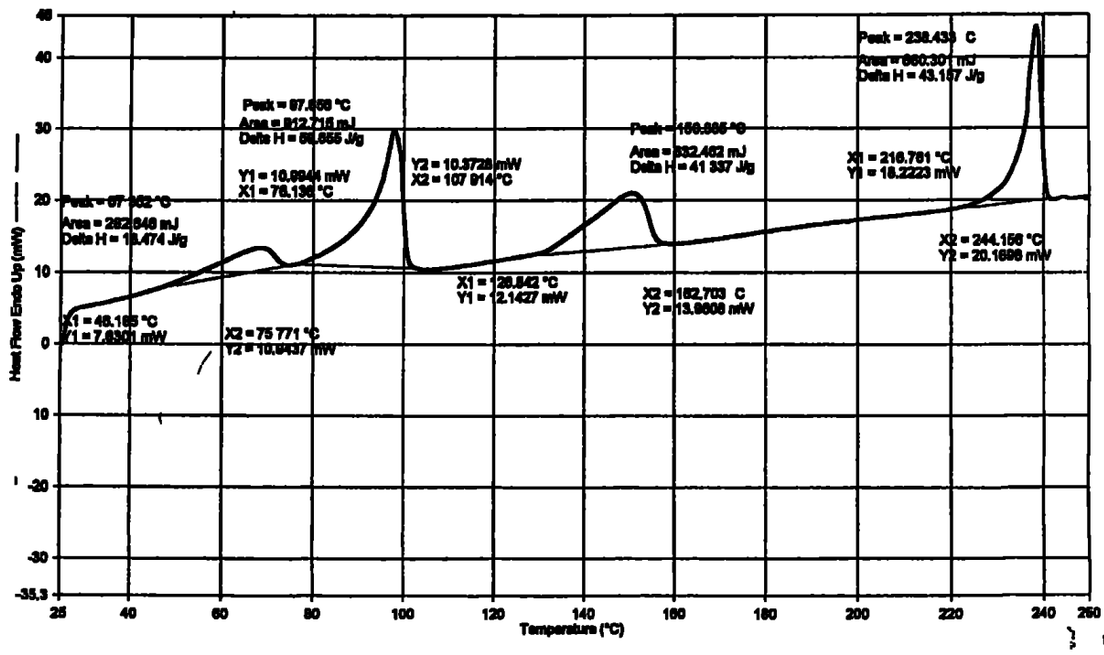
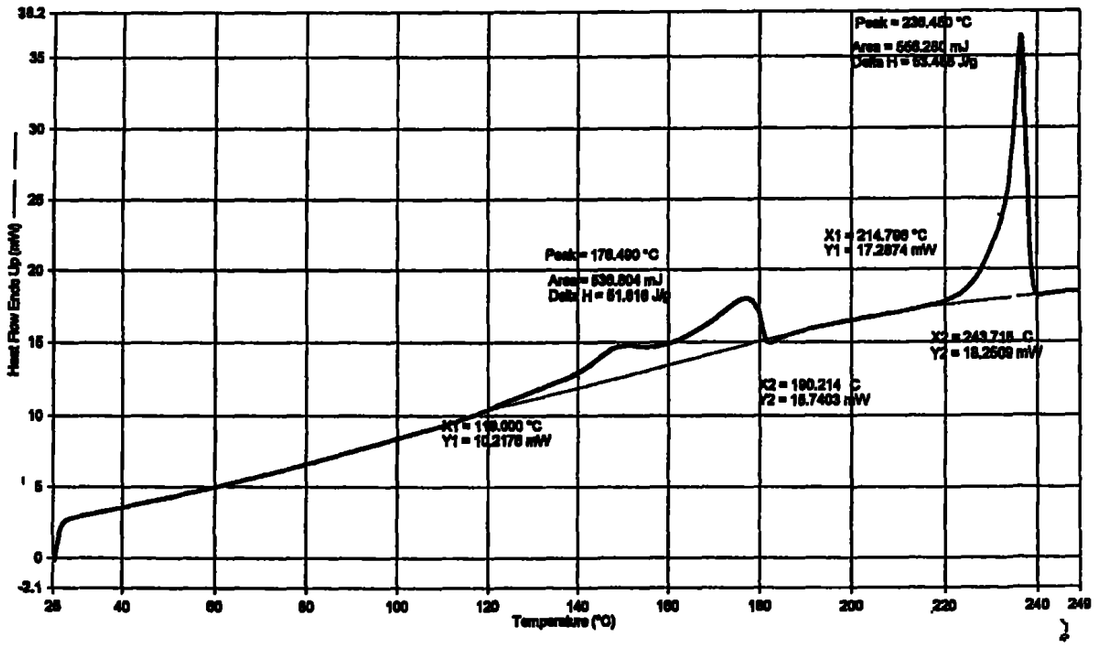


Figure 2-F

6680215 5569109

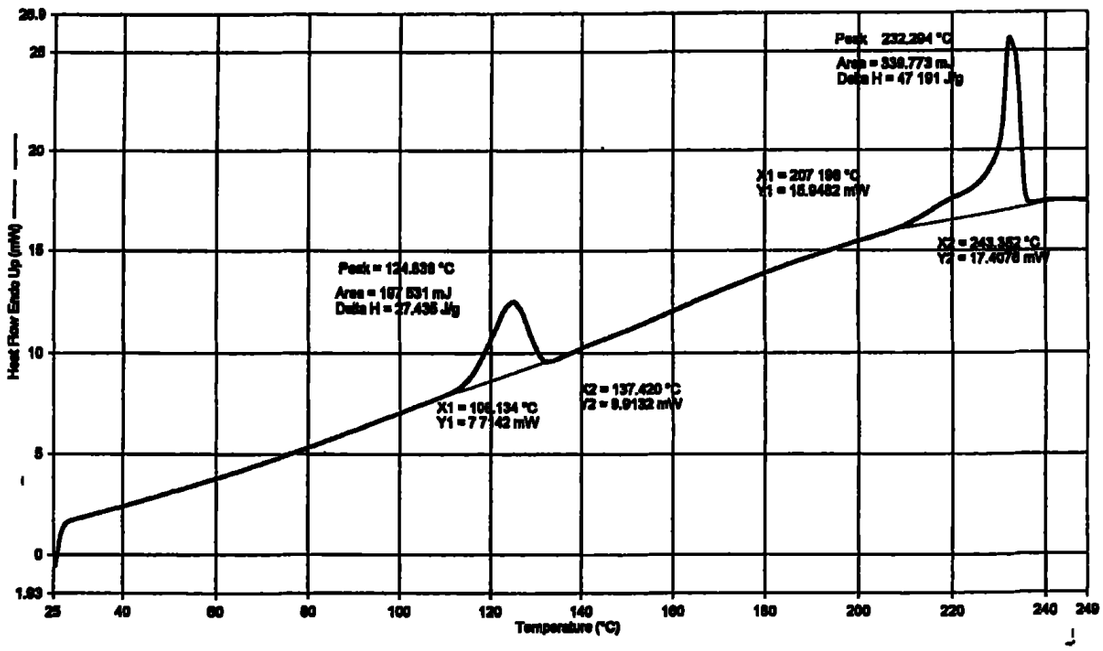
Sample Weight: 10.400 mg
Comment: THF solvate 1
unsealed pan



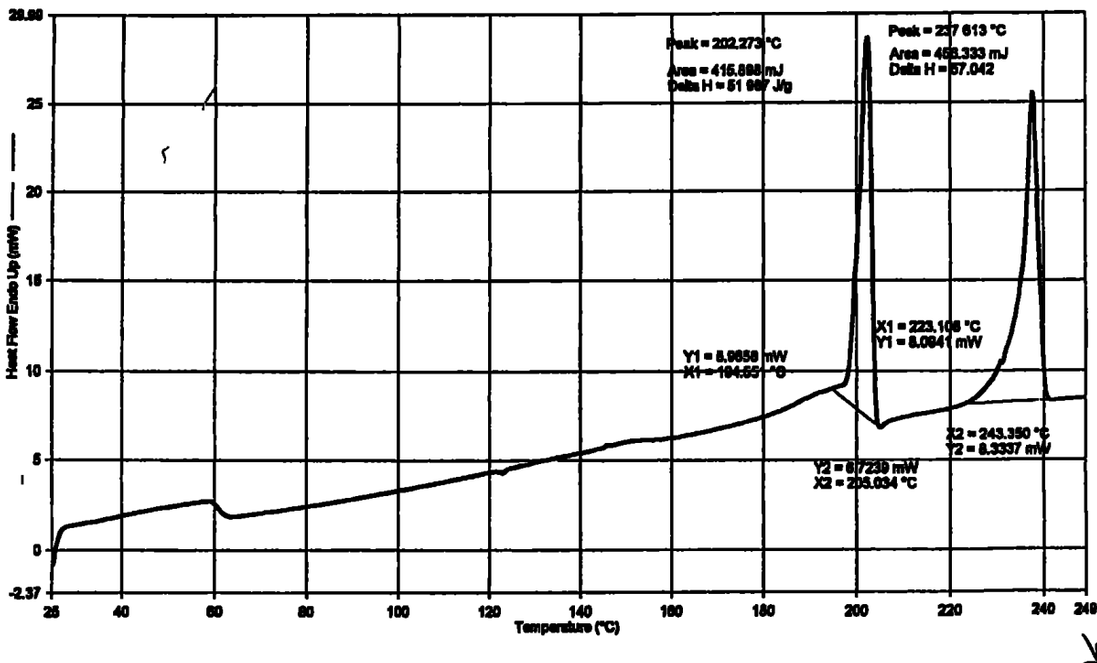
405

Figure 2-C

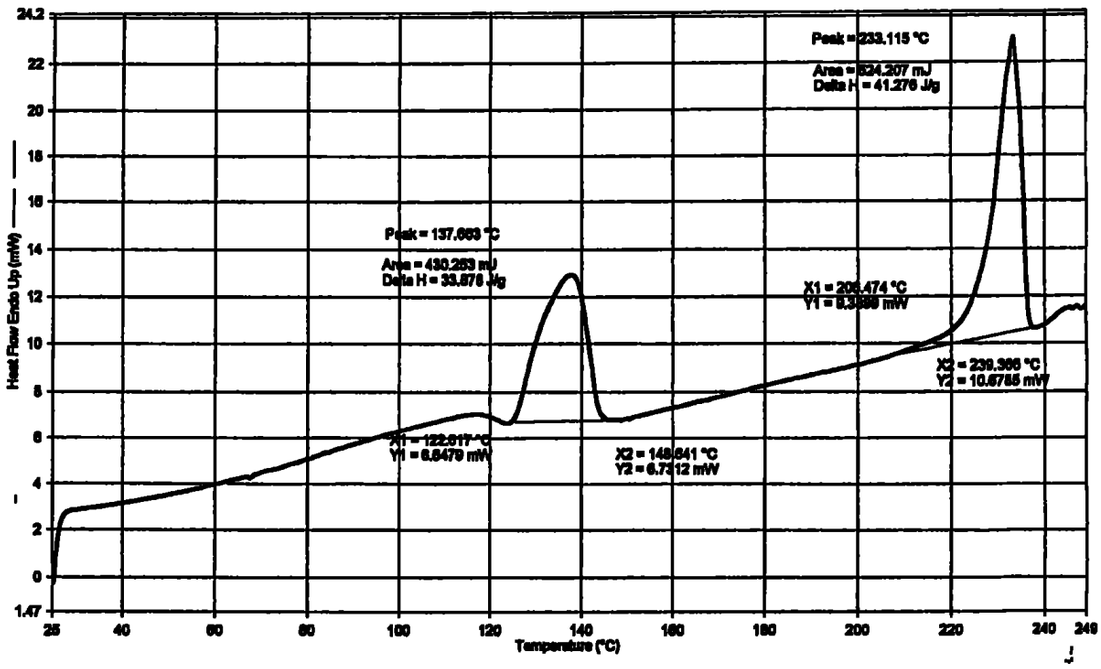
Sample Weight:	7.200 mg
Comment:	Lot 96K020-F3b
	Ethyl propionate solvate



Sample Weight: 8.000 mg
Comment: Acetic ACid solvate 1
unsealed pan

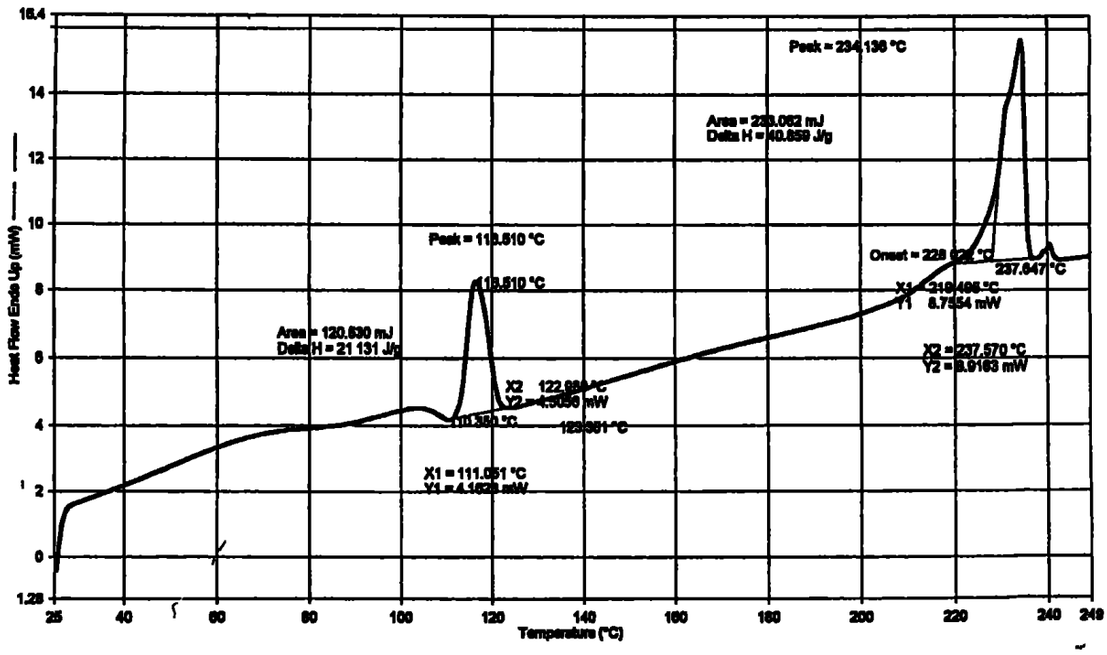


Sample Weight: 12.700 mg
Comment: Chloroform solvate 1
unsealed pan



404

Sample Weight: 6.704 mg
Comment: Acetone Solvate



404

Figure 2-k

668021 95569109

Sample Weight: 6.947 mg
Comment: toluene Solvate

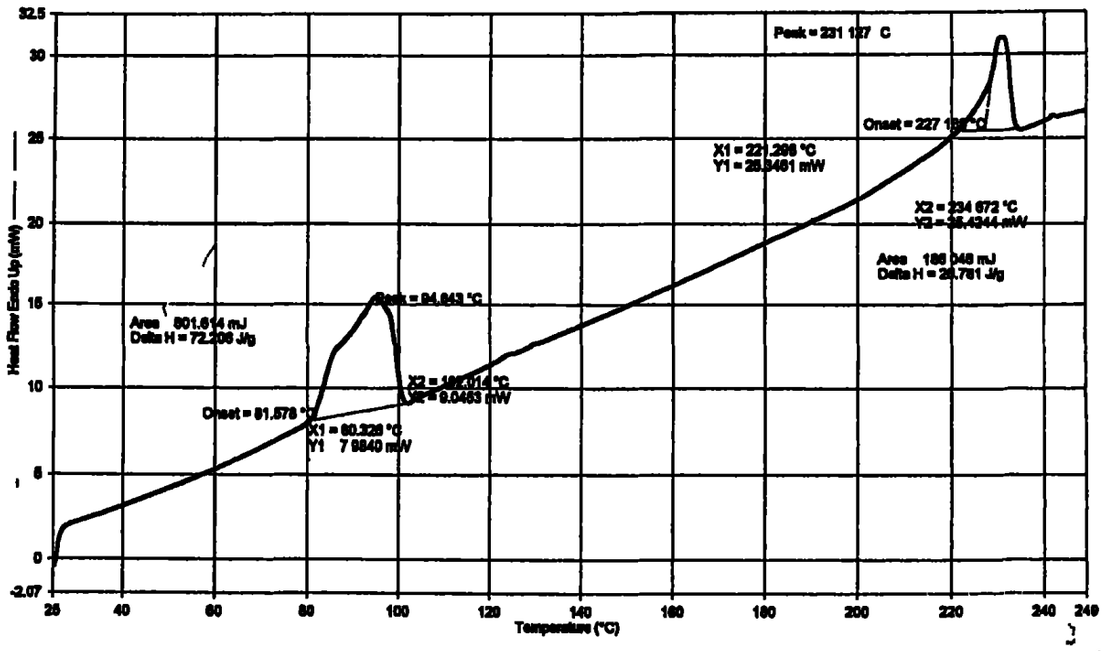


Figure 2-1

Sample Weight: 8.174 mg
Comment: Isopropanol Solvate

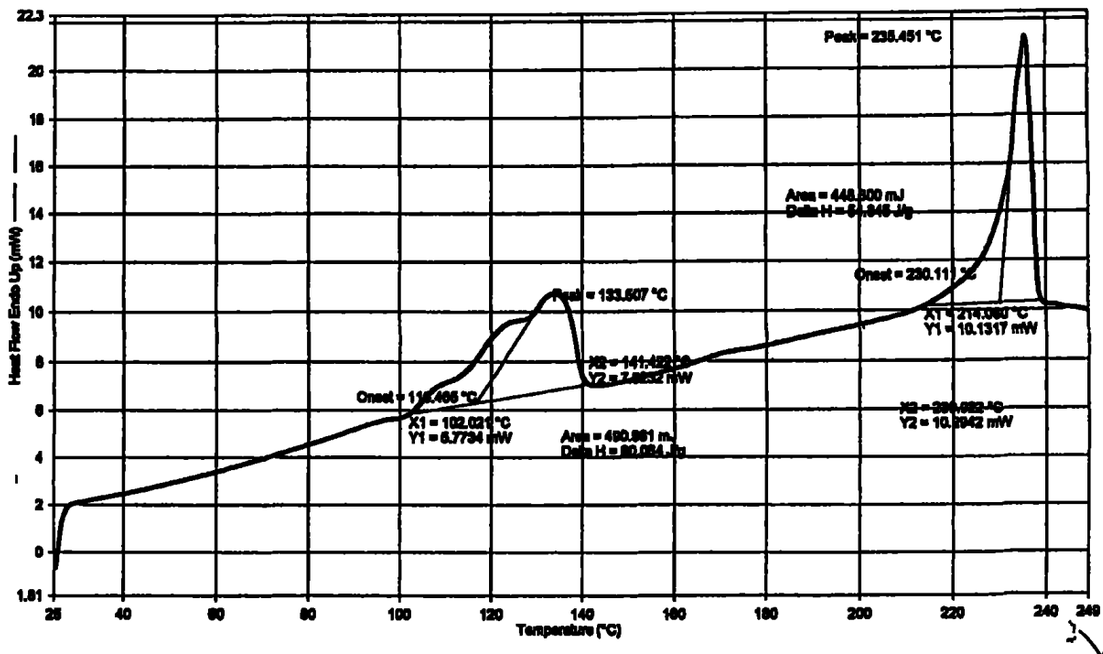
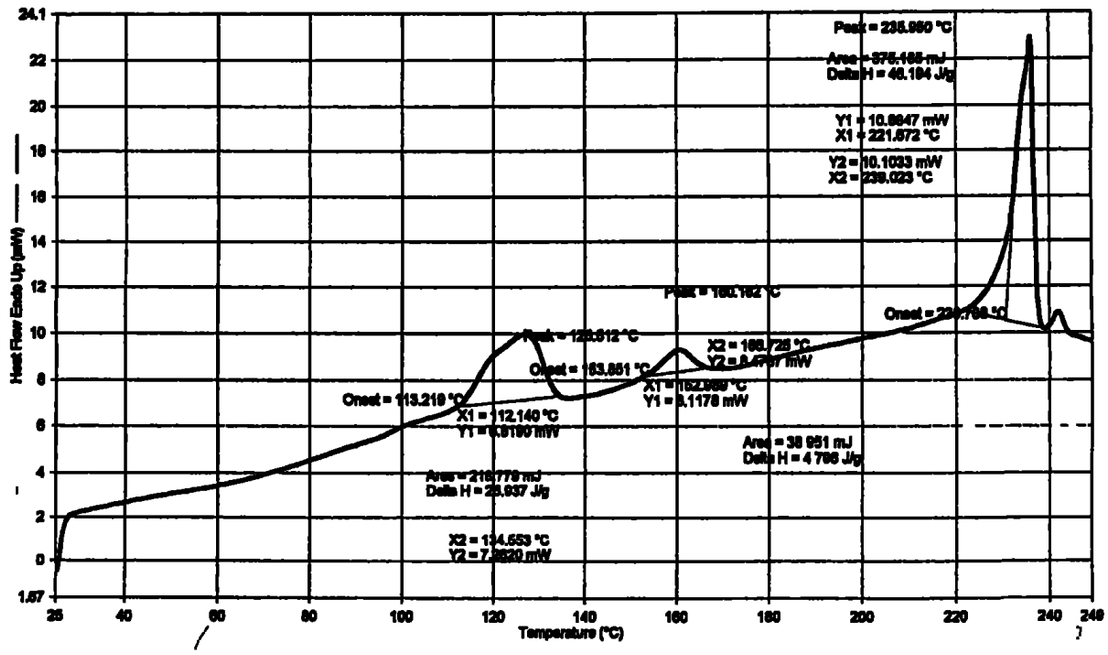


Figure 2 N

668021 [redacted] 5569T09

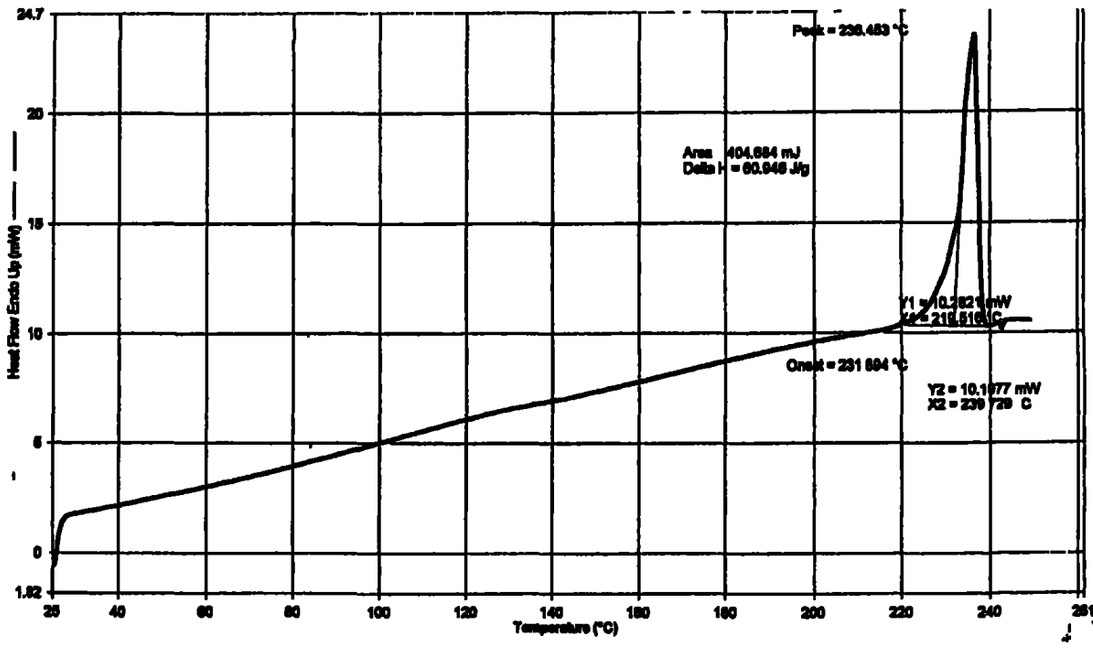
Sample Weight: 6.122 mg
Comment:
ethanol solvate
10 °C/min sealed pan



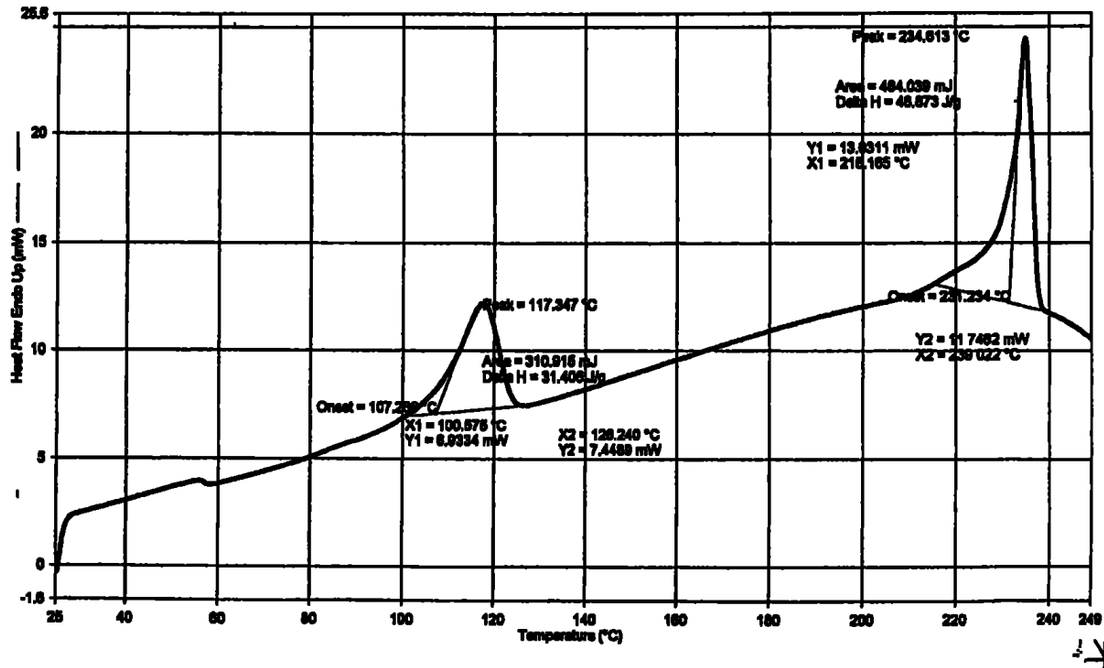
668021 Figure 2cN 60169109

408

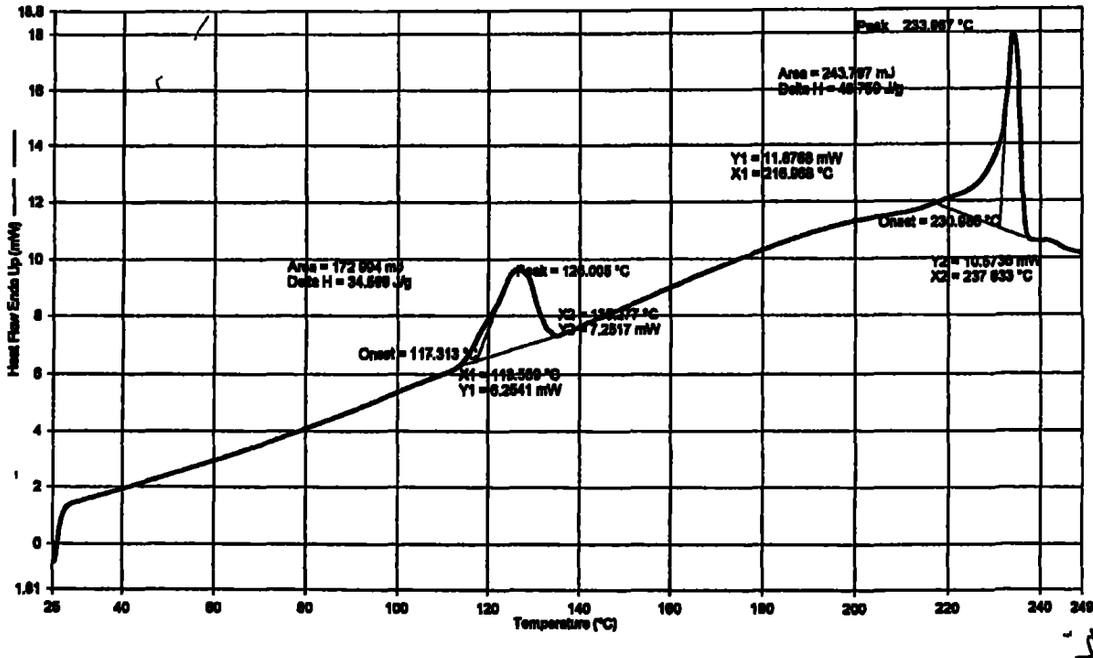
Sample Weight: 6.640 mg
Comment:
t-butyl acetate solvate
10 °C/min sealed pan



Sample Weight: 9.900 mg
Comment: isobutyl acetate activate
10 °C/min sealed pan



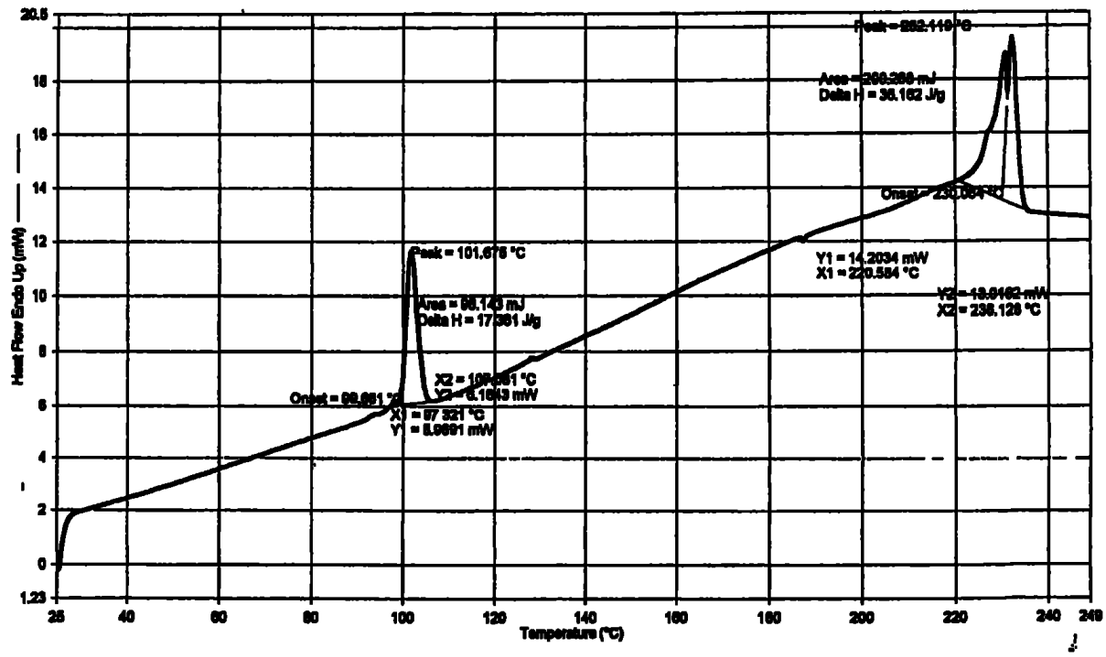
Sample Weight: 0.000 mg
Comment:
butyl acetate solvate
10 °C/min sealed pan



45

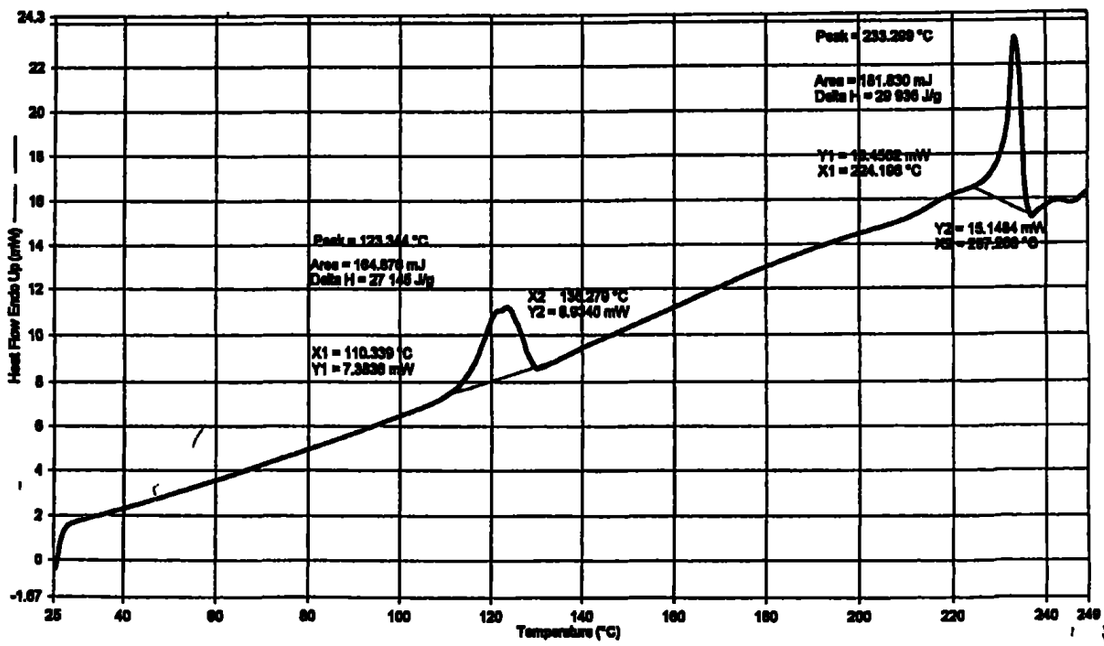
Figure 2-0

Sample Weight: 6.536 mg
Comment:
methyl acetate solvate 1
10 °C/min sealed pan

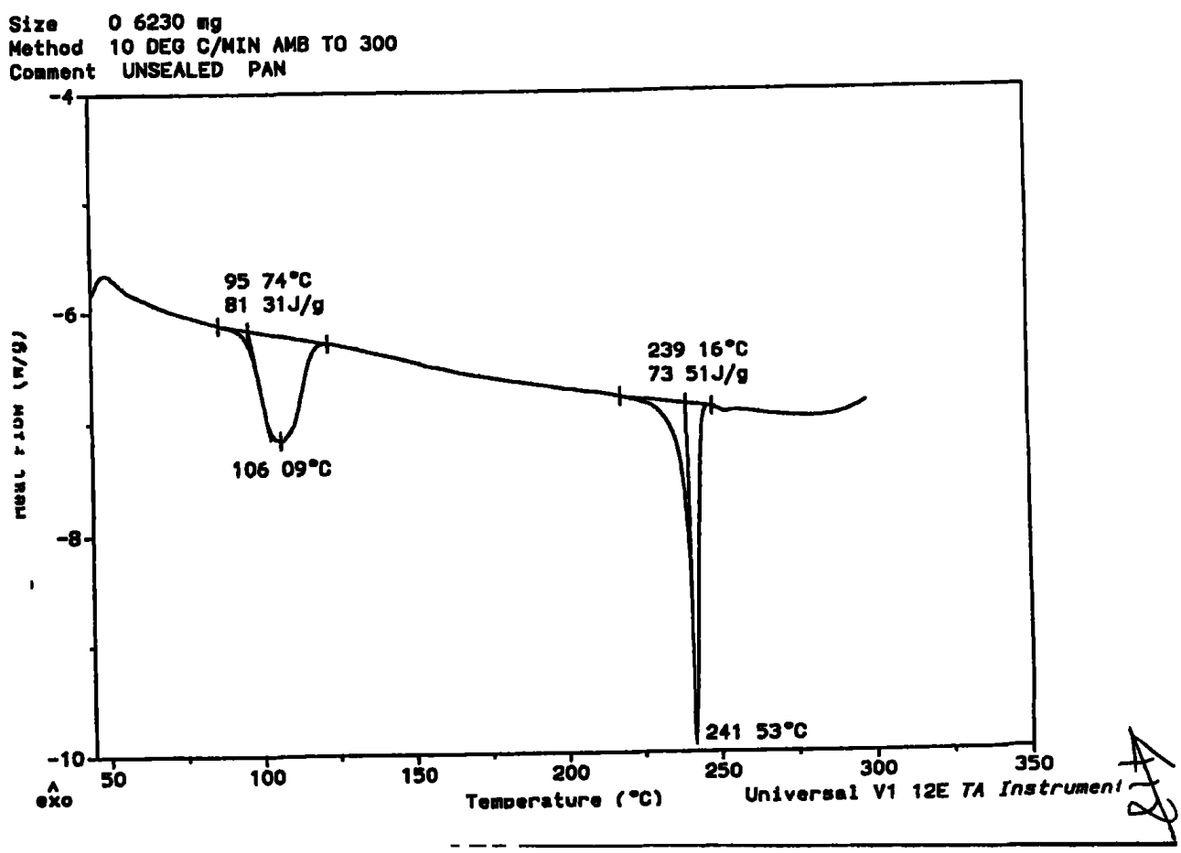


416

Sample Weight: 6.074 mg
Comment:
propyl acetate solvate 1
10 °C/min sealed pan



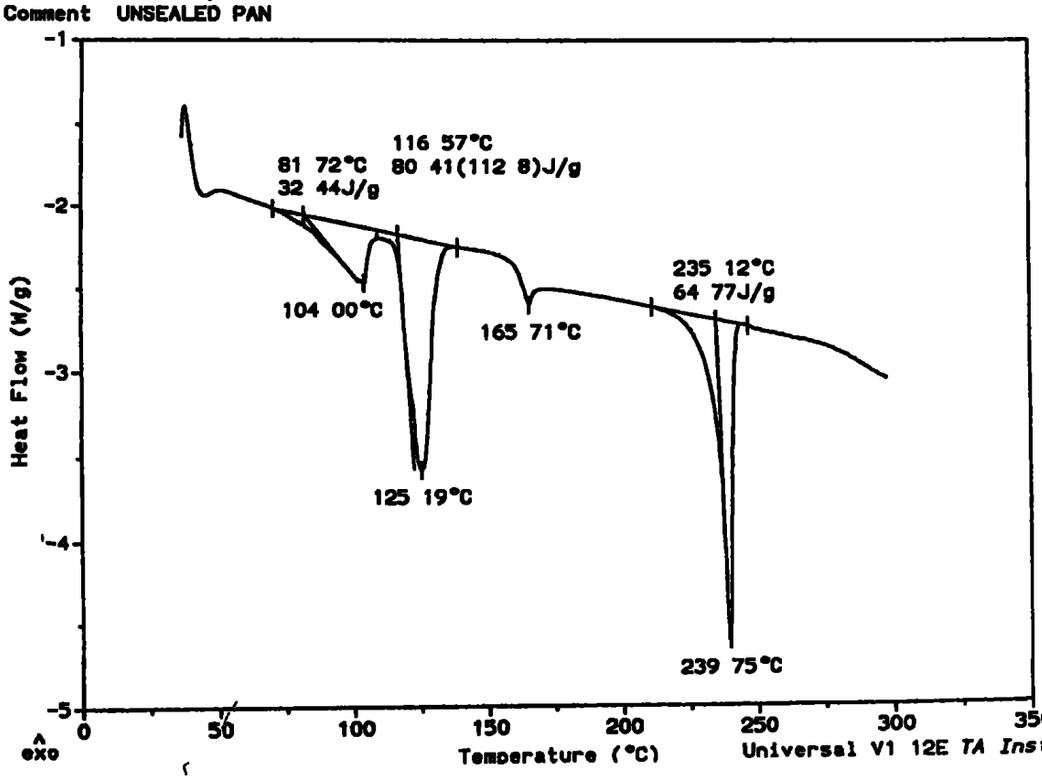
n-butanol solvate



668021 Figure 2 69T09

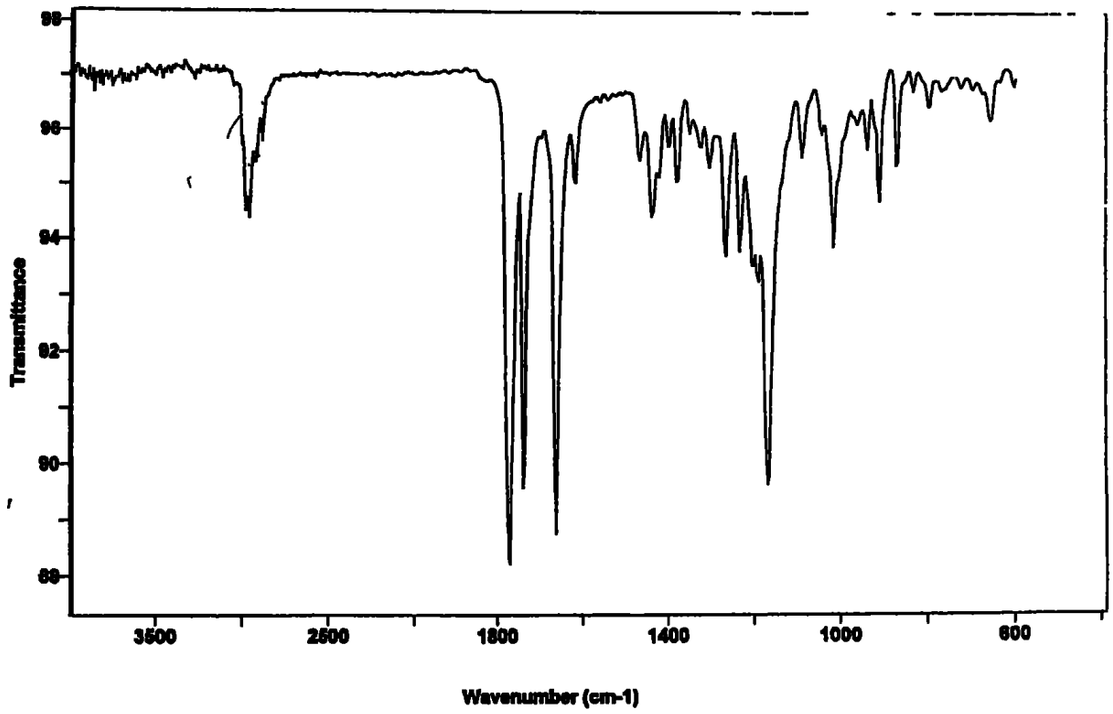
n-octanol solvate

Size 0 5220 mg
Method 10 DEG C/MIN AMB TO 300
Comment UNSEALED PAN

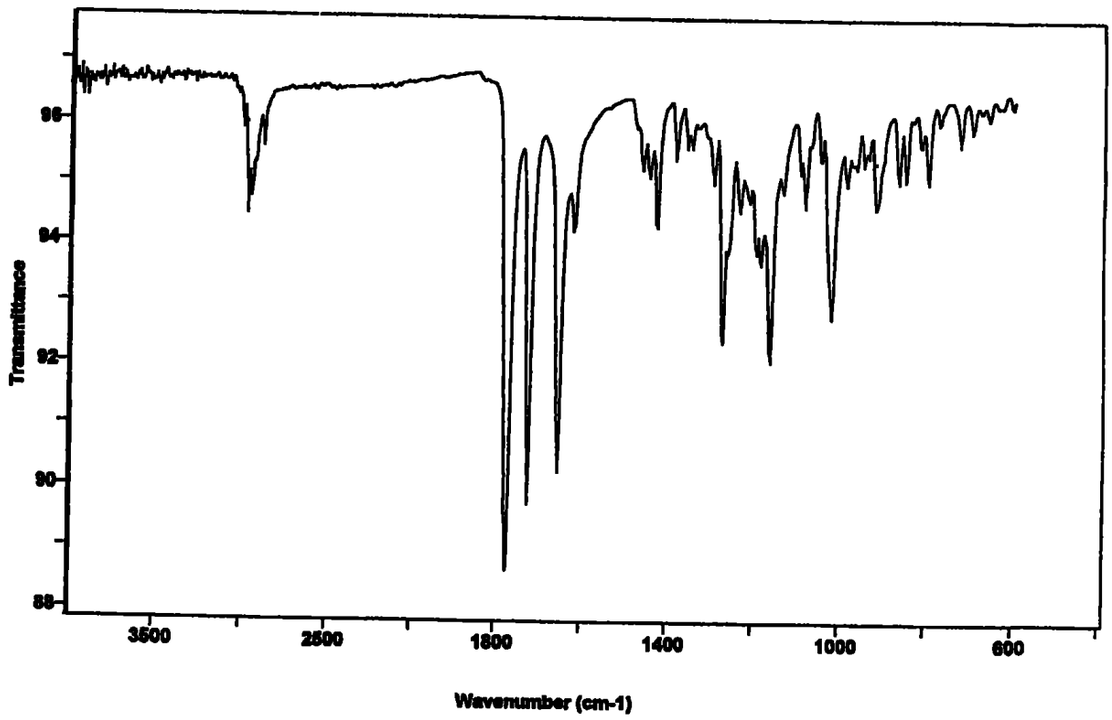


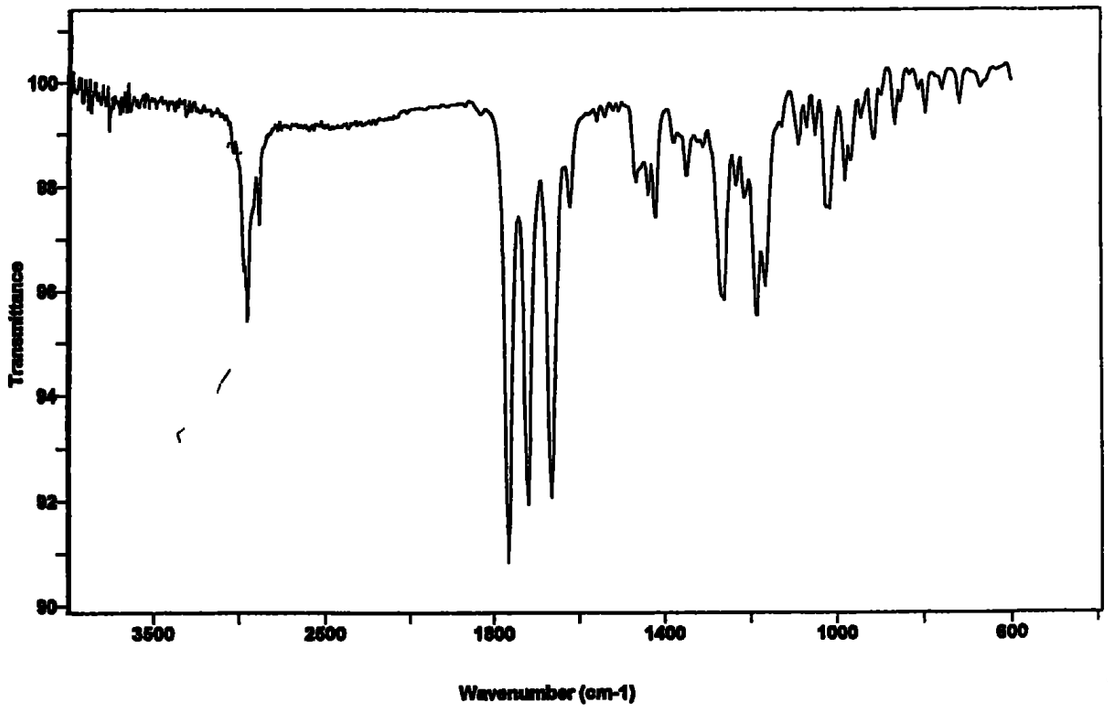
668021 6569709

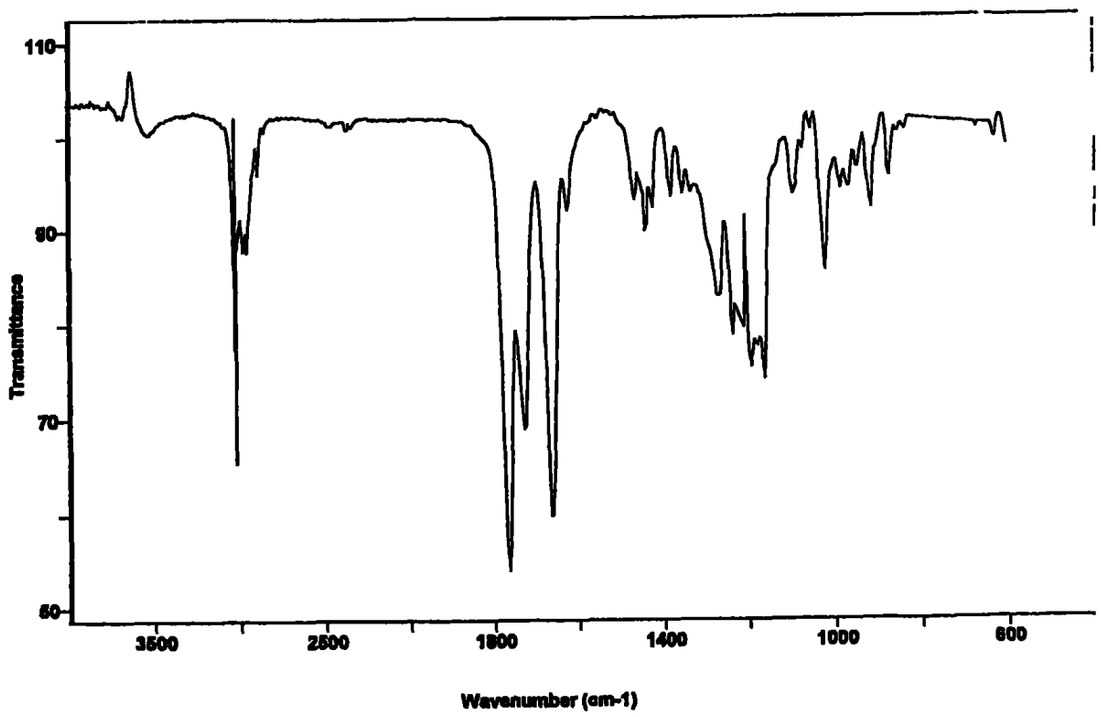
Figure 3-A



420

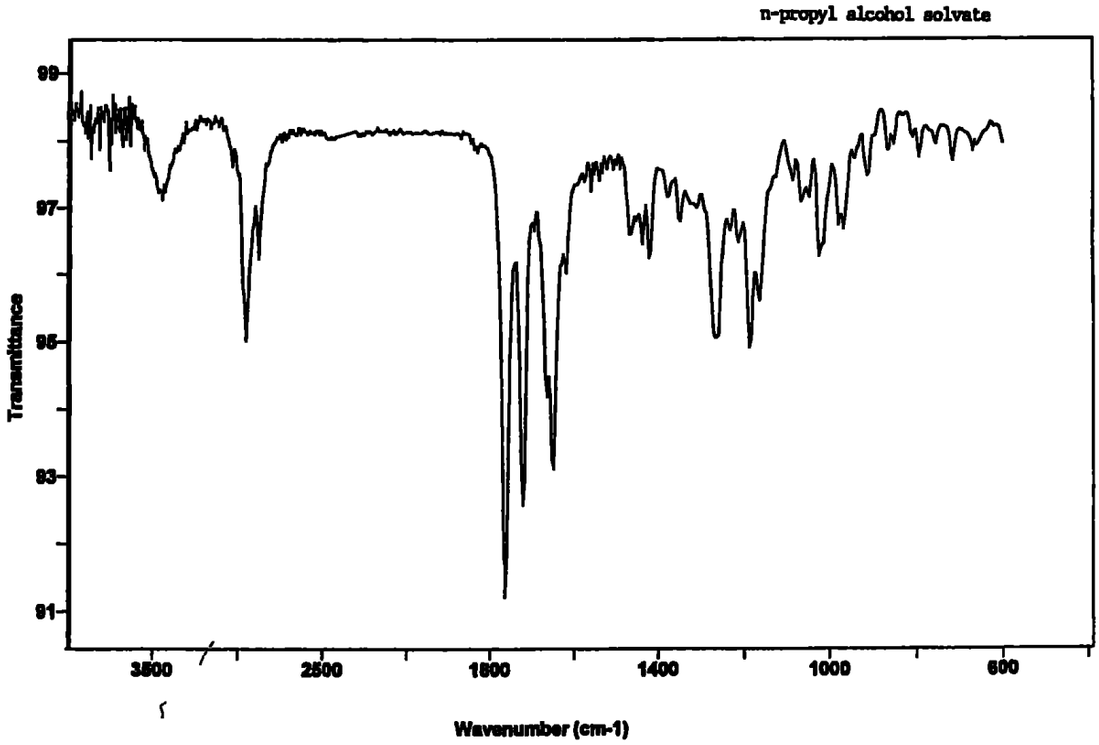

Transmittance
96
94
92
90
88
3500
2500
1800
1400
1000
600
Wavenumber (cm-1)

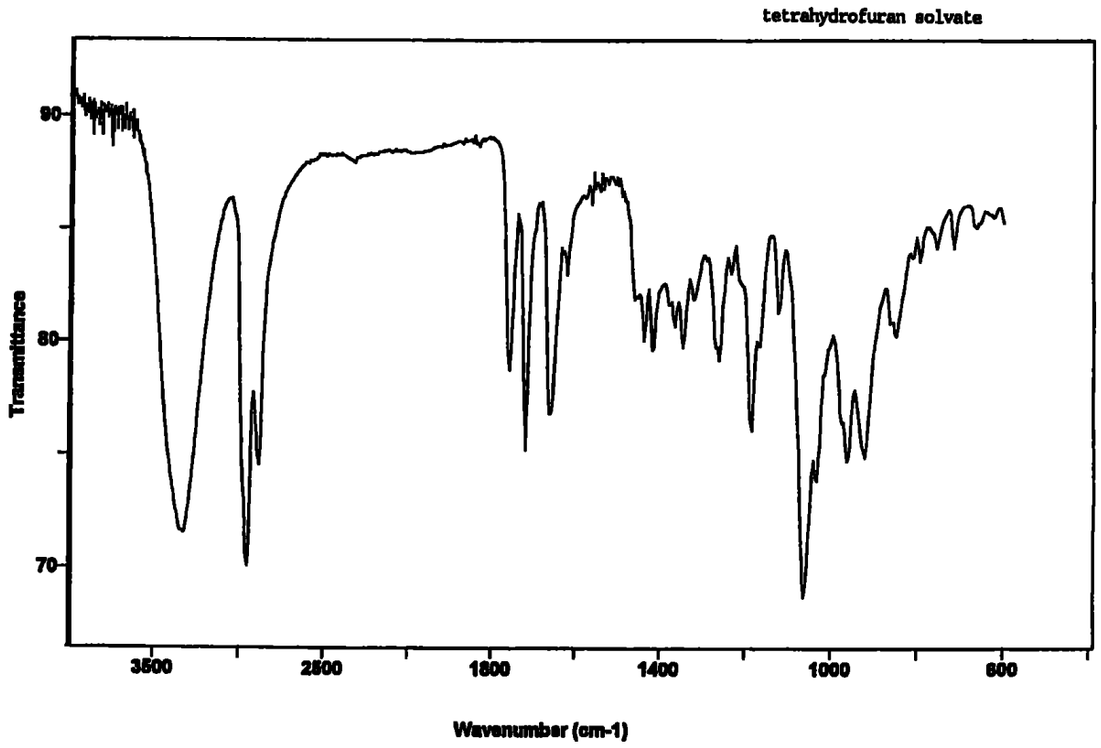

100
98
96
94
92
90
Transmittance
3500
2500
1800
1400
1000
600
Wavenumber (cm-1)


110
90
70
50
Transmittance
3500
2500
1900
1400
1000
600
Wavenumber (cm-1)

6688021 6569109

Figure 1F




tetrahydrofuran solvate
90
80
70
Transmittance
3500
2500
1800
1400
1000
800
Wavenumber (cm-1)

ethyl propionate solvate

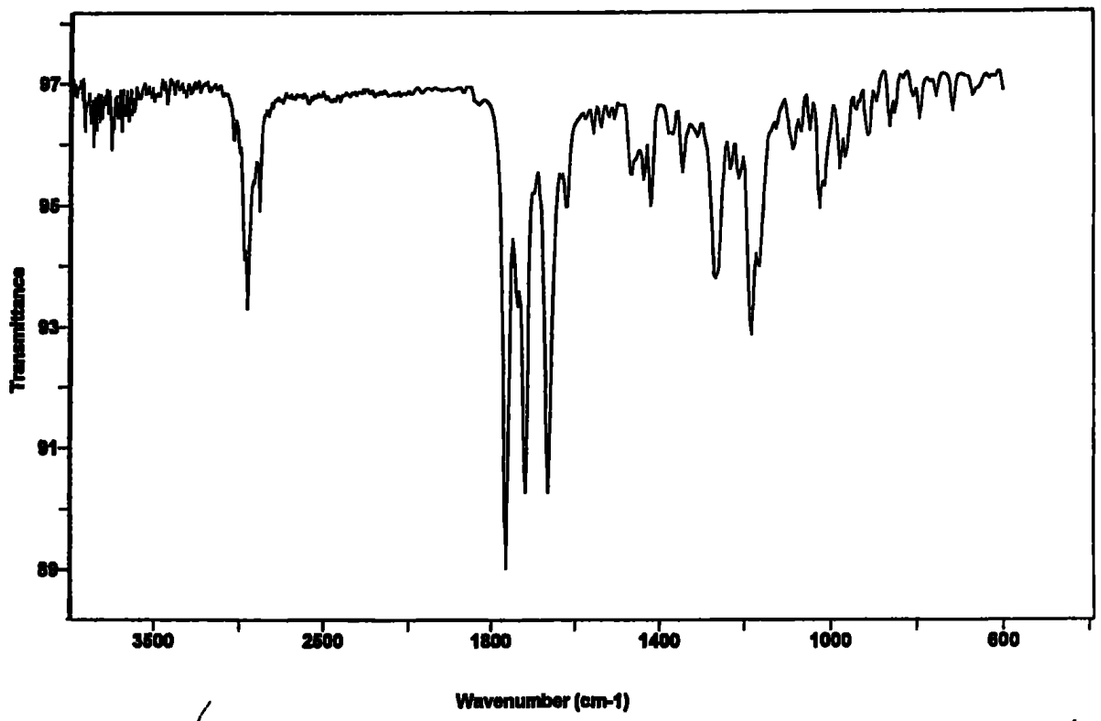
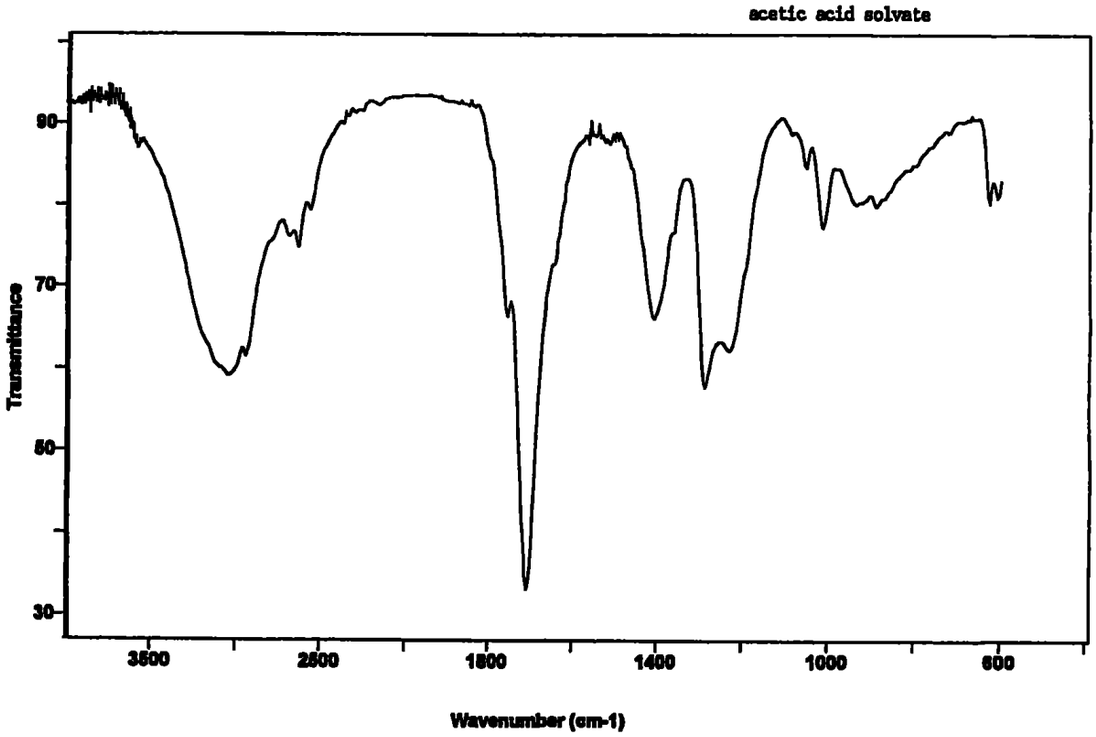


Fig 6

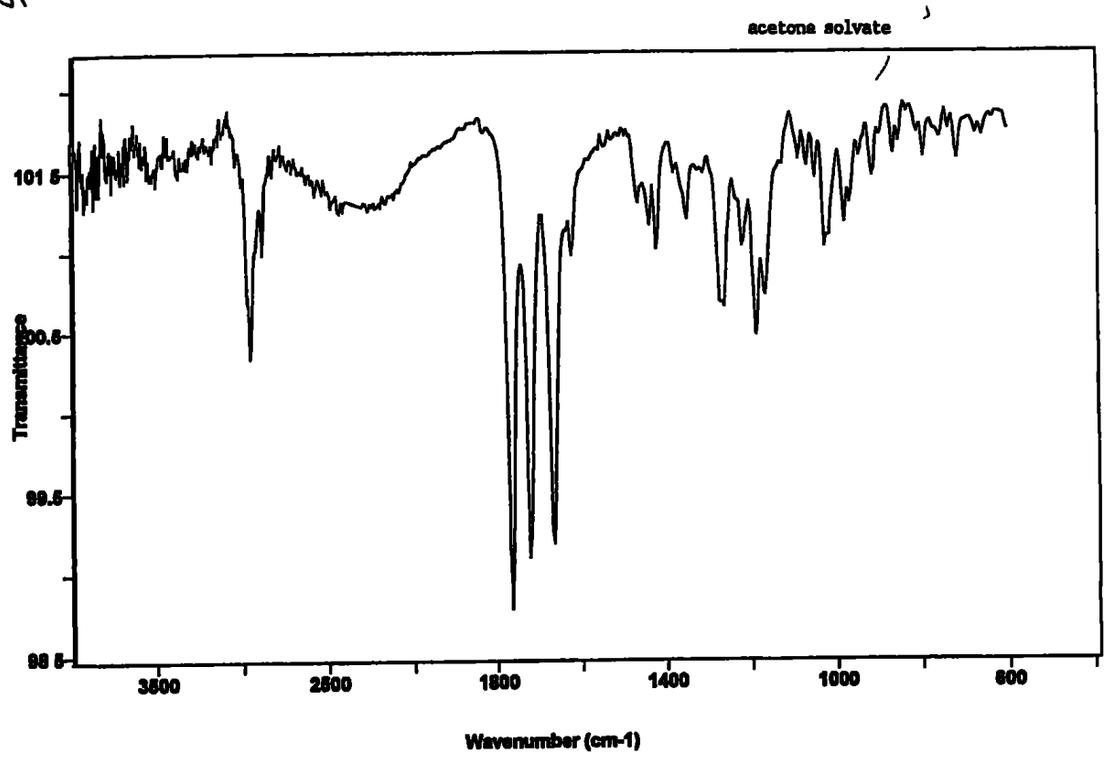
668021 6691709

Figure 2H



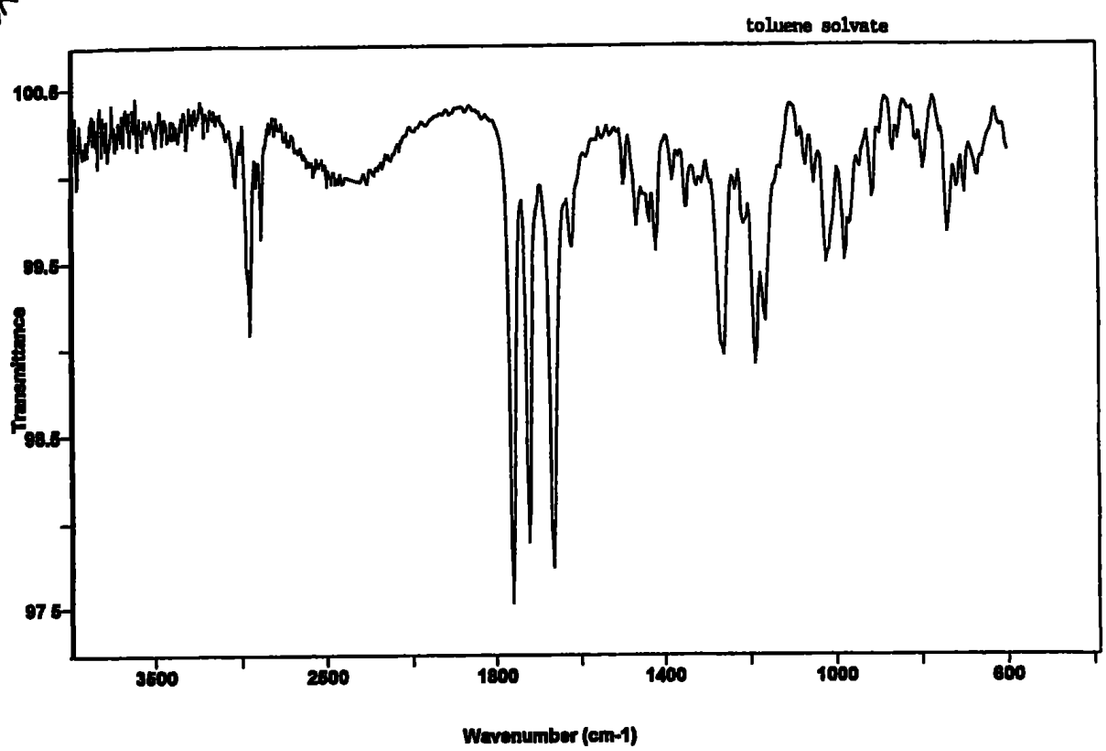
424

Figure 3-I



601695[redacted].120899

Figure 3-J

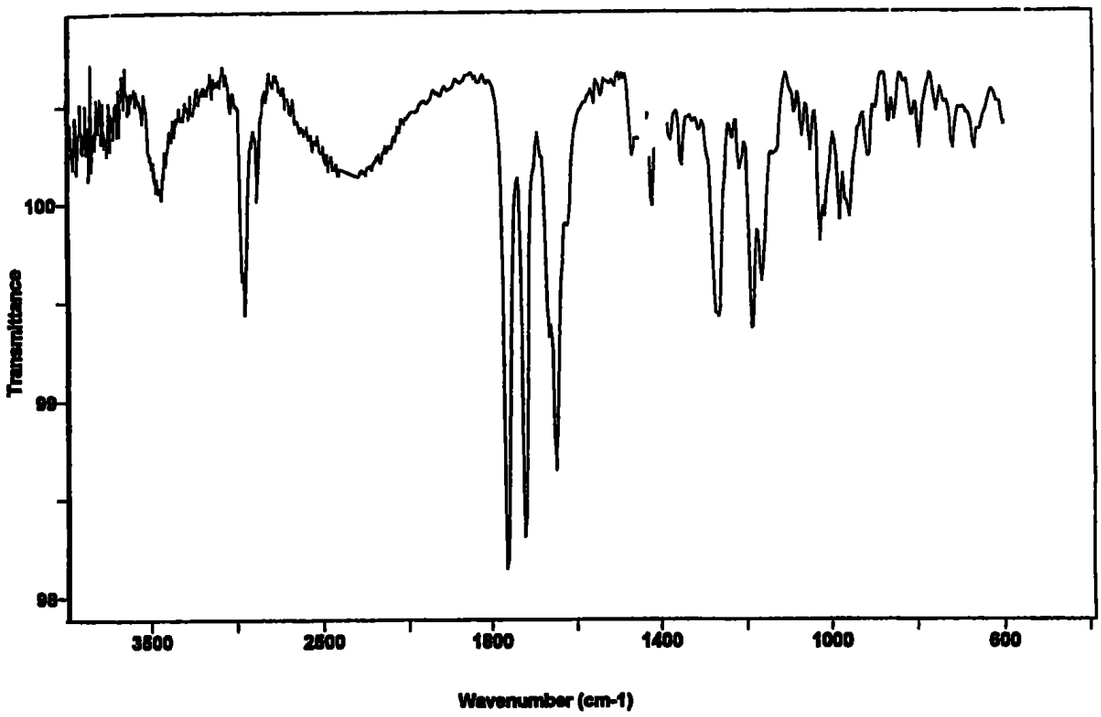


6016955B 120899

4330

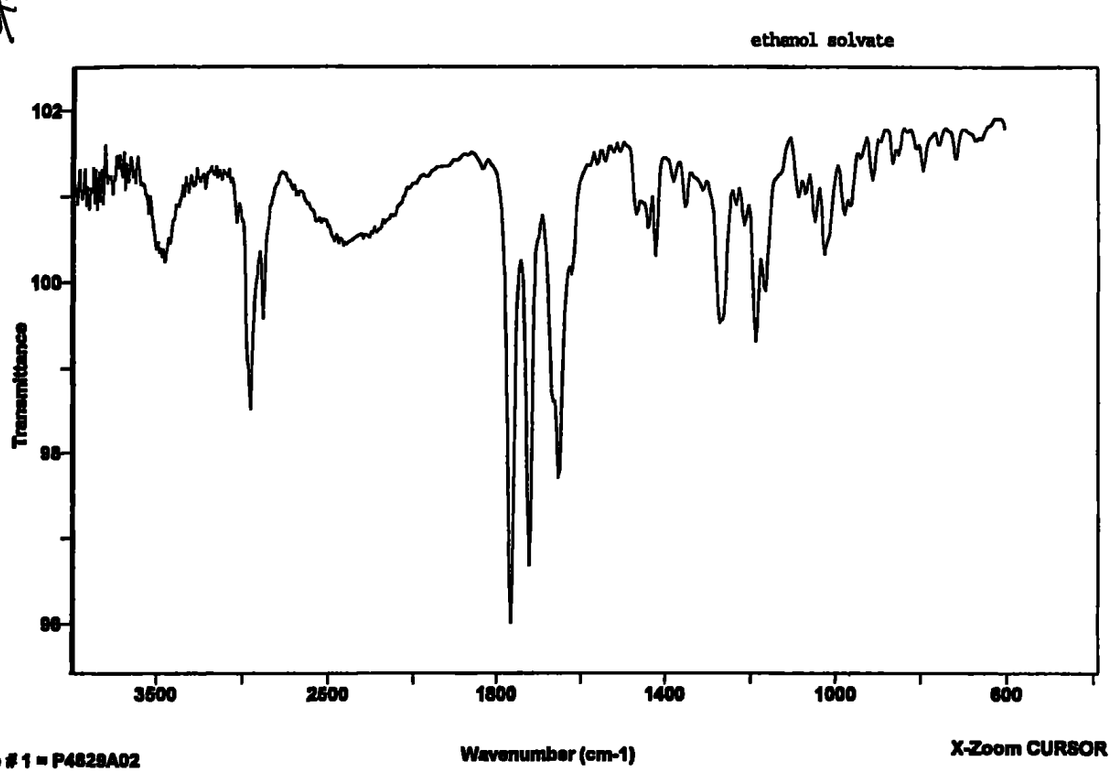
Figure 3-K

isopropanol solvate



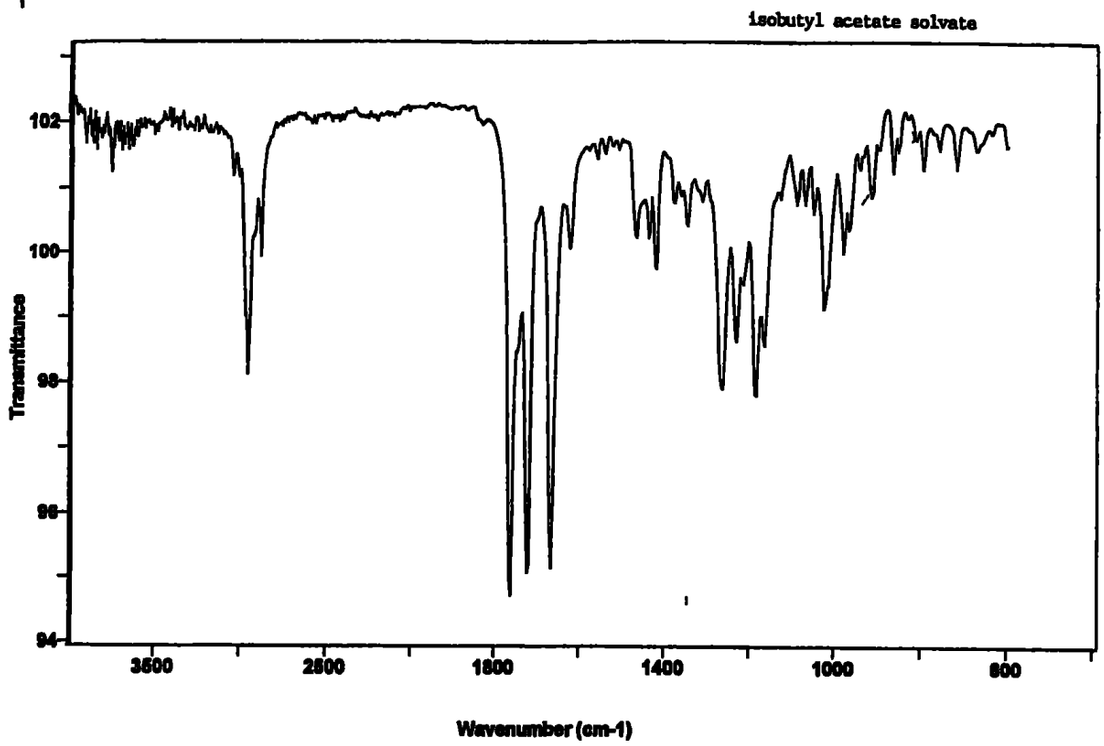
5016955 120899

Figure 3-L



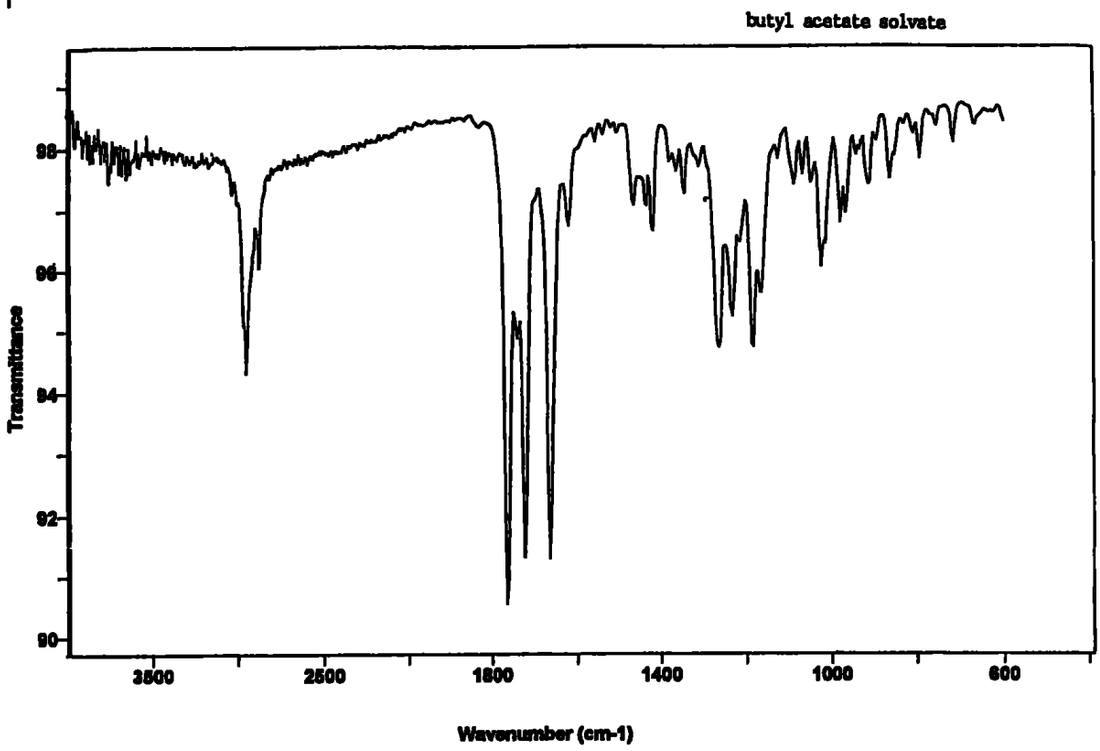
5016955 120899

Figure 3-M



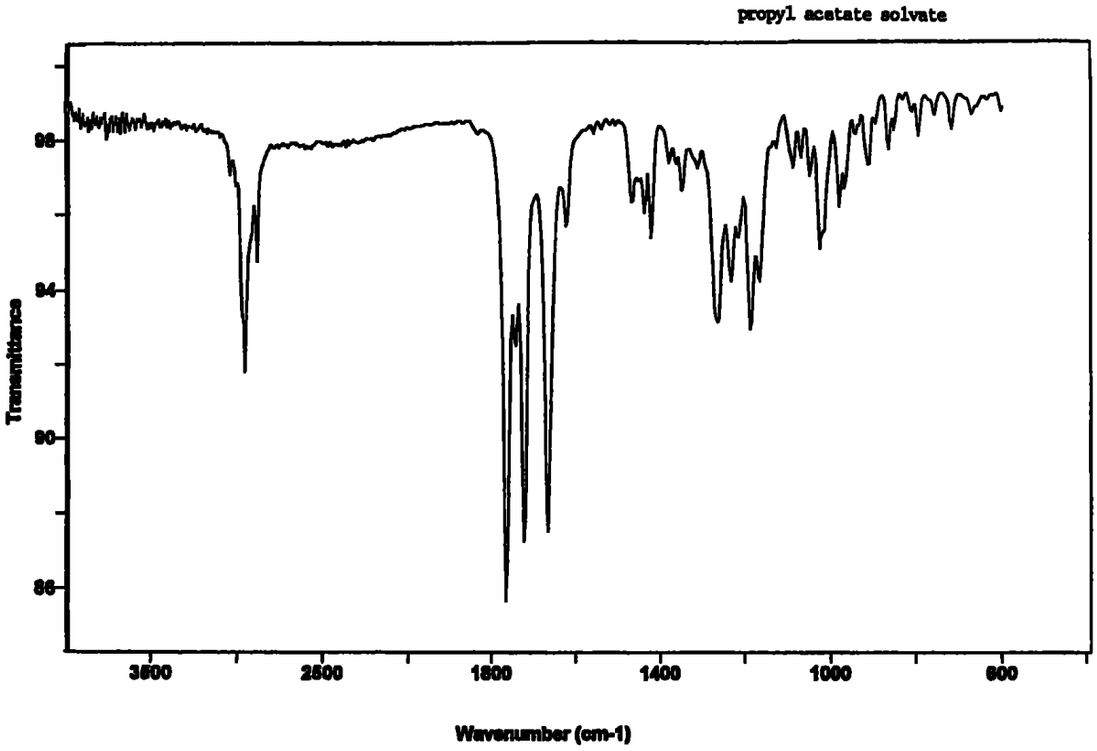
601695[redacted] 120899

Figure 3-N



601695[illegible] 120899

Figure 3-0



60169556 120899

435

Figure 3-P

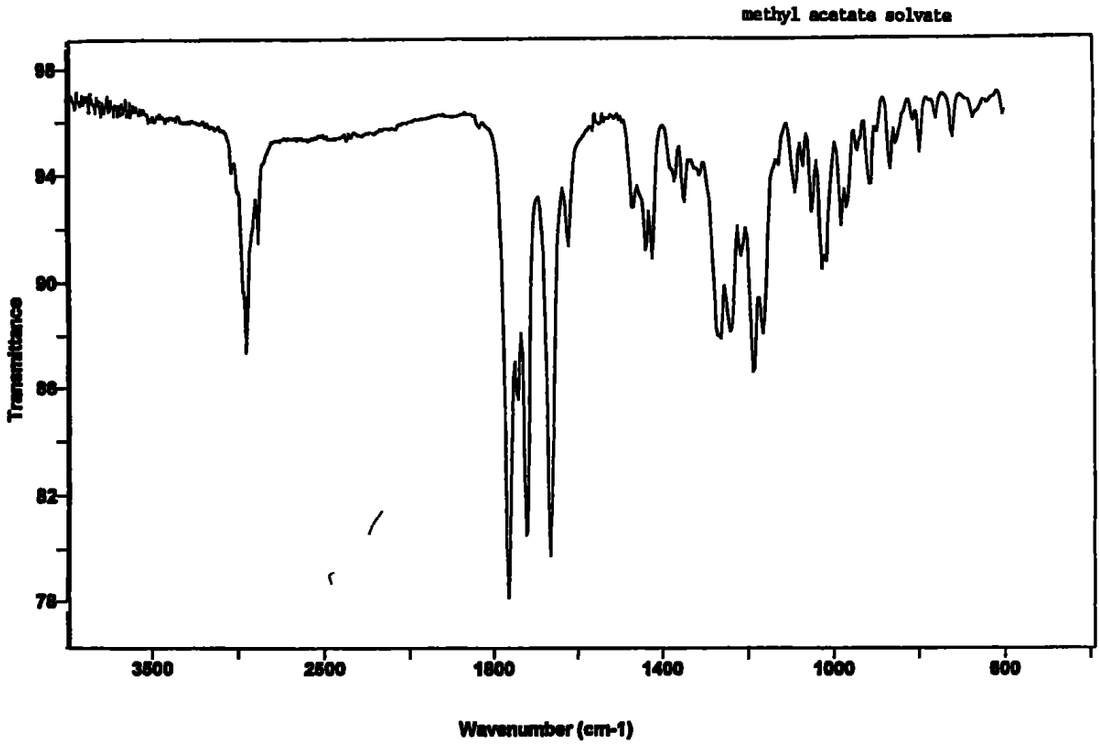


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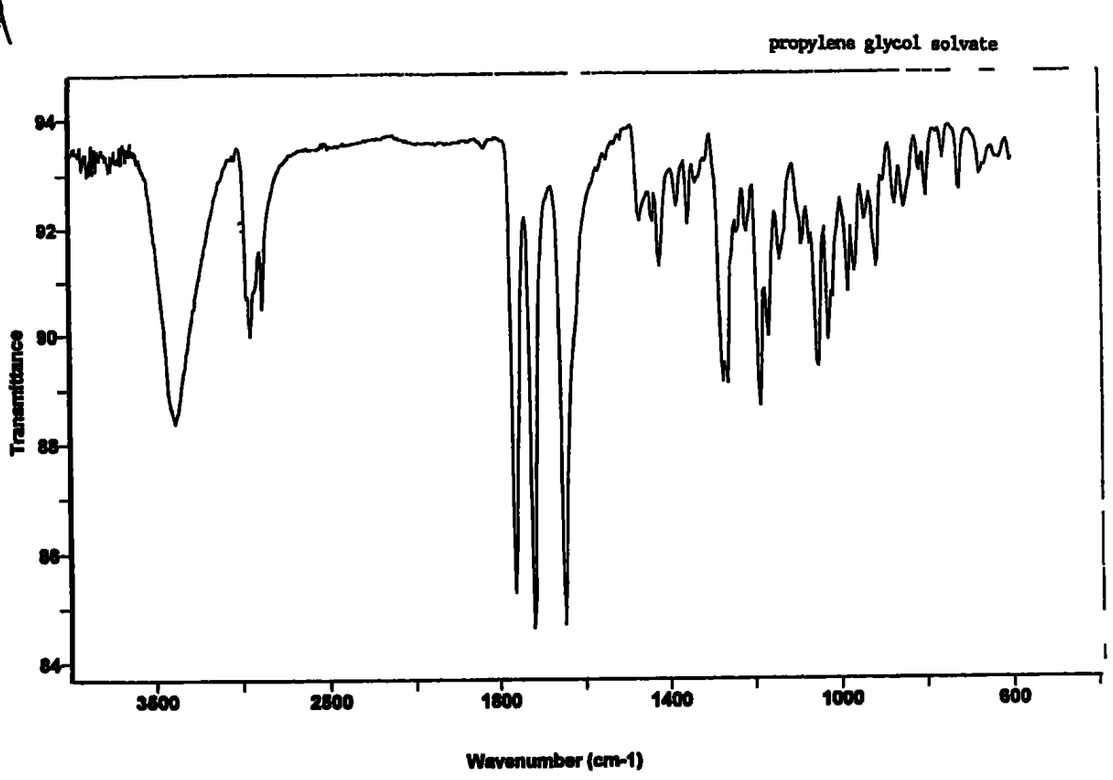
Figure 3-Q



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437

Figure 3-B

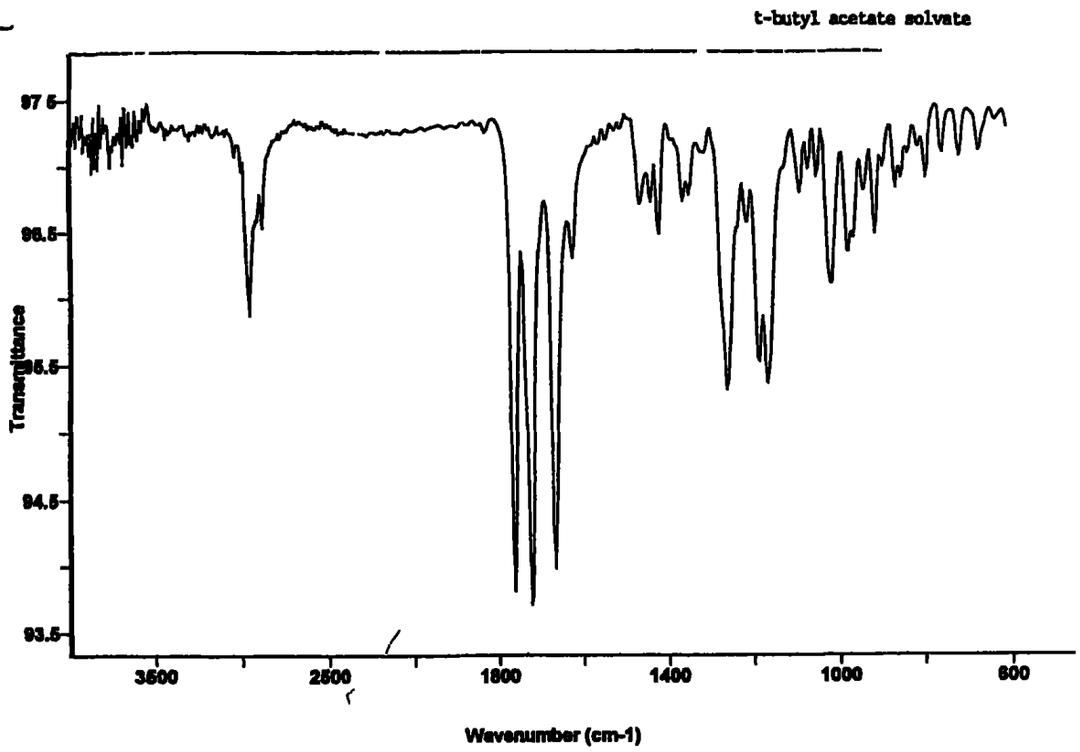

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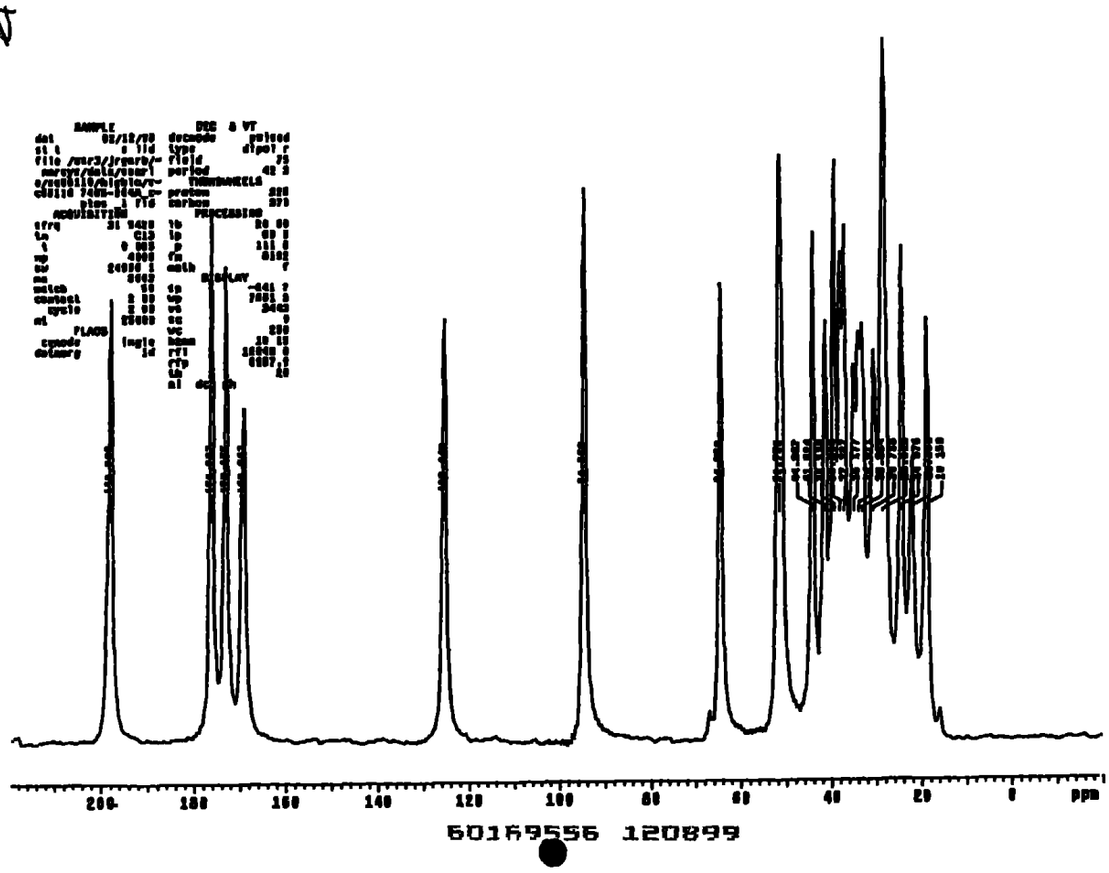
Figure 4



439

Figure 5

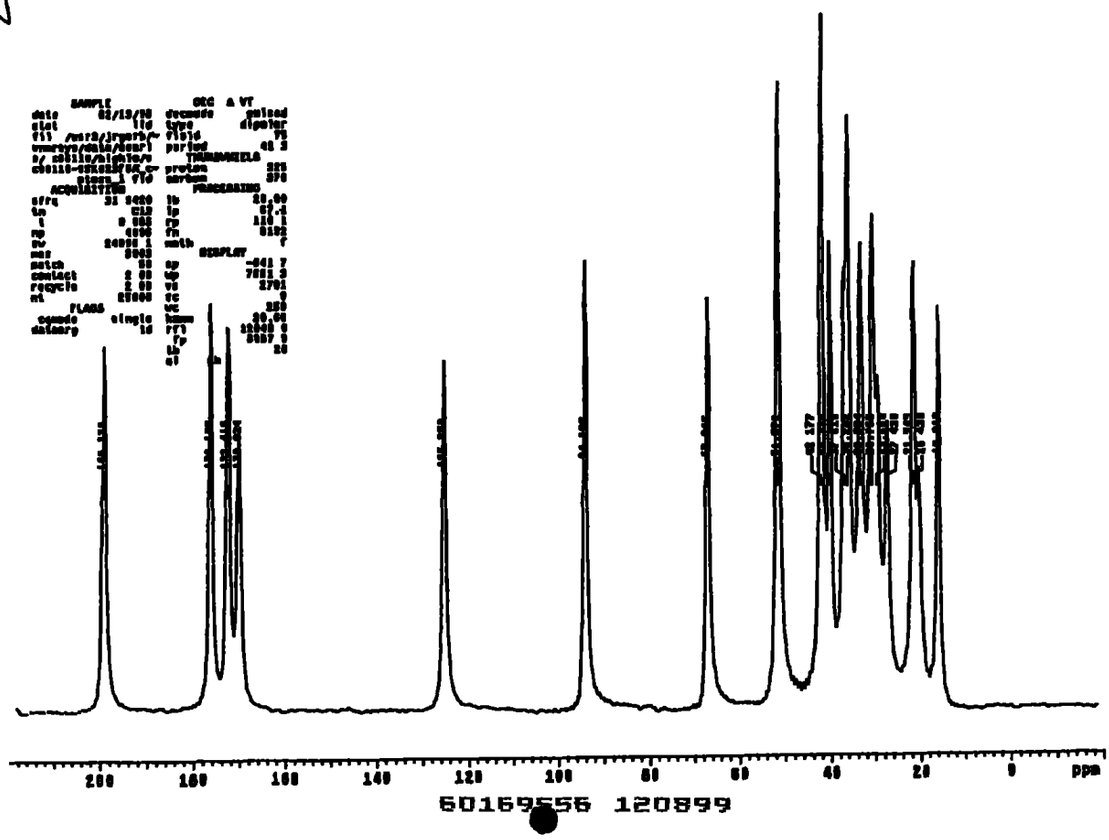


Figure 6-A

Size 3.3010 mg
Method 10 deg ramp to 300 deg

methyl ethyl ketone solvate

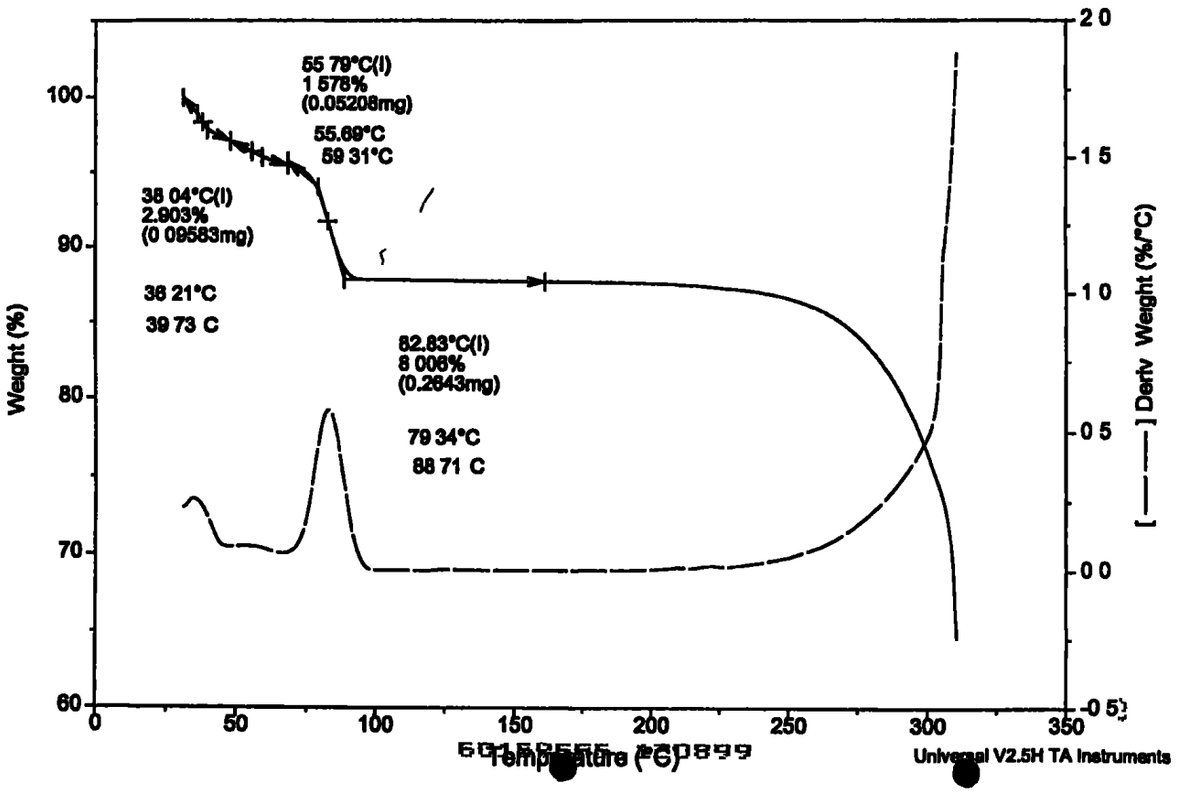


Figure 6-B

Size 13 4190 mg
Method 10 deg ramp to 300 deg

n-propyl alcohol solvate

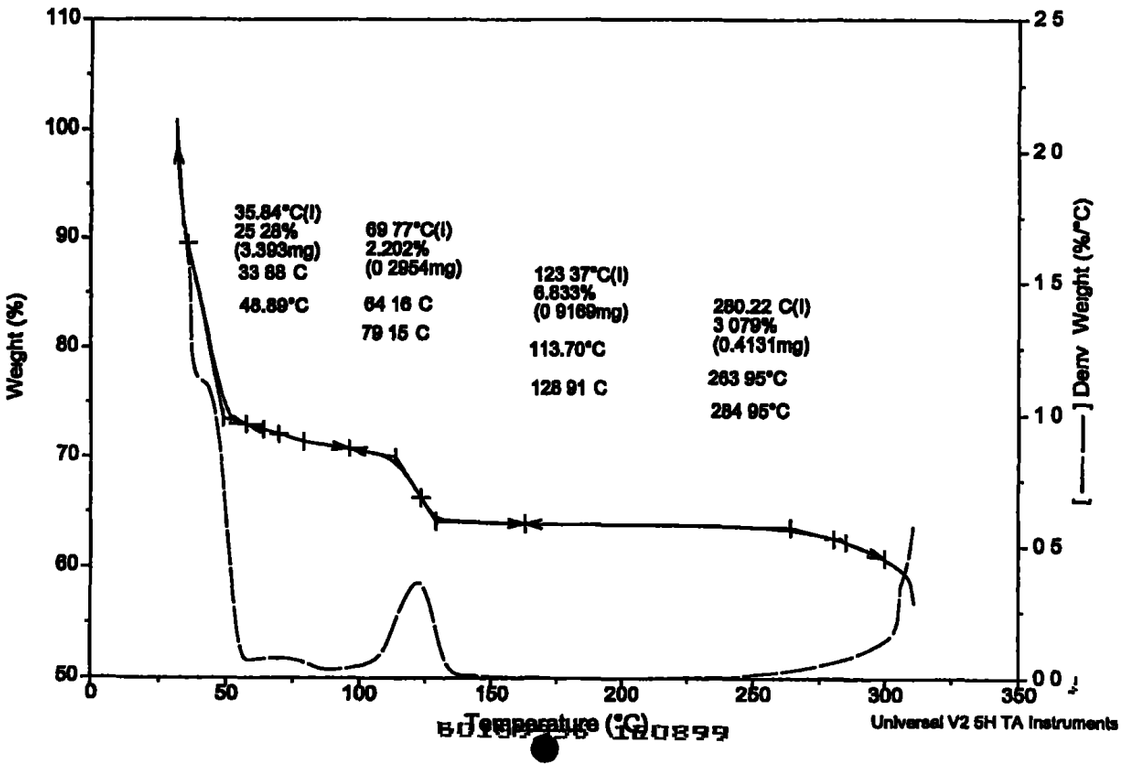


Figure 6-C

Size 14 9110 mg
Method 10 deg ramp to 300 deg

tetrahydrofuran solvate

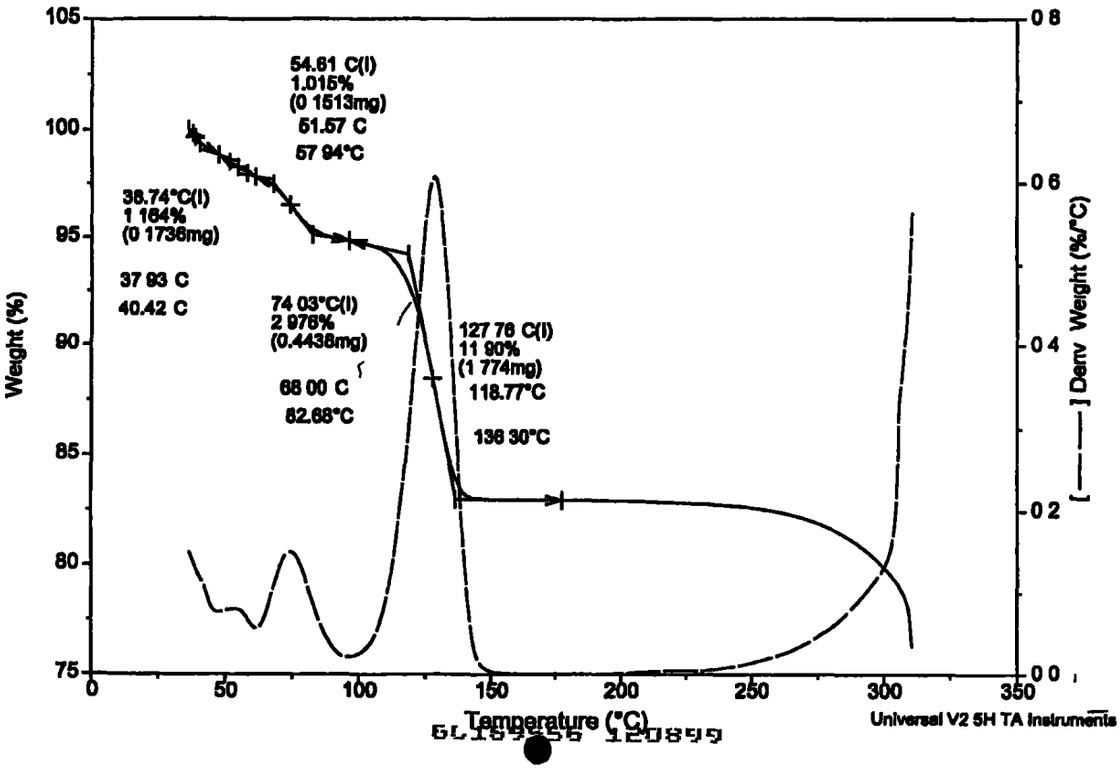


Figure 6 D

Size 9 5030 mg
Method 10 deg ramp to 300 deg

ethyl propionate solvate

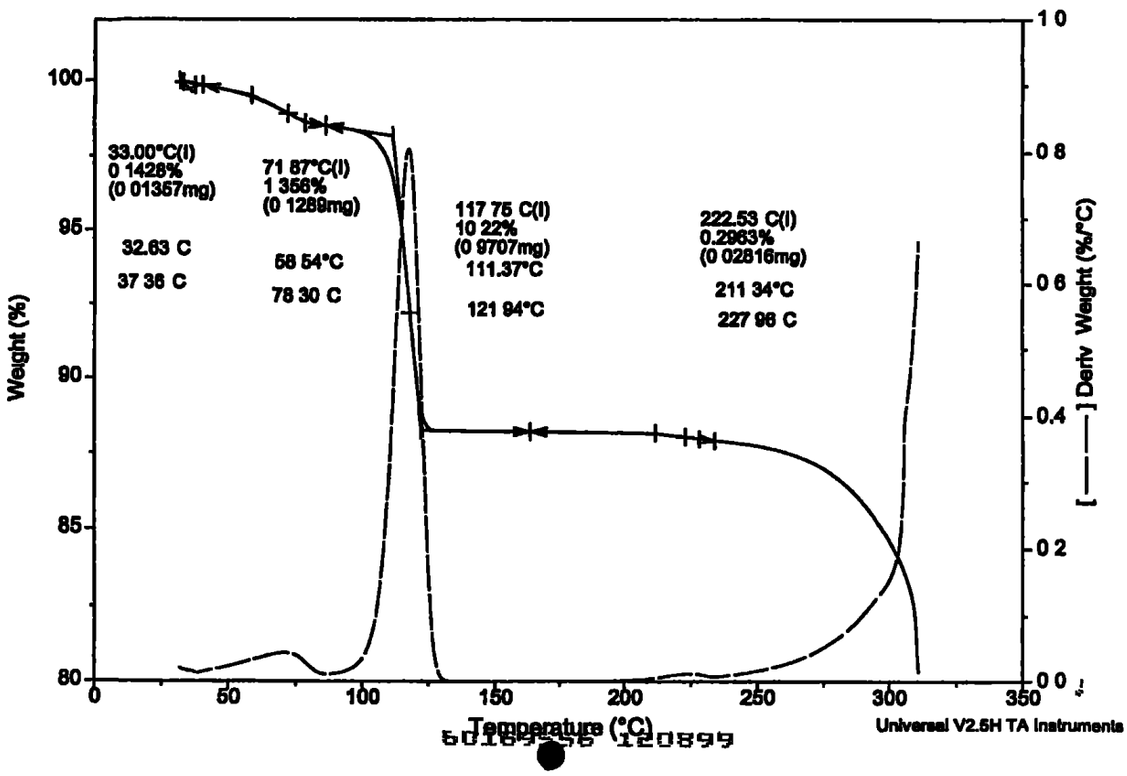


Figure 6-E

Size 8 4270 mg
Method 10 deg ramp to 300 deg

acetic acid solvate

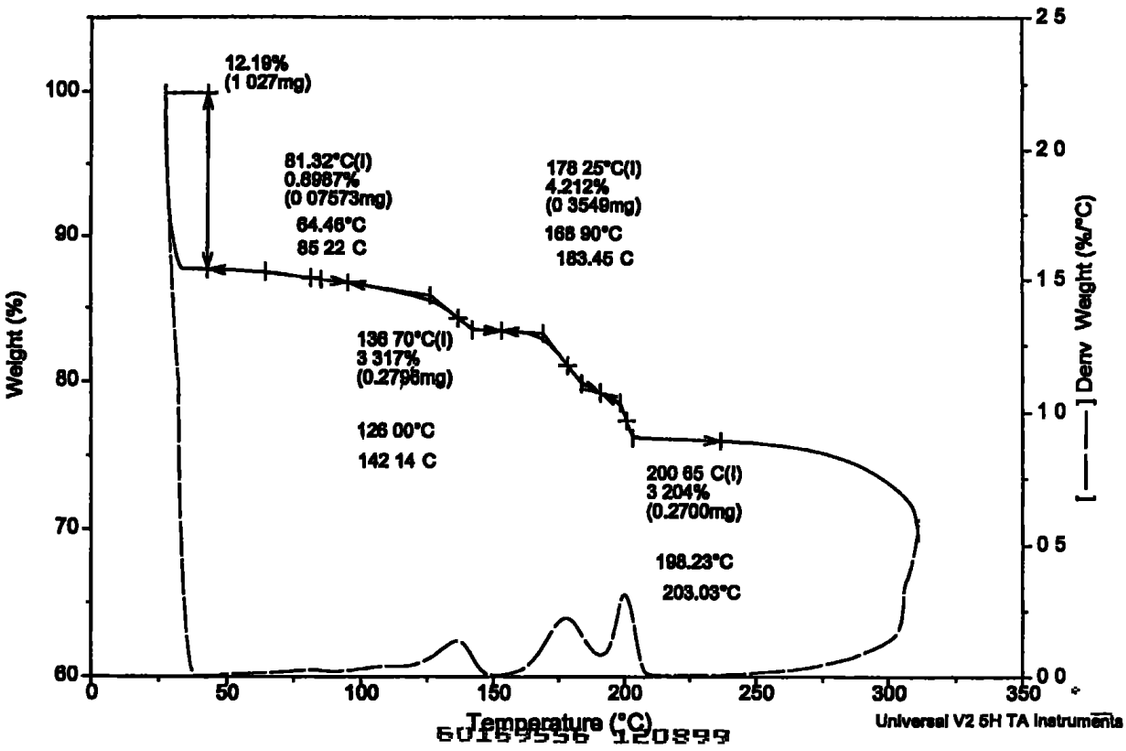


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Figure 6-F

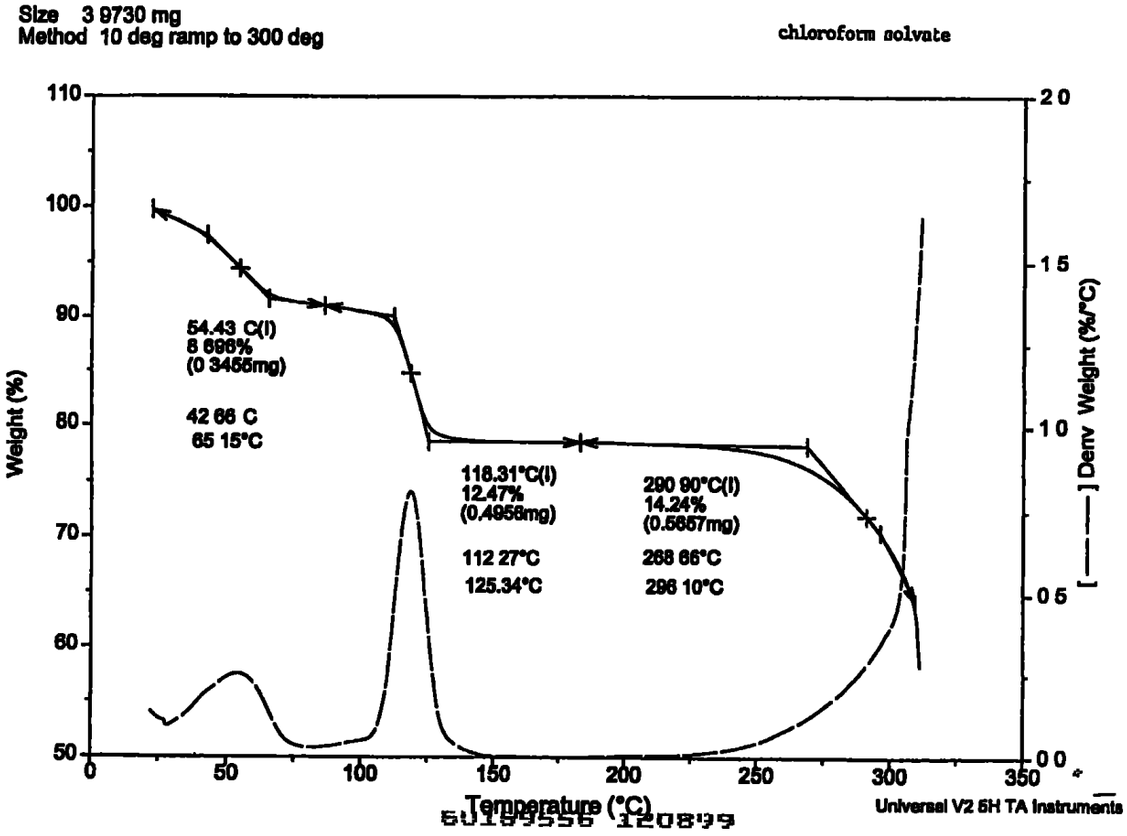
Size 3 9730 mg
Method 10 deg ramp to 300 deg
chloroform solvate
54.43 C(I)
8 666%
(0 3455mg)
42 66 C
65 15°C
118.31°C(I)
12.47%
(0.4956mg)
112 27°C
125.34°C
290 90°C(I)
14.24%
(0.5657mg)
268 66°C
296 10°C
Weight (%)
[———] Deriv Weight (%/°C)
Temperature (°C)
Universal V2 5H TA Instruments

Figure 6-G

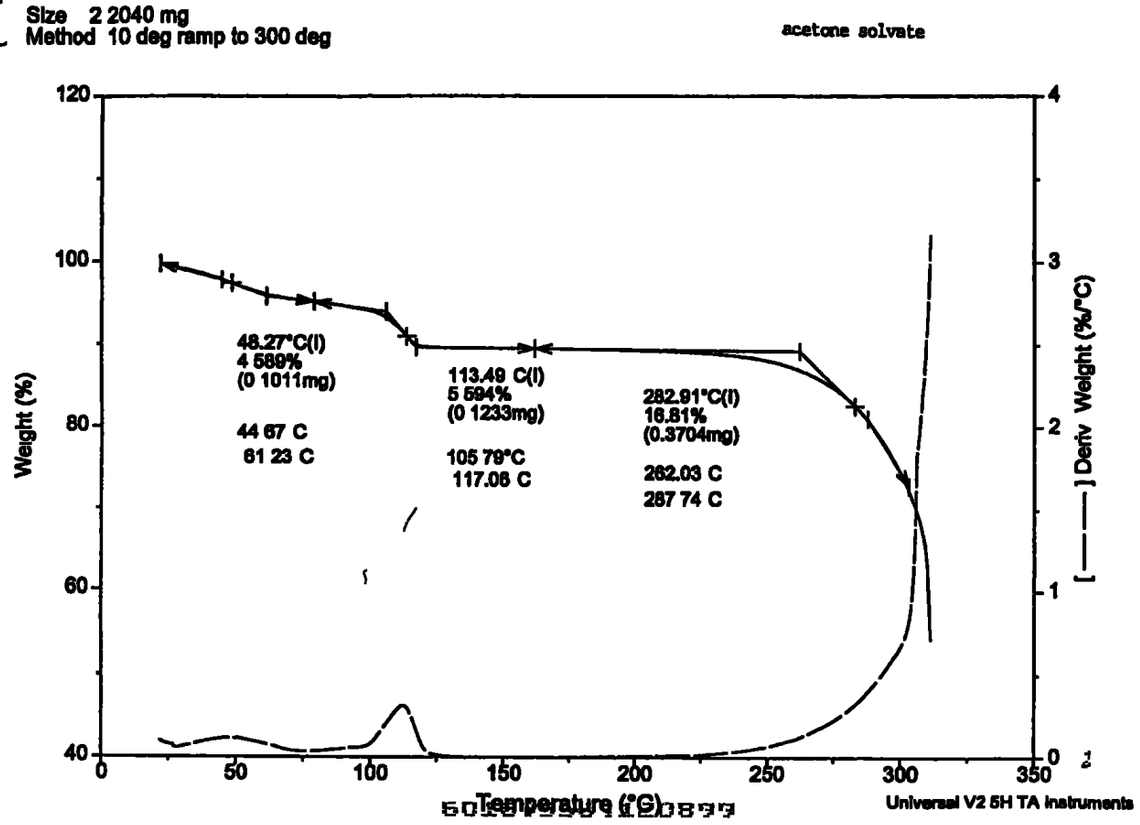
Size 2 2040 mg
Method 10 deg ramp to 300 deg
acetone solvate
48.27°C(I)
4 589%
(0 1011mg)
44 67 C
61 23 C
113.49 C(I)
5 594%
(0 1233mg)
105 79°C
117.06 C
282.91°C(I)
16.81%
(0.3704mg)
262.03 C
287 74 C
Weight (%)
[———] Deriv Weight (%/°C)
Temperature (°C)
Universal V2 5H TA Instruments

Figure 6-H

Size 1 7480 mg
Method 10 deg ramp to 300 deg

toluene solvate

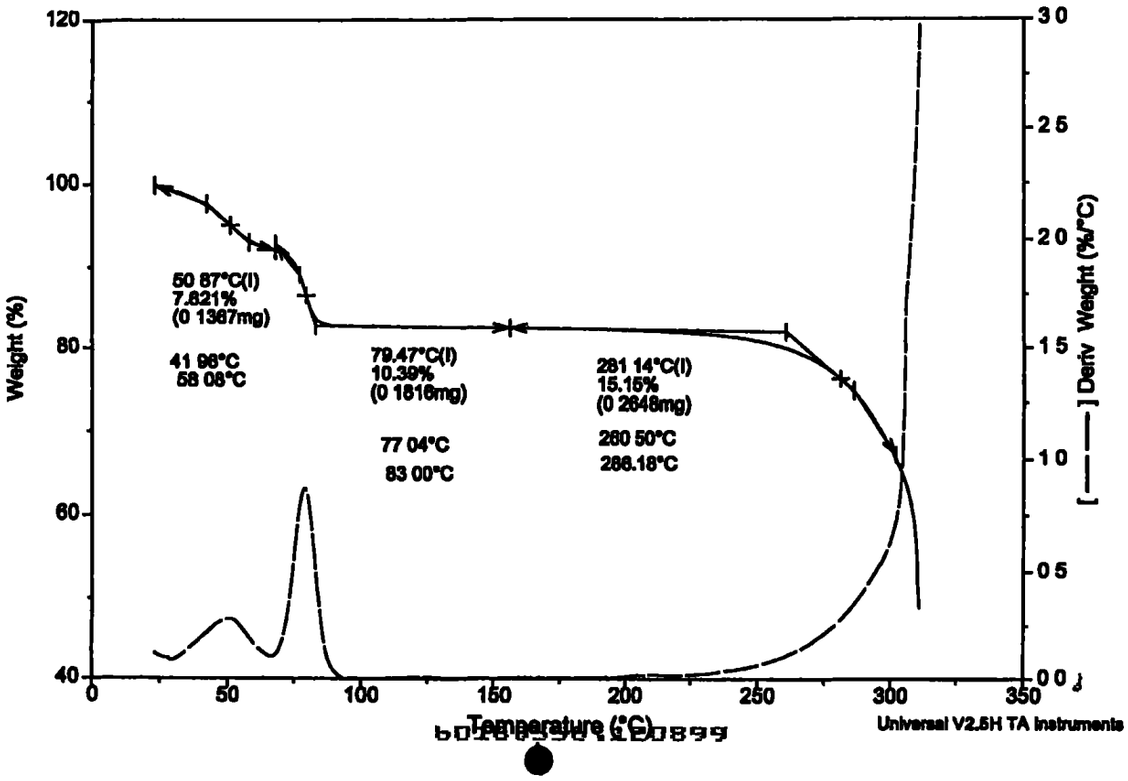

Figure 6-I

Size 6 4350 mg
Method 10 deg ramp to 300 deg

isopropanol solvate

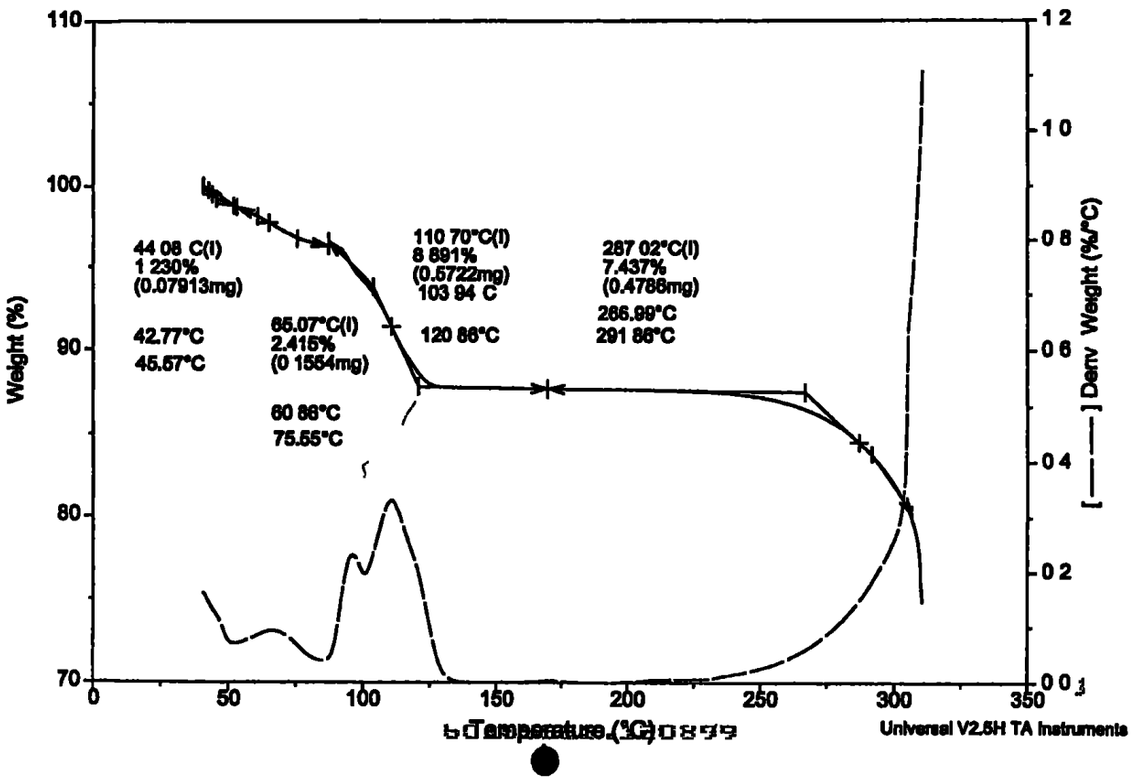

Figure 6-J

449

Size 6 1160 mg
Method 10 deg ramp to 300 deg

ethanol solvate

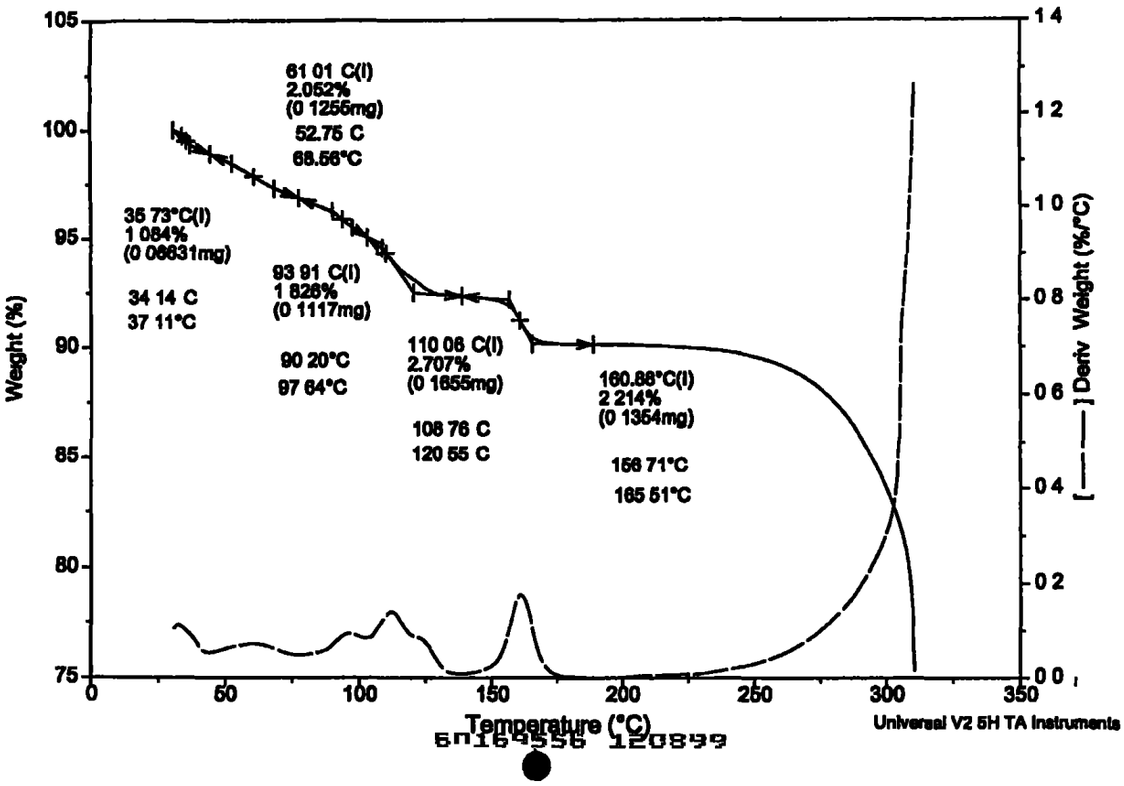

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Figure 6-K

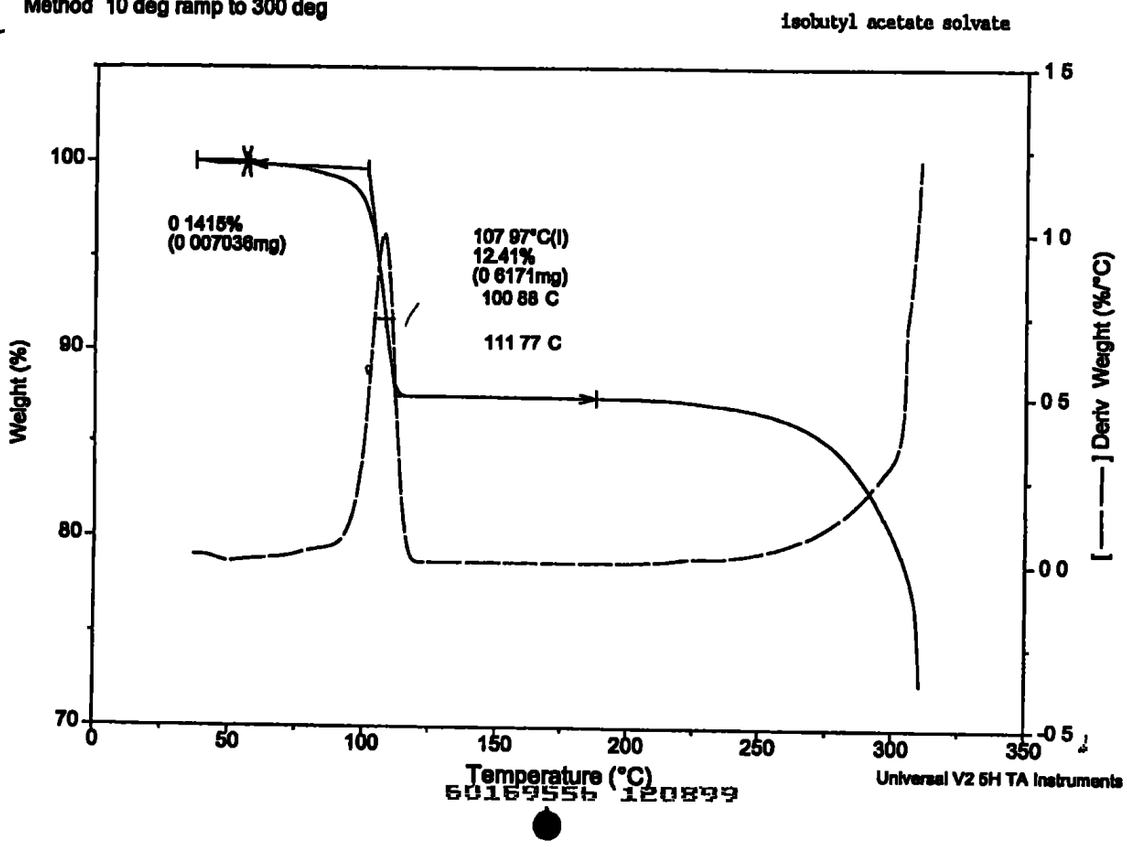
Size 4 9710 mg
Method 10 deg ramp to 300 deg
isobutyl acetate solvate
0 1415%
(0 007036mg)
107 97°C(I)
12.41%
(0 6171mg)
100 88 C
111 77 C
Weight (%)
[——] Deriv Weight (%/°C)
Temperature (°C)
Universal V2 5H TA Instruments
60169556 120899

Figure 6 L

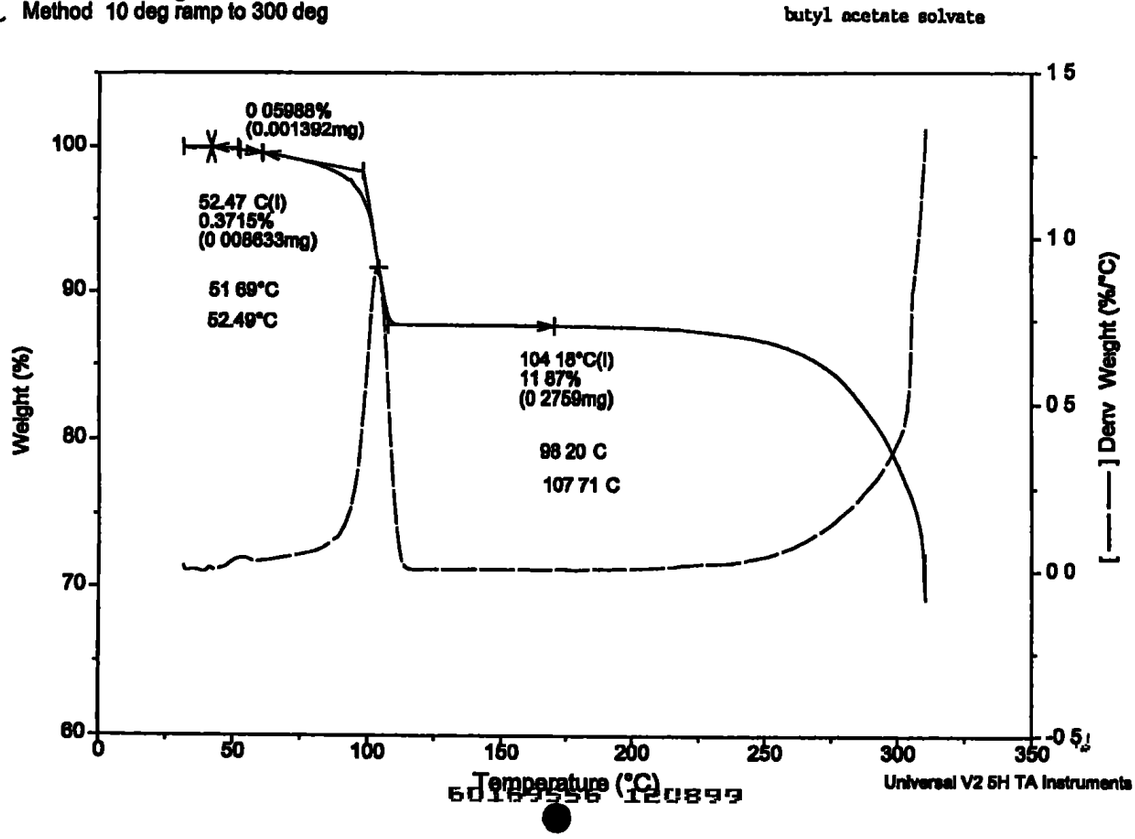
Size 2.3240 mg
Method 10 deg ramp to 300 deg
butyl acetate solvate
0 05988%
(0.001392mg)
52.47 C(I)
0.3715%
(0 008633mg)
51 69°C
52.49°C
104 18°C(I)
11 87%
(0 2759mg)
98 20 C
107 71 C
Weight (%)
[——] Deriv Weight (%/°C)
Temperature (°C)
Universal V2 5H TA Instruments

Figure 6-M

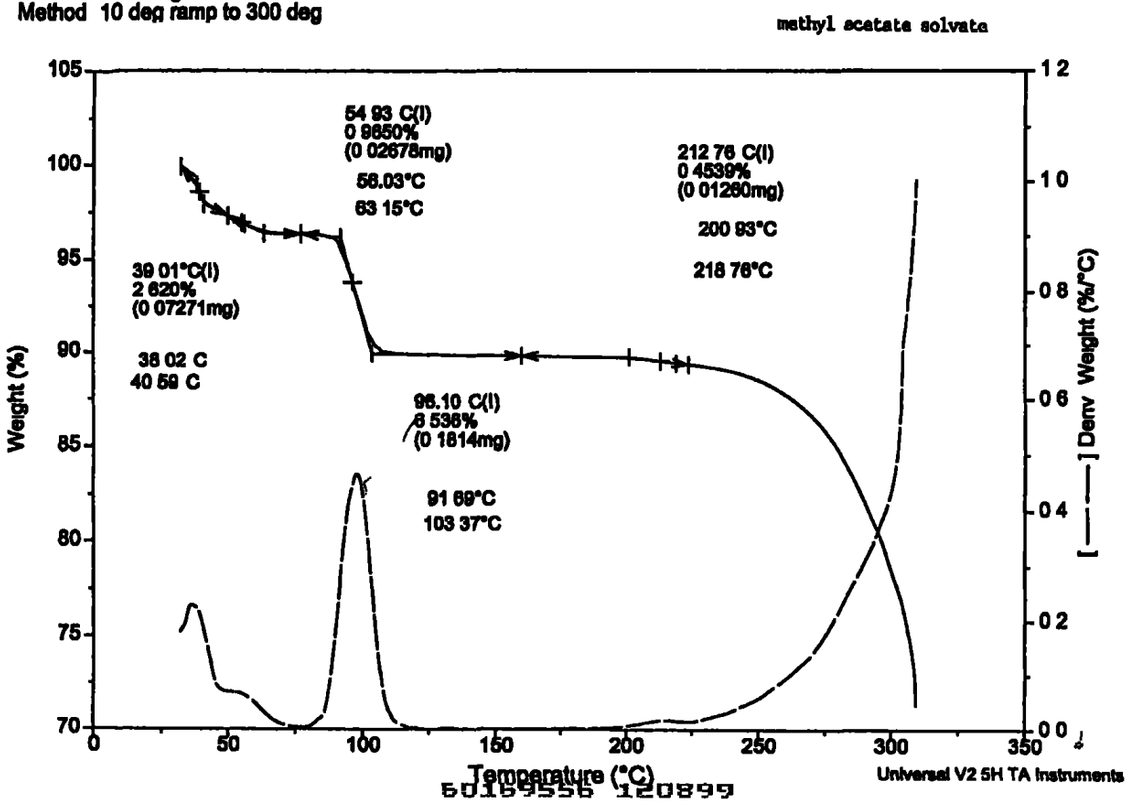
Size 2 7750 mg
Method 10 deg ramp to 300 deg
methyl acetate solvate
54 93 C(I)
0 9650%
(0 02678mg)
56.03°C
63 15°C
212 76 C(I)
0 4539%
(0 01260mg)
200 93°C
218 76°C
39 01°C(I)
2 620%
(0 07271mg)
38 02 C
40 59 C
96.10 C(I)
6 536%
(0 1814mg)
91 69°C
103 37°C
Weight (%)
[——] Deriv Weight (%/°C)
Temperature (°C)
Universal V2 5H TA Instruments

Figure 6-N

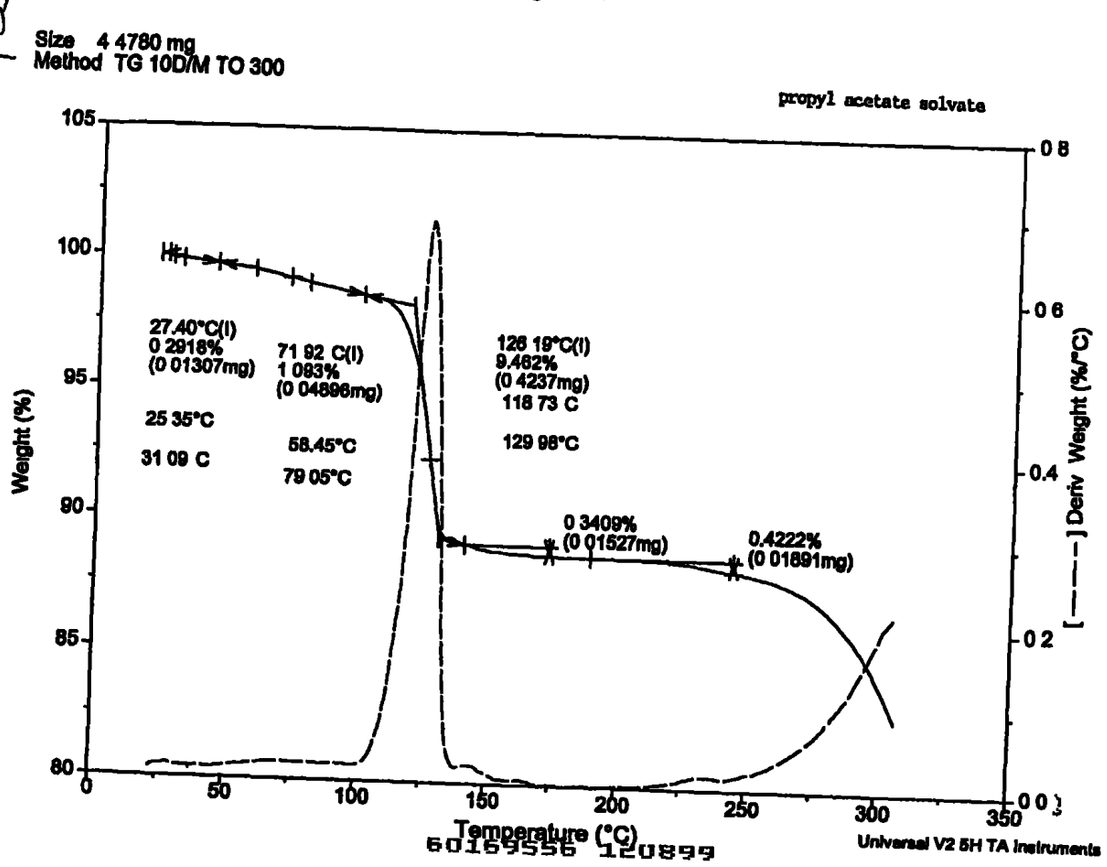
Size 4 4780 mg
Method TG 10D/M TO 300
propyl acetate solvate
27.40°C(I)
0 2916%
(0 01307mg)
25 35°C
31 09 C
71 92 C(I)
1 093%
(0 04896mg)
58.45°C
79 05°C
126 19°C(I)
9.462%
(0 4237mg)
118 73 C
129 98°C
0 3409%
(0 01527mg)
0.4222%
(0 01891mg)
Weight (%)
[——] Deriv Weight (%/°C)
Temperature (°C)
60169556 120899
Universal V2 5H TA Instruments

Figure 6-0

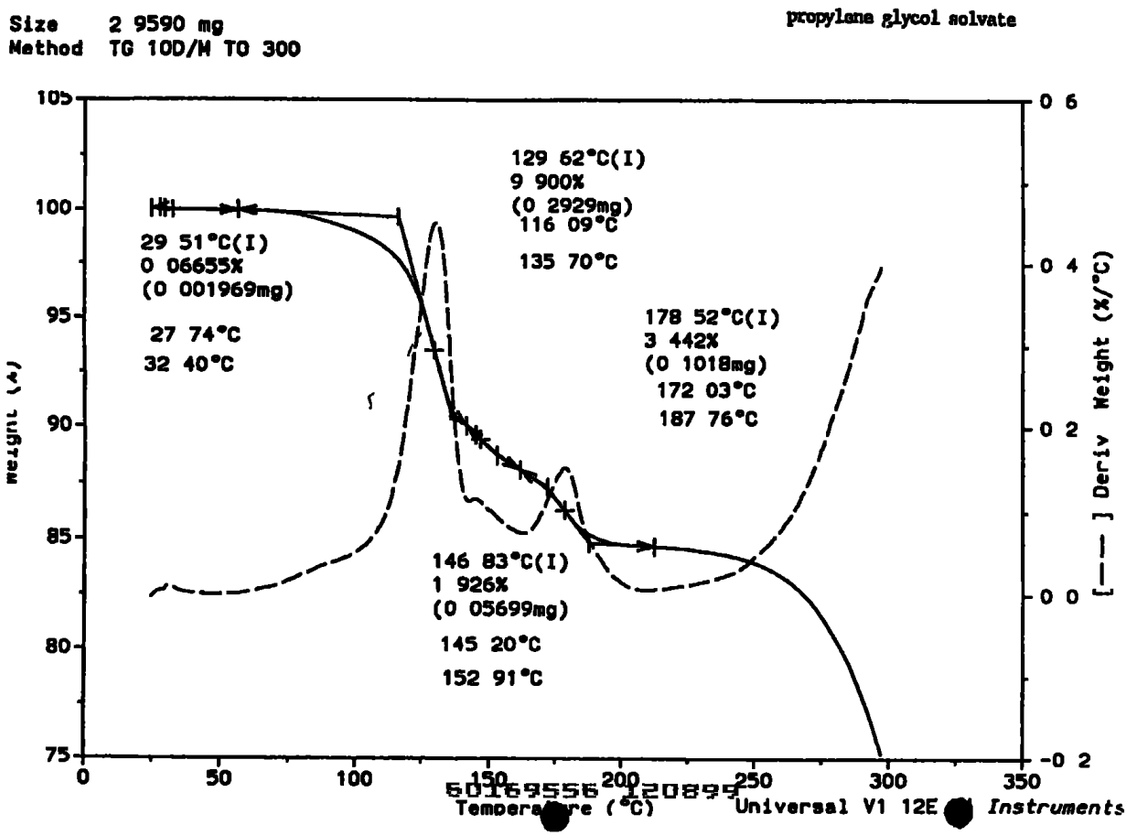
Size 2 9590 mg
Method TG 10D/M TO 300
propylene glycol solvate
129 62°C(I)
9 900%
(0 2929mg)
116 09°C
135 70°C
29 51°C(I)
0 06655%
(0 001969mg)
27 74°C
32 40°C
178 52°C(I)
3 442%
(0 1018mg)
172 03°C
187 76°C
146 83°C(I)
1 926%
(0 05699mg)
145 20°C
152 91°C
Weight (%)
[——] Deriv Weight (%/°C)
Temperature (°C)
Universal V1 12E
Instruments

Figure 6-P

Size 2 7050 mg
Method TG 10D/M TO 300

n-butanol solvate

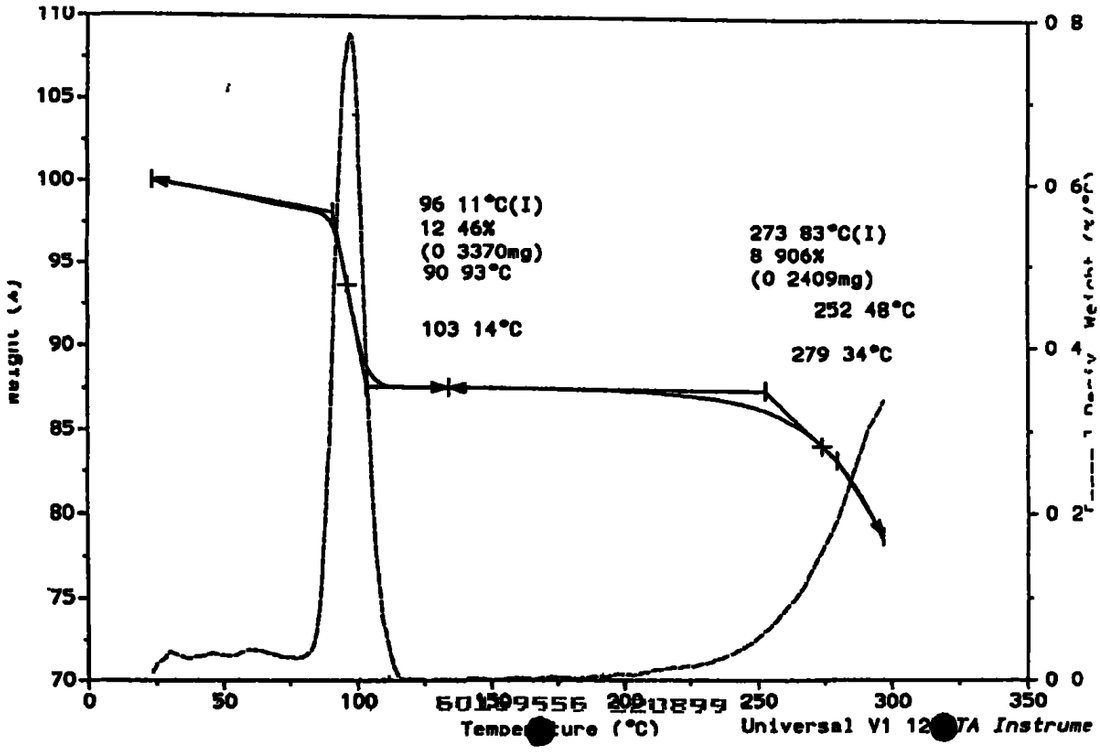

Figure 6 Q

Size 2 2420 mg
Method TG 10D/M TO 300

n-octanol solvate

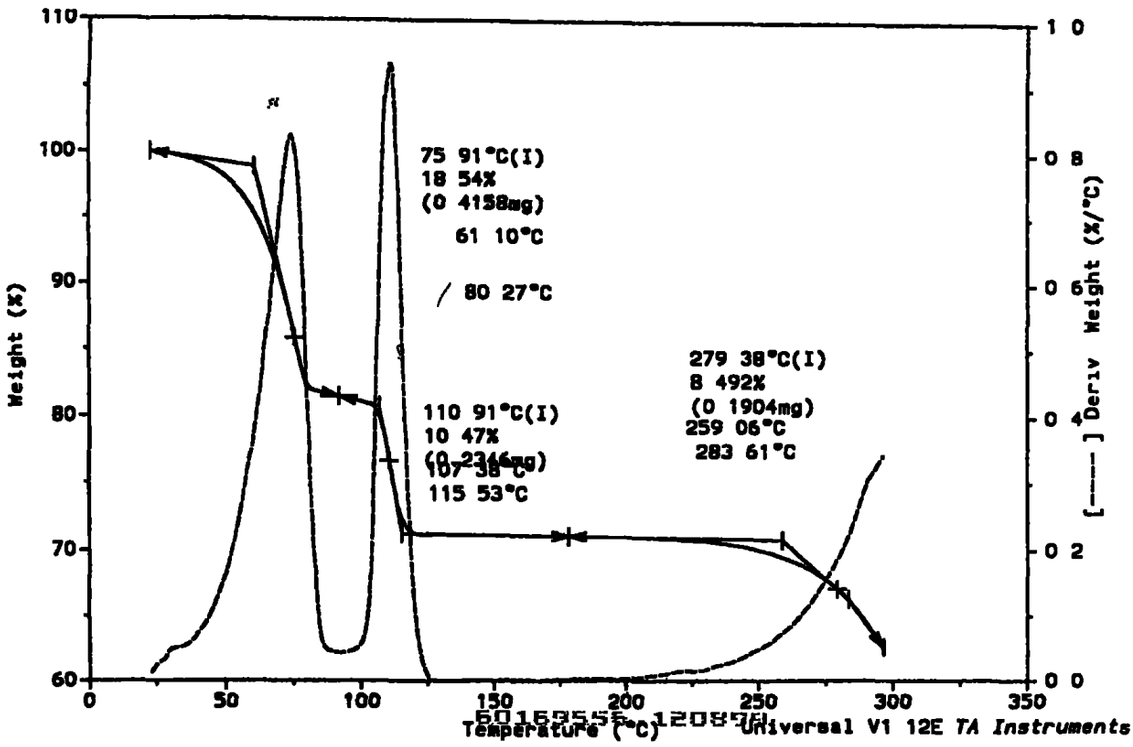

Figure 6 R

Size 2 8630 mg
Method TG 10D/M TO 300

t-butyl acetate solvate

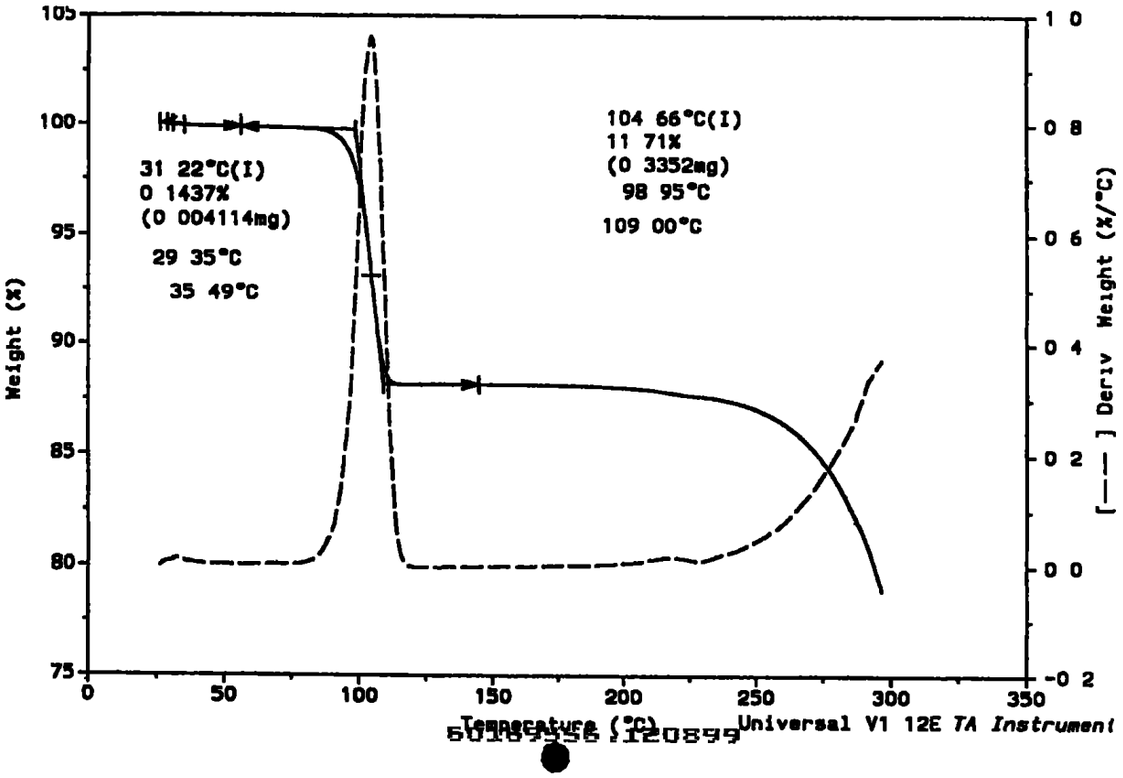

Figure 7

Figure 8

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457

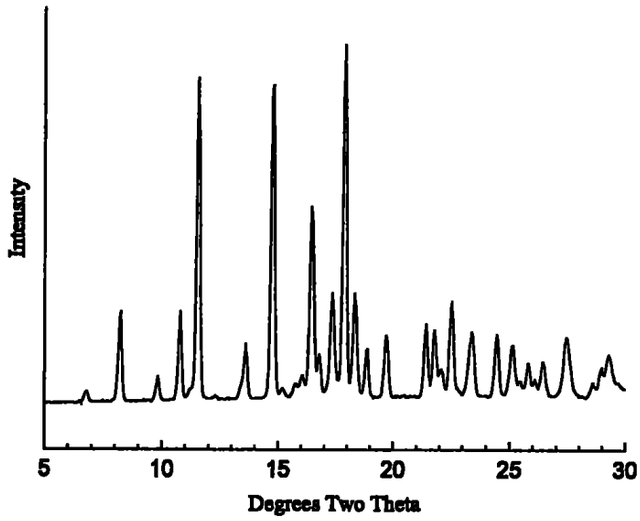


Figure 9

60169556 120899

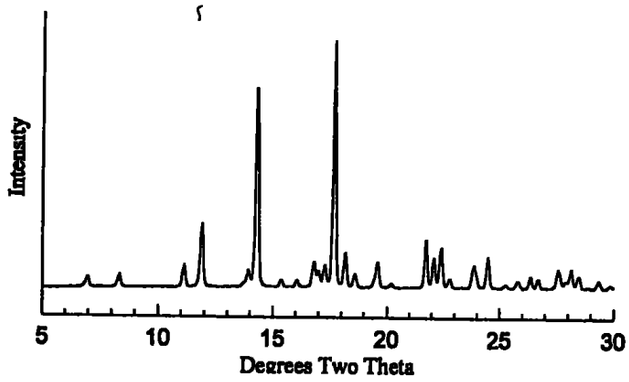


Figure 10

60169556 120899

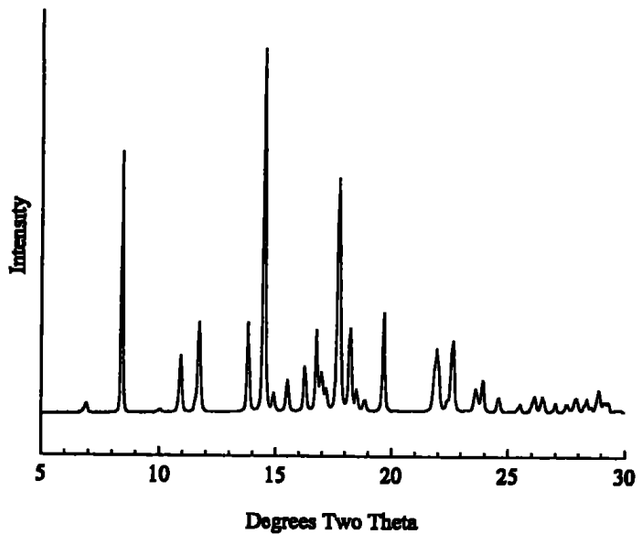


Figure 11

50169556 120899

5011555 .12008 99

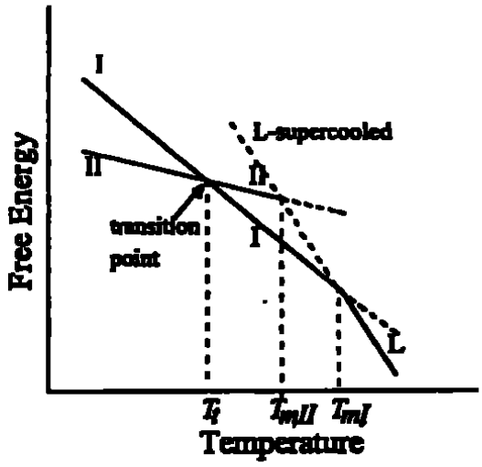


Figure 12:

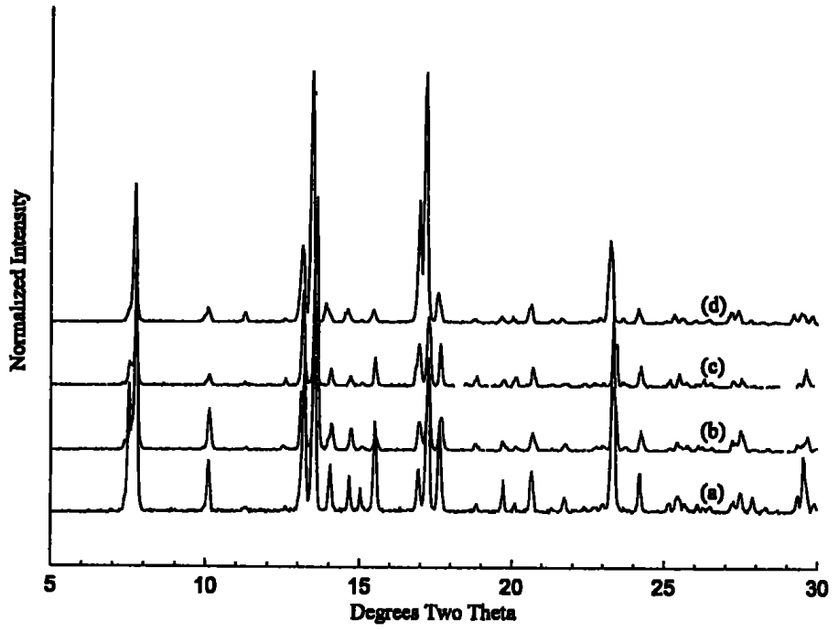


Figure 13

60169556 120849

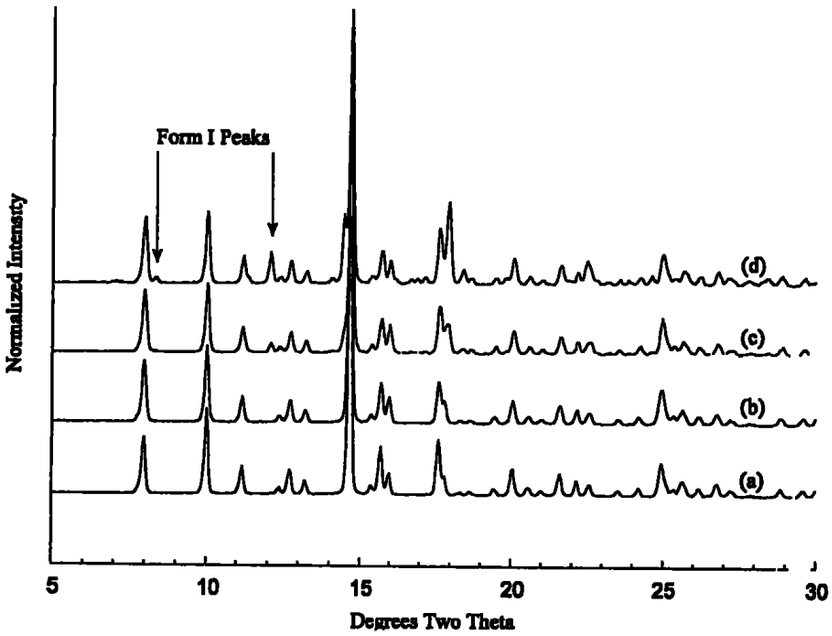


Figure 14

60169556 120899

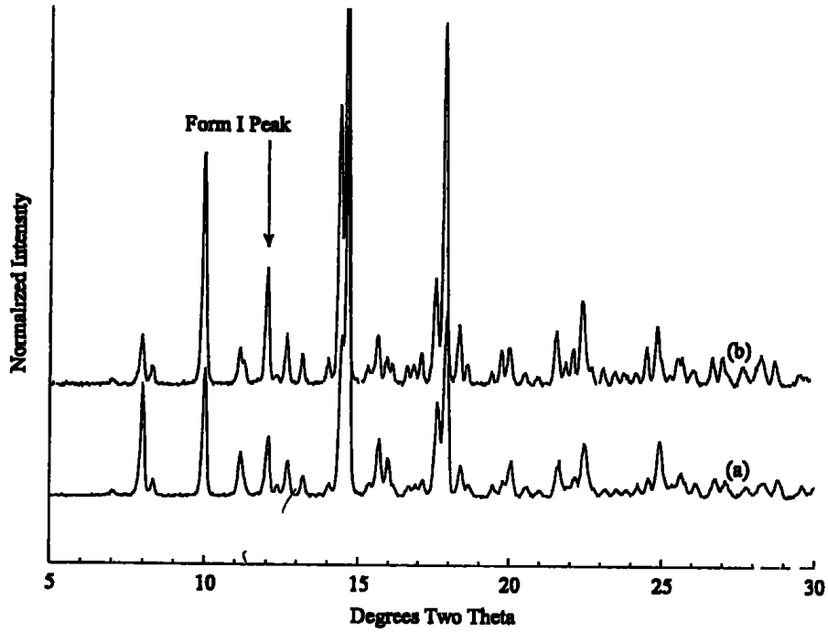


Figure 15

50169556 120899

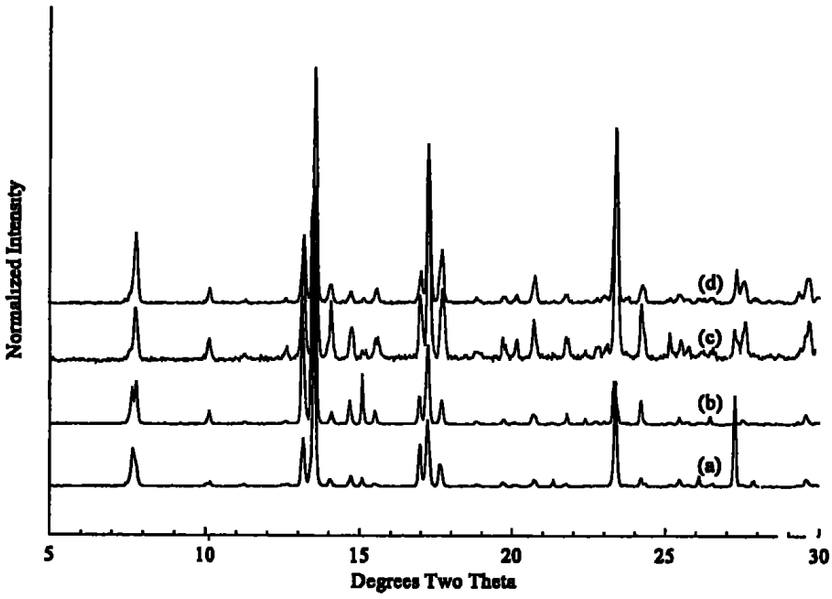


Figure 16

60169556 120899

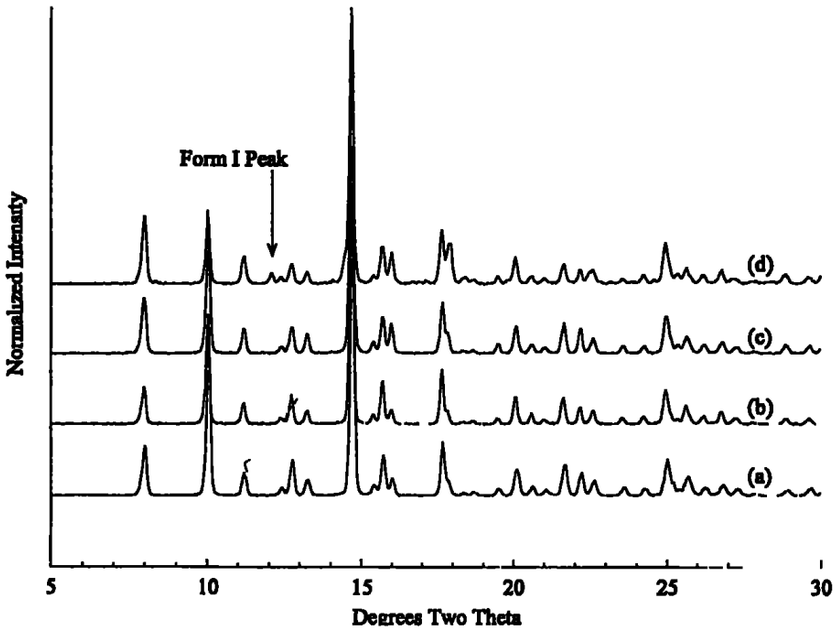


Figure 17

60169556 120899

Figure 18

468
963

6680271.9556931105

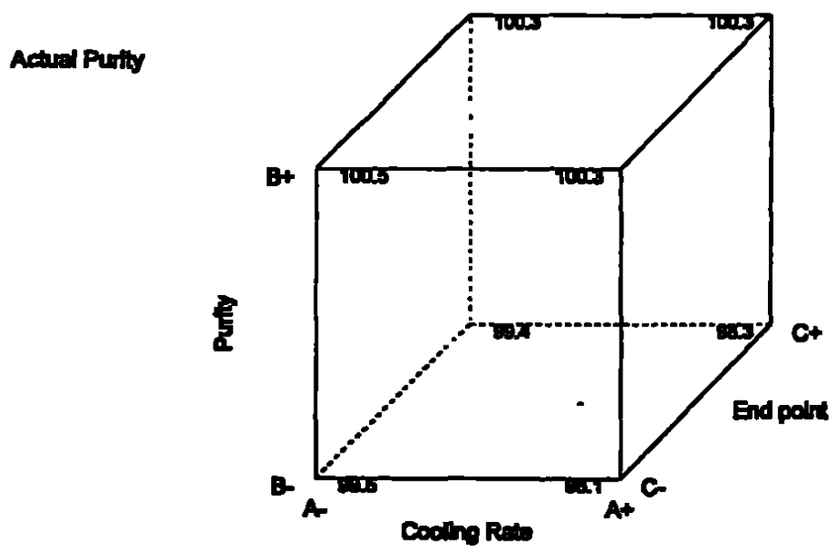
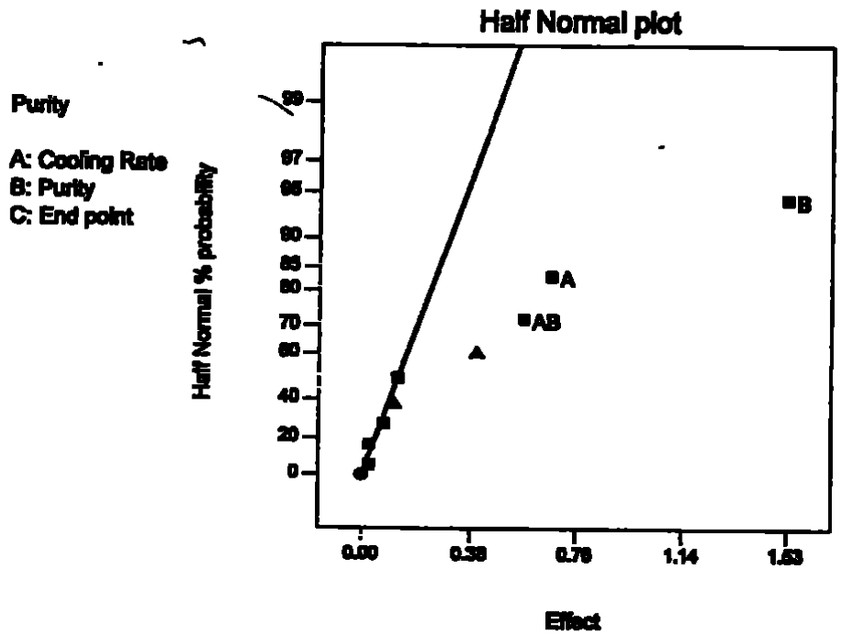


Figure 19

469
964



6016935556 :120899

Figure 20

470
465

6016955.12D899

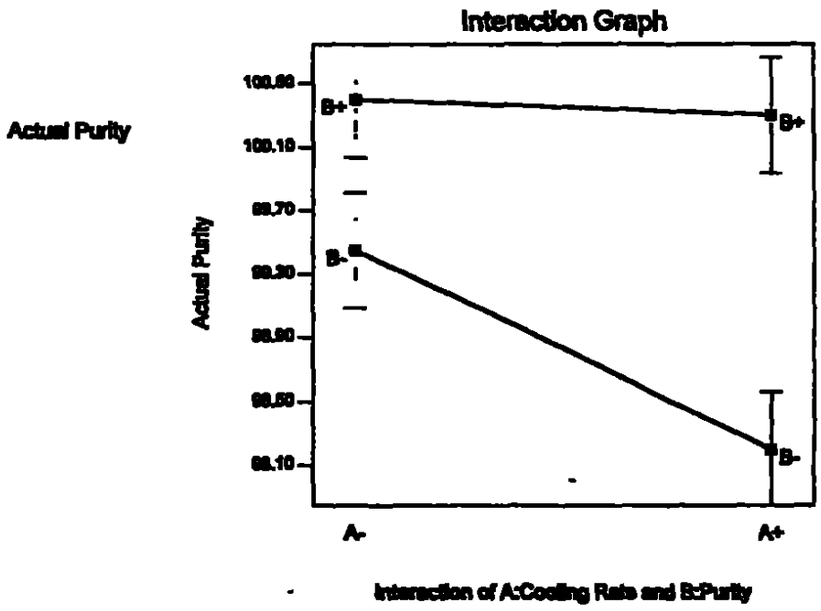


Figure 21

6016955556 .120899

Actual Form

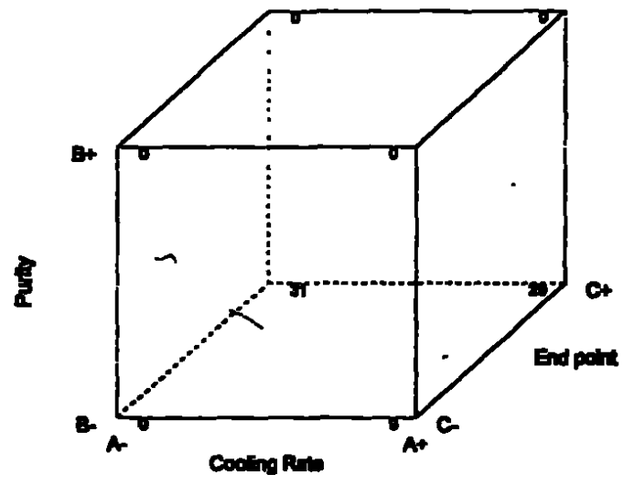
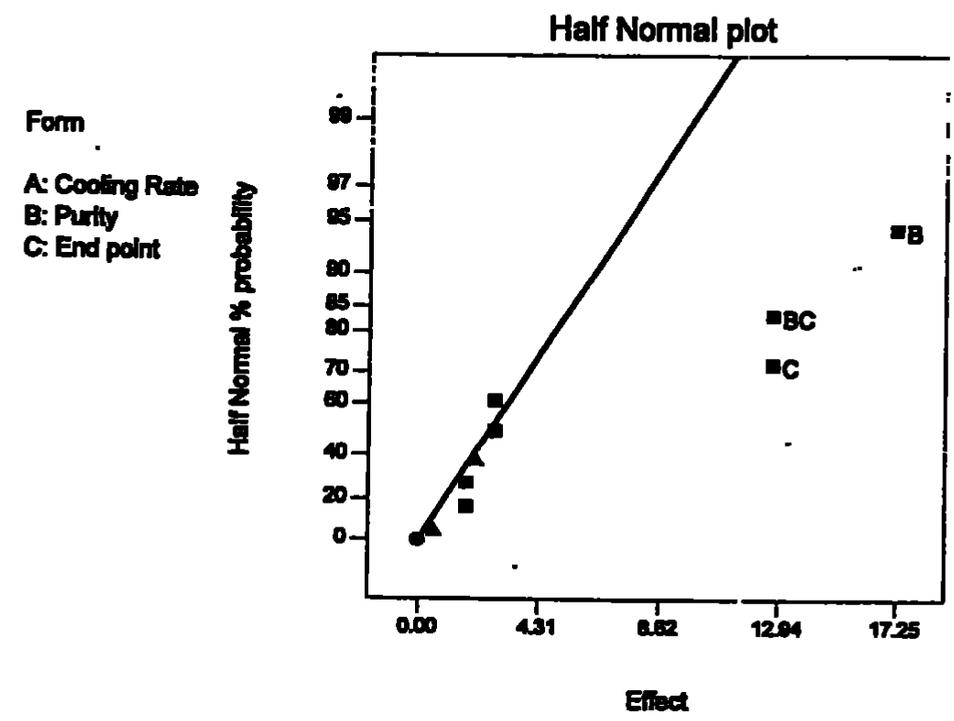


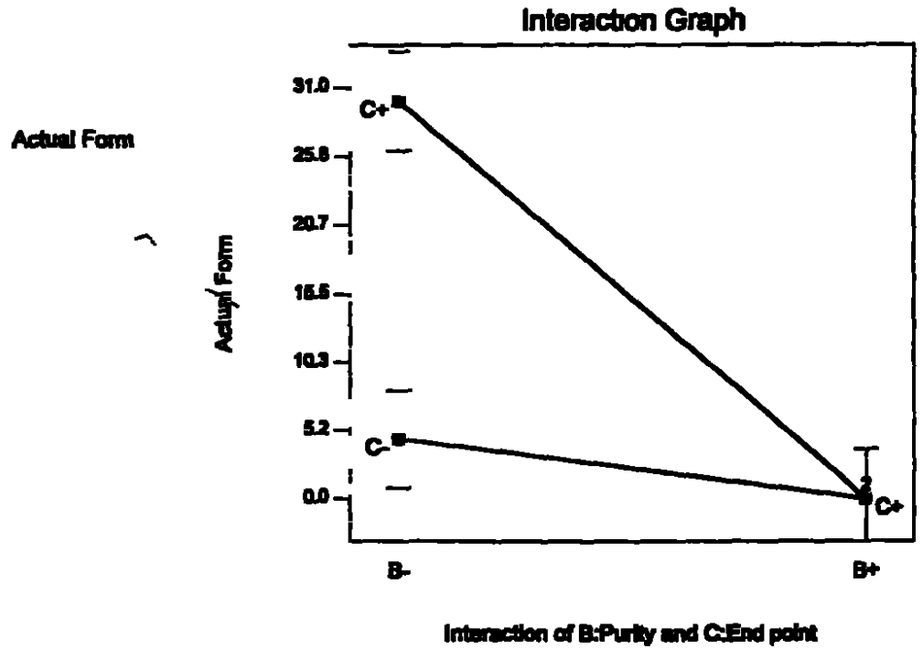
Figure 22



60169556.1208899

Figure 23

473
468



6016955 12089

Figure 24

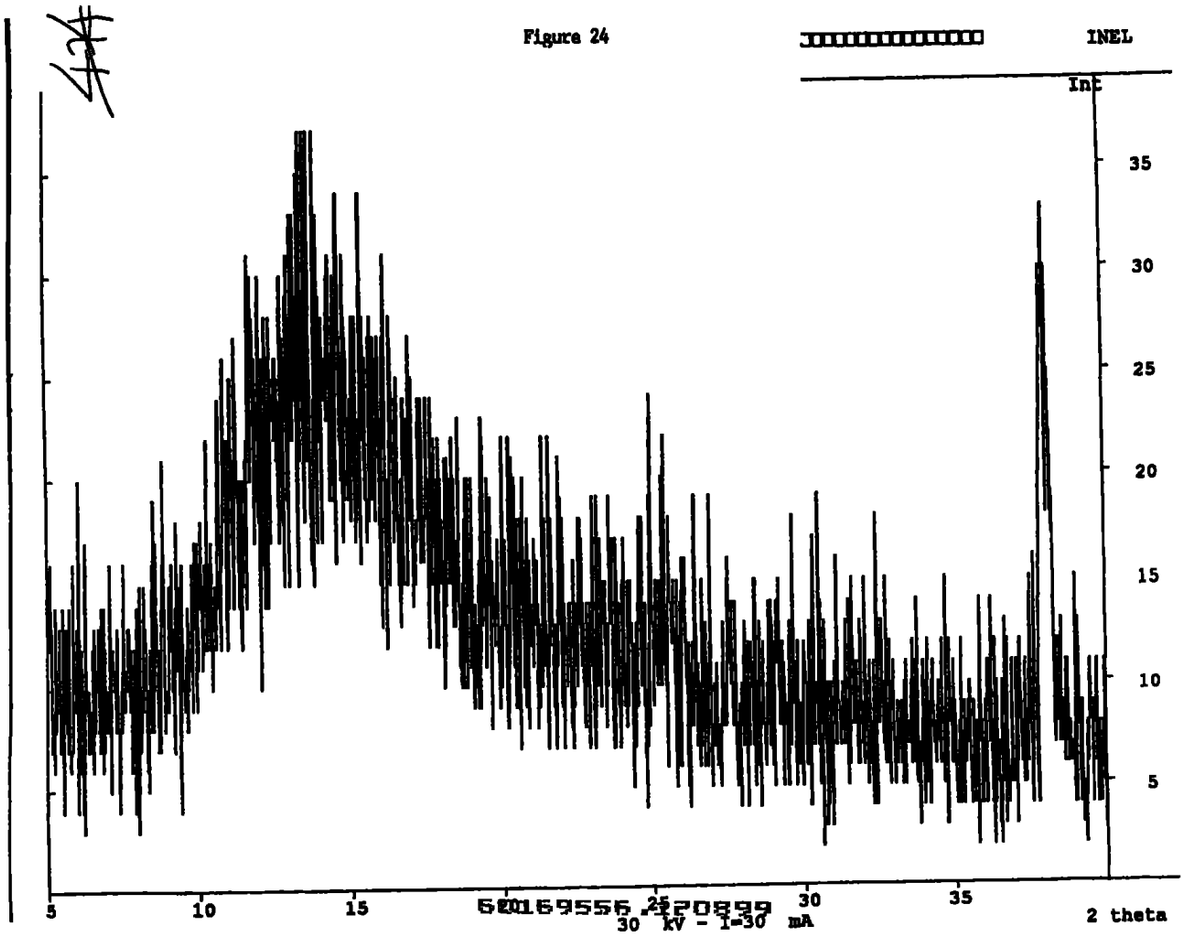
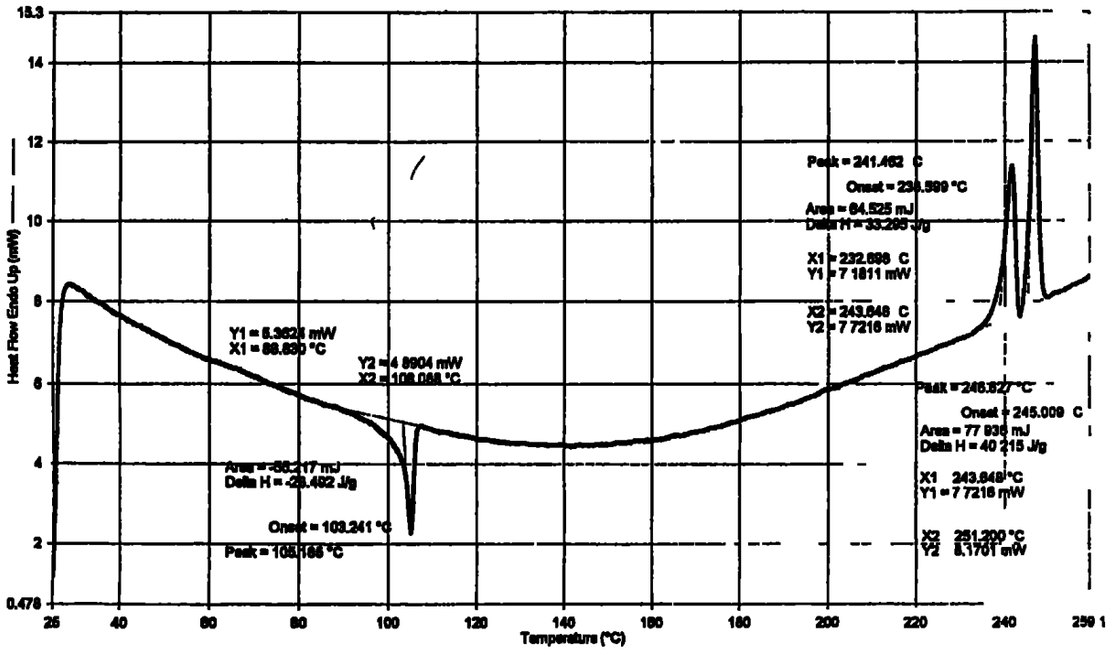


Figure 25

Sample Weight: 1 938 mg
Comment: Eplerenone
Amorphous by freeze dry in water



50169556 120899

6680217 95569105

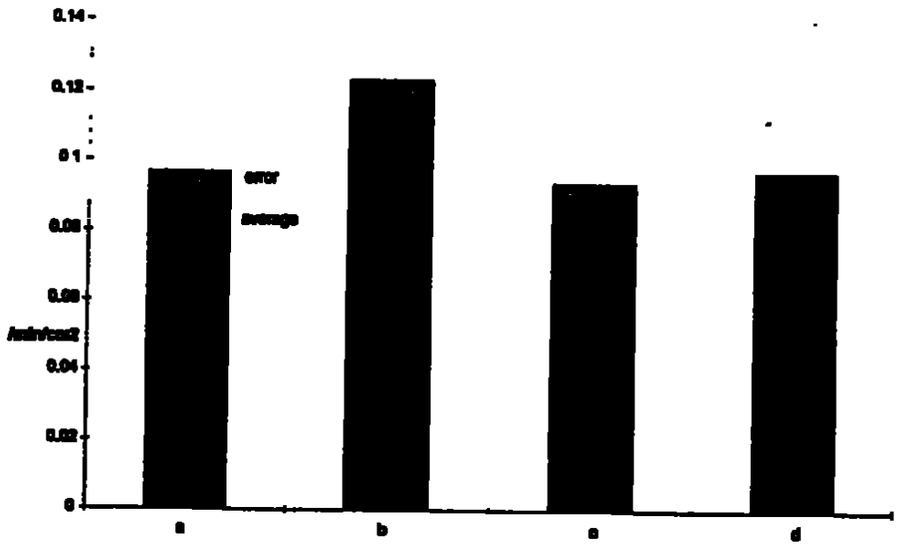


Figure 26:

478
972

PR 12564?

LOS ESTADOS UNIDOS DE AMERICA

A TODOS AQUELLOS A QUIENES LA PRESENTE CONCIERNE:
DEPARTAMENTO DE COMERCIO DE LOS ESTADOS UNIDOS

Oficina de Patentes y Marcas de los Estados Unidos

5 de enero de 2001

LA PRESENTE ES PARA CERTIFICAR QUE LA ANEXA A ESTA ES UNA COPIA FIEL DE LOS REGISTROS DE LA OFICINA DE PATENTES Y MARCAS DE LOS ESTADOS UNIDOS DE LOS DOCUMENTOS DE LA SOLICITUD DE PATENTE IDENTIFICADA MAS ADELANTE QUE SATISFACEN LOS REQUISITOS PARA QUE SE OTORGUE UNA FECHA DE PRIORIDAD BAJO 35 USC 111..

SOLICITUD NUMERO: 60/169,556

FECHA DE PRESENTACION: ***8 de diciembre de 1999***

Por la Autoridad del
COMISIONADO DE PATENTES Y MARCAS

Firmado por

H. PHILLIPS

Funcionario Certificador

493
479

REIVINDICACIONES

1.Una combinación que comprende partículas que comprenden una primera forma de estado sólido de eplerenona y partículas que comprenden una segunda forma de estado sólido de eplerenona,
donde dichas formas de estado sólido de eplerenona están selecciona de:

la forma cristalina de Forma I de eplerenona;

la forma cristalina de Forma II de eplerenona;

una forma cristalina de eplerenona que comprende un solvente;

una eplerenona amorfa; y donde al menos 90% de dichas partículas tiene un tamaño de partícula menor a 15 micras.

2.La combinación de la reivindicación 1 donde dichas formas en estado sólido de eplerenona se seleccionan de

la forma cristalina de Forma I de eplerenona;

la forma cristalina de Forma II de eplerenona;

una forma cristalina de eplerenona que comprende un solvente, donde dicha forma no comprende metilen cloruro, etil éter o isopropanol; y eplerenona amorfa.

3.La combinación de la reivindicación 1 donde dichas formas de estado sólido de eplerenona se seleccionan de :

la forma cristalina de forma I de eplerenona

la forma cristalina de Forma II de eplerenona;

una forma cristalina de eplereno que comprende un solvente, donde dicha forma no comprende metilen cloruro, etil éter, isopropanol, metil etil cetona o etanol; y eplerenona amorfa.

4. La combinación de la reivindicación 1 donde la relación de peso de dicha primera forma de estado sólido a dicha segunda forma de estado sólido es al menos 1:1.

5.La combinación de la reivindicación 4 donde la relación peso es al menos 2:1.

6.La combinación de la reivindicación 4 donde la relación peso es al menos 5:1.

7.La combinación de la reivindicación 4 donde la relación peso es al menos 9:1.

8.La combinación de la reivindicación 1 donde la primera forma de estado sólido es la forma cristalina de Forma II de eplerenona, y la segunda forma de estado sólido es la forma cristalina de Forma I de eplerenona.

9. la combinación de la reivindicación 8 donde la relación peso de dicha primera forma de estado sólido a dicha segunda forma de estado sólido es al menos de 1:9.

10.La combinación de la reivindicación 8 donde la relación en peso es al menos 1:1.

11.La combinación de la reivindicación 8 donde la relación en peso es al menos de 2:1.

12.La combinación de la reivindicación 8 donde la relación en peso es al menos 5:1.

13.La combinación de la reivindicación 8 donde la relación en peso es al menos 9:1

14.La combinación de la reivindicación 1 donde al menos 90% de dichas partículas tienen un tamaño de partícula menor a 1 micra.

15.La combinación de la reivindicación 14 donde al menos 90% de dichas partículas tiene un tamaño de partícula menor a 800 nm.

16.La combinación de la reivindicación 14 donde al menos 90% de dichas partículas tienen un tamaño de partícula desde 10 nm hasta 1 micra.

17.la combinación de la reivindicación 14 donde al menos 90% de dichas partículas tienen un tamaño de partícula desde 10 nm hasta 800 nm.

18.La combinación de la reivindicación 14 donde al menos 90% de dichas partículas tiene un tamaño de partícula desde 200 nm hasta 800 nm.

19.La combinación de la reivindicación 14 donde al menos 90% de dichas partículas tienen un tamaño de partículas desde 400 nm hasta 800 nm.

20.Una composición farmacéutica que comprenden partículas que comprenden una primera forma de estado sólido de eplerenona y partículas que comprenden una segunda forma de estado sólido de eplerenona en asociación con al menos un portador, adyuvante o diluyente farmacéuticamente aceptable;

donde dichas formas de estado sólido de eplerenona se seleccionan de:

la forma cristalina de Forma I de eplerenona;

la forma cristalina de Forma II de eplerenona;

una forma cristalina de eplerenona que comprende un solvente; y

eplerenona amorfa; y donde dicha primera forma de estado sólido y dicha segunda forma de estado sólido en combinación comprenden una cantidad terapéuticamente efectiva de eplerenona; y donde al menos 90% de dichas partículas tiene un tamaño de partícula inferior a 15 micras.

21.La composición farmacéutica de la reivindicación 20 donde dichas formas de estados sólidos de eplerenona se seleccionan de:

la forma cristalina de forma I de eplerenona;

la forma cristalina de forma II de eplerenona;

una forma cristalina de eplerenona que comprende un solvente donde dicha forma no comprende metilen cloruro, etil éter, o isopropanol ; y

eplerenona amorfa.

22.La composición farmacéutica de la reivindicación 20 donde dichas formas de estado sólido de eplerenona se seleccionan de:

la forma cristalina de Forma I de eplerenona;

la forma cristalina de Forma II de eplerenona;

una forma cristalina de eplerenona que comprende un solvente, donde dicha forma no comprende metilen cloruro, etil éter, isopropanol, metil etil cetona o etanol y eplerenona amorfa.

23.La composición farmacéutica de la reivindicación 20 donde la proporción en peso de dicha primera forma en estado sólido con respecto a dicha segunda forma en estado sólido es al menos 1:9.

24.La composición farmacéutica de la reivindicación 23 donde la proporción en peso es al menos 1:1.

25.La composición farmacéutica de la reivindicación 23 donde la proporción en peso es al menos 9:1.

26.La composición farmacéutica de la reivindicación 20 donde la primera forma de estado sólido es la forma cristalina de Forma II de eplerenona, y la segunda forma de estado sólido es la forma cristalina de Forma I de eplerenona.

27.La composición farmacéutica de la reivindicación 26 donde la proporción en peso de dicha primera forma de estado sólido con relación a dicha segunda forma de estado sólido es al menos 1:9.

28.La composición farmacéutica de la reivindicación 27 donde la proporción en peso es al menos 1:1

29.La composición farmacéutica de la reivindicación 27 donde la proporción en peso es al menos 2:1

30.La composición farmacéutica de la reivindicación 27 donde la proporción en peso es al menos 5:1

31.La composición farmacéutica de la reivindicación 27 donde la proporción en peso es al menos 9:1.

32.La composición farmacéutica de la reivindicación 20 donde al menos 90% de dichas partículas tienen un tamaño de partícula menor a una micra.

33.La composición farmacéutica de la reivindicación 32 donde al menos 90% de dichas partículas tienen un tamaño de partícula menor a 800 nm.

34.La composición farmacéutica de la reivindicación 32 donde al menos 90% de dichas partículas tienen un tamaño de partícula desde 10 nm hasta 1 micra.

35.la composición farmacéutica de la reivindicación 32 donde al menos 90% de dichas partículas tienen un tamaño de partícula desde 100 nm hasta 800 nm.

36.La composición farmacéutica de la reivindicación 32 donde al menos 90% de dichas partículas tiene un tamaño de partícula desde 200 nm hasta 800 nm.

37.La composición farmacéutica de la reivindicación 32 donde al menos 90% de dichas partículas tienen un tamaño de partícula desde 400 nm hasta 800 nm.

38.El método de tratar o prevenir una condición o desorden mediada por aldosterona en un sujeto, dicho método comprende administrar a un sujeto que tiene o es susceptible a dicha condición o desorden una combinación que comprende partículas las cuales comprenden una primera forma de estado sólido de eplerenona y partículas que comprenden una segunda forma de estado sólido de eplerenona,

donde dichas formas de estado sólido de eplerenona son seleccionadas de:

la forma cristalina de Forma I de eplerenona;

la forma cristalina de Forma II de eplerenona;

la forma cistalina de eplerenona que comprende un solvente;

y eplerenona amorfa; y

donde dicha primera forma de estado sólido y dicha segunda forma de estado sólido en combinación comprenden una cantidad terapéuticamente efectiva de eplerenona; y

donde al menos 90% de dichas partículas tienen un tamaño de partículas inferior a 15 micras.

39.El método de la reivindicación 38 que comprende administrar a un sujeto que es susceptible a esa condición o desorden una combinación que comprende partículas las cuales comprenden una primera forma de estado sólido de eplerenona y partículas las cuales comprenden una segunda forma de estado sólido de eplerenona en asociación con al menos un portador, adyuvante o diluyente farmacéuticamente aceptable; donde dichas formas de estado sólido de eplerenona se seleccionan de:

la forma cristalina de Forma I de eplerenona;

la forma cristalina de Forma II de eplerenona;

una forma cristalina de eplerenona que comprende un solvente;

y

eplerenona amorfa; y

donde dichas primera forma de estado sólido y una segunda forma de estado sólido en combinación comprenden una cantidad terapéuticamente efectiva de eplerenona; y

donde al menos 90% de dichas partículas tienen un tamaño de partícula menor a 15 micras.

40. El método de la reivindicación 39 para ser usado en el tratamiento de hiperaldosteronismo.

41.El método de la reivindicación 39 para ser usado en el tratamiento de hipertensión.

42.El método de la reivindicación 39 para ser usado en el tratamiento de fallas de corazón.

43.El método de la reivindicación 39 para ser usado en el tratamiento de cirrosis del hígado.

44.El método de la reivindicación 39 para ser usado en el tratamiento de una condición seleccionada de un grupo consistente de exceso de colágeno/ fibrosis, hipertrofia benigna de próstata, y depresión.

45.Partículas que comprenden una forma amorfa de eplerenona que tienen un patrón de difracción en polvo en rayos X sustancialmente carente de cualquier pico característico, donde al menos 90% de dichas partículas tienen un tamaño de partícula menor a 15 micras.

46.Las partículas de la reivindicación 45 donde al menos 90% de dichas partículas tienen un tamaño de partícula menor a 1 micra.

47. Las partículas de la reivindicación 45 donde al menos 90% de dichas partículas tienen un tamaño de partícula menor a 800 nm.

48.Las partículas de la reivindicación 45 donde al menos 90% de dichas partículas tienen un tamaño de partícula entre 10 nm hasta 1 micra.

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49.Las partículas de la reivindicación 45 donde al menos 90% de dichas partículas tienen un tamaño de partícula entre 100 nm hasta 800 nm.

50.Las partículas de la reivindicación 45 donde al menos 90% de dichas partículas tienen un tamaño de partícula entre 200 nm hasta 800 nm.

51. Las partículas de la reivindicación 45 donde al menos 90% de dichas partículas tienen un tamaño de partícula entre 400 nm hasta 800 nm.

COLO 12482-801-001-5

JJC-ID-9

PTO 340533

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

January 05, 2001

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE UNDER 35 USC 111.

APPLICATION NUMBER: ***60/169,608***
FILING DATE: ***December 08, 1999***

By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

W. MONTGOMERY
Certifying Officer

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

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Title of the Invention: NANOPARTICLES COMPRISING A SOLVATED CRYSTALLINE FORM OF EPLERENONE

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The application includes:

[x] Specification - Number of Pages 98

[x] Drawings - Number of Sheets 92

[ ] Small Entity Statement

[x] Return Receipt Postcard

[ ] Other _____

Method of Payment of Filing Fee:

[X] A check in the amount of $ 150.00 is enclosed to cover the Provisional Filing Fee.

[ ] The Commissioner is hereby authorized to charge filing fees and credit Deposit Account No. 19-1345.

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

[X] No
[ ] Yes, the name of the U.S. Government agency and the Government contract number are: _____

Respectfully submitted,

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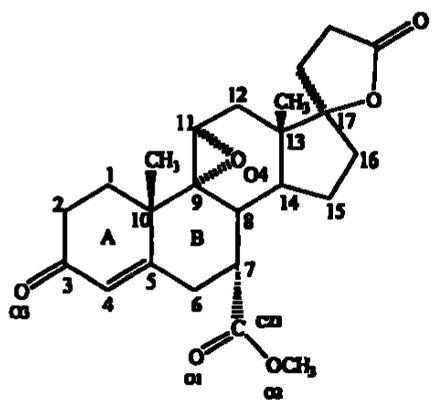
NANOPARTICLES COMPRISING A SOLVATED CRYSTALLINE FORM OF EPLERENONE

Field of the Invention

This invention is in the field of aldosterone receptor antagonist pharmaceutical agents and specifically relates to novel nanoparticles comprising a solvated crystalline form of eplerenone, methods of preparing these eplérenone nanoparticles, pharmaceutical compositions comprising these eplerenone nanoparticles, and methods for the treatment and/or prophylaxis of aldosterone-mediated conditions and/or disorders, including conditions and disorders associated with hyperaldosteronism such as hypertension.

Background of the Invention

The compound methyl hydrogen 9,11-epoxy-17-hydroxy-3-oxopregn-4-ene-7,21-dicarboxylate, γ-lactone 1 (also referred to herein as "eplerenone") was first reported in Grob et al., U.S. Patent 4,559,332 which discloses a class of 9,11-epoxy steroid compounds and their salts. Eplerenone is an aldosterone receptor antagonist that can be administered in a therapeutically effective amount where use of an aldosterone receptor antagonist is indicated, such as in the treatment of pathological conditions associated with hyperaldosteronism including hypertension, heart failure including cardiac insufficiency, and cirrhosis of the liver.



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U.S. Patent 4,559,332 generally discloses the preparation of eplerenone and the preparation of pharmaceutical formulations comprising eplerenone.

Additional processes for the preparation of 9,11-epoxy steroid compounds and their salts, including eplerenone, are disclosed in Ng et al., WO97/21720 and Ng et al., WO98/25948.

Grob et al., "Steroidal Aldosterone Antagonists: Increased Selectivity of 9α,11-Epoxy Derivatives", Helvetica Chimica Acta, Vol. 80, pp. 566-585 (1997), discloses an X-ray crystal structure analysis of a solvate of eplerenone prepared by crystallizing eplerenone from a methylene chloride/diethyl ether solvent system.

De Gasparo et al., "Antialdosterones: Incidence and Prevention of Sexual Side Effects", J. Steroid Biochem., Vol. 32, No. 13, pp. 223-227 (1989), discloses the use of non-formulated eplerenone having a 20 micron particle size in a single dose study of eplerenone.

Spironolactone 2, a 20-spiroxane-steroid having activity as an aldosterone receptor antagonist, is commercially available for the treatment of hypertension. Spironolactone, however, has antiandrogenic activity that can result in gynecomastia and impotence in men. It also has weak progestational activity that can produce menstrual irregularities in women. Accordingly, there is interest in the development of additional active aldosterone receptor antagonists such as eplerenone that do not interact with other steroid receptor systems such as glucocorticoid, progestin and androgen steroid receptor systems and/or that provide for a broader range of treatment.

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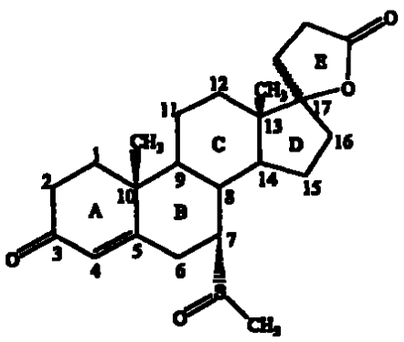

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Agafonov et al., "Polymorphism of Spironolactone", J. Pharm. Sciences, Vol. 80, No. 2, pp 181-185 (1991), discloses an acetonitrile solvate, an ethanol solvate, an ethyl acetate solvate, a methanol solvate and two non-solvated polymorphic crystalline forms of spironolactone. Brittan, Polymorphism in Pharmaceutical Solids, pp. 114-116, 207, 235, and 261 (Marcel Dekker, Inc. 1999), likewise discloses these solid state forms of spironolactone.

Summary of the Invention

A first aspect of the invention comprises novel particles comprising a solvated crystalline form of eplerenone wherein at least 90% of the particles have a particle size less than about 15 microns. These eplerenone particles possess activity as an aldosterone receptor antagonist and have unique properties relative to other solid state particulate forms of eplerenone.

A second aspect of the invention comprises substantially phase pure particles comprising a solvated crystalline form of eplerenone wherein at least 90% of the particles have a particle size less than about 15 microns.

A third aspect of the invention comprises pharmaceutical compositions comprising particles comprising a solvated crystalline form of eplerenone wherein at least 90% of the particles have a particle size less than about 15 microns.

A fourth aspect of the invention comprises a method for the prophylaxis and/or treatment of an aldosterone-mediated condition and/or disorder comprising administering to a subject a therapeutically-effective amount of particles comprising a solvated crystalline form of eplerenone wherein at least 90% of the particles have a particle size less than about 15 microns.

A fifth aspect of the invention comprises methods for the preparation of particles comprising a solvated crystalline form of eplerenone wherein at least 90% of the particles have a particle size less than about 15 microns.

Additional aspects of the invention are discussed throughout the specification of this application.

Brief Description of the Drawings

Fig. 1-A shows X-ray powder diffraction patterns of Form I eplerenone.

Fig. 1-B shows X-ray powder diffraction patterns of Form II eplerenone.

Fig. 1-C shows X-ray powder diffraction patterns of the methyl ethyl ketone solvate of eplerenone.

Figs. 1-D through 1-O show X-ray powder diffraction patterns for the following eplerenone solvates: n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, acetone solvate, toluene solvate, isopropanol solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, methyl acetate

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solvate, and propyl acetate solvate, respectively.

Fig. 2-A shows a differential scanning calorimetry (DSC) thermogram of non-milled Form II directly crystallized from methyl ethyl ketone.

Fig. 2-B shows a differential scanning calorimetry (DSC) thermogram of non-milled Form II prepared by desolvation of a solvate obtained by crystallization of a high purity eplerenone from methyl ethyl ketone.

Fig. 2-C shows a differential scanning calorimetry (DSC) thermogram of Form II prepared by crystallizing a solvate from a solution of high purity eplerenone in methyl ethyl ketone, desolvating the solvate to yield Form II, and milling the resulting Form II.

Fig. 2-D shows a differential scanning calorimetry (DSC) thermogram of non-milled Form I prepared by desolvation of a solvate obtained by digestion of low purity eplerenone from appropriate solvents.

Figs. 2-E through 2-T show DSC thermograms for the following eplerenone solvates: n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, chloroform solvate, acetone solvate, toluene solvate, isopropanol solvate, t-butyl acetate solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, methyl acetate solvate, propyl acetate solvate, n-butanol solvate, and n-octanol solvate, respectively.

Fig. 3-A shows the infrared spectra (diffuse reflectance, DRIFTS) of Form I eplerenone.

Fig. 3-B shows the infrared spectra (diffuse reflectance, DRIFTS) of Form II eplerenone.

Fig. 3-C shows the infrared spectra (diffuse reflectance, DRIFTS) of the methyl ethyl ketone solvate of

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eplerenone.

Fig. 3-D shows the infrared spectra (diffuse reflectance, DRIFTS) of eplerenone in chloroform solution.

Figs. 3-E through 3-Q show infrared absorption spectra for the following eplerenone solvates: n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, acetone solvate, toluene solvate, isopropanol solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, propyl acetate solvate, methyl acetate solvate, propylene glycol solvate, and t-butyl acetate solvate respectively.

Fig. 4 shows ^{13}C NMR spectra for Form I of eplerenone.

Fig. 5 shows ^{13}C NMR spectra for Form II of eplerenone.

Figs. 6-A through 6-R show thermogravimetry analysis profiles for the following eplerenone solvates: methyl ethyl ketone solvate, n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, chloroform solvate, acetone solvate, toluene solvate, isopropanol solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, methyl acetate solvate, propyl acetate solvate, propylene glycol solvate, n-butanol solvate, n-octanol solvate, and t-butyl acetate solvate, respectively.

Fig. 7 is a scanning electron micrograph of Form II eplerenone prepared by desolvation of the methyl ethyl ketone solvate.

Fig. 8 is a scanning electron micrograph of Form II eplerenone prepared by direct crystallization from ethyl acetate.

Fig. 9 shows an X-ray powder diffraction pattern of a crystalline form of 7-methyl hydrogen 4α,5α:9α,11α-diepoxy-

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17-hydroxy-3-oxo-17α-pregnane-7α,21-dicarboxylate, γ-lactone isolated from methyl ethyl ketone.

Fig. 10 shows an X-ray powder diffraction pattern of the crystalline form of 7-methyl hydrogen 11α,12α-epoxy-17-hydroxy-3-oxo-17α-pregn-4-ene-7α,21-dicarboxylate, γ-lactone isolated from isopropanol.

Fig. 11 shows an X-ray powder diffraction pattern of the crystalline form of 7-methyl hydrogen 17-hydroxy-3-oxo-17α-pregna-4,9(11)-diene-7α,21-dicarboxylate, γ-lactone isolated from n-butanol.

Fig. 12 illustrates the Gibbs free energy-temperature relationship for enantiotropically related polymorphs.

Fig. 13 shows the X-ray powder diffraction patterns for the wet cake (methyl ethyl ketone solvate) obtained from (a) 0%, (b) 1%, (c) 3%, and (d) 5% diepoxide-doped methyl ethyl ketone crystallizations.

Fig. 14 shows the X-ray powder diffraction patterns for the dried solids obtained from (a) 0%, (b) 1%, (c) 3%, and (d) 5% diepoxide-doped methyl ethyl ketone crystallizations.

Fig. 15 shows the X-ray powder diffraction patterns for the dried solids from the methyl ethyl ketone crystallization with 3% doping of diepoxide (a) without grinding of the solvate prior to drying, and (b) with grinding of the solvate prior to drying.

Fig. 16 shows the X-ray powder diffraction patterns for the wet cake (methyl ethyl ketone solvate) obtained from (a) 0%, (b) 1%, (c) 5%, and (d) 10% 11,12-epoxide-doped methyl ethyl ketone crystallizations.

Fig. 17 shows the X-ray powder diffraction patterns for the dried solids obtained from (a) 0%, (b) 1%, (c) 5%, and

(d) 10% 11,12-epoxide-doped methyl ethyl ketone crystallizations.

Fig. 18 shows a cube plot of product purity, starting material purity, cooling rate and endpoint temperature based on the data reported in Table X-7A.

Fig. 19 shows a half normal plot prepared using the cube plot of Fig. 18 to determine those variables having a statistically significant effect on the purity of the final material.

Fig. 20 is an interaction graph based on the results reported in Table X-7A showing the interaction between starting material purity and cooling rate on final material purity.

Fig. 21 shows a cube plot of Form I weight fraction, starting material purity, cooling rate and endpoint temperature based on the data reported in Table X-7A.

Fig. 22 shows a half normal plot prepared using the cube plot of Fig. 21 to determine those variables having a statistically significant effect on the purity of the final material.

Fig. 23 is an interaction graph based on the results reported in Table X-7A showing the interaction between starting material purity and endpoint temperature on final material purity.

Fig. 24 shows an X-ray diffraction pattern of amorphous eplerenone.

Fig. 25 shows a DSC thermogram of amorphous eplerenone.

Fig. 26 shows the dissolution rates measured for four polymorph samples.

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Detailed Description of the Preferred Embodiments

As with all pharmaceutical compounds and compositions, the chemical and physical properties of eplerenone are important in its commercial development. These properties include, but are not limited to: (1) packing properties such as molar volume, density and hygroscopicity, (2) thermodynamic properties such as melting temperature, vapor pressure and solubility, (3) kinetic properties such as dissolution rate and stability (including stability at ambient conditions, especially to moisture and under storage conditions), (4) surface properties such as surface area, wettability, interfacial tension and shape, (5) mechanical properties such as hardness, tensile strength, compactibility, handling, flow and blend; and (6) filtration properties. These properties can affect, for example, the processing and storage of pharmaceutical compositions comprising eplerenone. Solid state forms of eplerenone that provide an improvement in one or more of these properties relative to other solid state forms of eplerenone are desirable.

The present invention comprises such novel solid state forms of eplerenone. Specifically, these novel solid state forms include solvated crystalline forms, non-solvated and non-hydrated crystalline forms (designated "Form I" and "Form II"), and an amorphous form of eplerenone. Each solid state form of eplerenone described in the present application possesses one or more of the above-described advantageous chemical and/or physical properties relative to the other solid state forms described herein or otherwise disclosed in the literature.

In one embodiment of the invention, the solid state form comprises Form I eplerenone. Form I exhibits a faster dissolution rate (approximately 30% higher) at lower temperatures (i.e., temperatures below the enantiotropic transition temperature as later discussed) than, for example, Form II. Where dissolution of eplerenone in the gastrointestinal tract is the rate-controlling step for delivery of the eplerenone to the target cells, faster dissolution generally results in improved bioavailability. Form I, therefore, can provide an improved bioavailability profile relative to Form II. In addition, selection of a solid state form of eplerenone having a faster dissolution rate likewise provides greater flexibility in the selection of excipients for, and in the formulation of, immediate release pharmaceutical compositions relative to other solid state forms having a slower dissolution rate.

In another embodiment of the invention, the solid state form comprises Form II eplerenone. Form II possesses greater physical stability at lower temperatures (i.e., at temperatures below the enantiotropic transition temperature as later discussed) than, for example, Form I. Solid state forms of eplerenone such as Form II that do not require the use of special processing or storage conditions, and that avoid the need for frequent inventory replacement, are desirable. For example, selection of a solid state form of eplerenone that is physically stable during the manufacturing process (such as during milling of eplerenone to obtain a material with reduced particle size and increased surface area) can avoid the need for special processing conditions and the increased costs generally associated with such special processing conditions. Similarly, selection of a

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solid state form of eplerenone that is physically stable at different conditions of storage (especially considering the different possible storage conditions during the lifetime of the eplerenone product) can help avoid polymorphic or other degradative changes in the eplerenone that can lead to product loss or deterioration of product efficacy. Therefore, the selection of a solid state form of eplerenone such as Form II having greater physical stability provides a meaningful benefit over less stable eplerenone forms.

In another embodiment of the invention, the solid state form of eplerenone comprises a solvated crystalline form of eplerenone. Such solvated crystalline forms are useful as intermediates in the preparation of Form I and Form II eplerenone. An additional benefit from the use of a solvated crystalline form as an intermediate is the "intrinsic micronizing" of the crystal that results upon desolvation as discussed later in this application. Such "intrinsic micronizing" can reduce or eliminate milling requirements. Further, where additional milling is still required, it is easier to mill certain solvates before the desolvation step than to mill Form I or Form II after desolvation of the solvate.

Pharmaceutically acceptable solvated crystalline forms also can be used directly in pharmaceutical compositions. These solvated crystalline forms preferably do not comprise methylene chloride, isopropanol or ethyl ether; and, in yet another embodiment, do not comprise methylene chloride, isopropanol, ethyl ether, methyl ethyl ketone or ethanol; and, in yet another embodiment, do not comprise methylene chloride, isopropanol, ethyl ether, methyl ethyl ketone, ethanol, ethyl acetate or acetone. More preferably, the solvated crystalline

forms are substantially exclusive of solvents that are not pharmaceutically-acceptable solvents. Because Form I and Form II typically are more physically stable than the crystalline solvates at room temperature and under atmospheric pressure, the solvated crystalline forms used in such compositions generally comprise a pharmaceutically acceptable higher boiling and/or hydrogen bonding solvent such as, but not limited to, butanol. It is believed that the solvated crystalline forms collectively can offer a range of different dissolution rates and, where dissolution of eplerenone in the gastrointestinal tract is the rate-controlling step for delivery of the eplerenone to the target cells, bioavailabilities relative to Form I and Form II.

In another embodiment of the invention, the solid state form of eplerenone comprises an amorphous form of eplerenone. Amorphous eplerenone is useful as an intermediate in the preparation of Form I and Form II eplerenone. In addition, it is believed that amorphous eplerenone possesses a different dissolution rate and, where dissolution of eplerenone in the gastrointestinal tract is the rate-controlling step for delivery of the eplerenone to the target cells, bioavailability relative to Form I and Form II.

In another embodiment, the invention comprises combinations of solid state eplerenone forms selected from the group consisting of Form I eplerenone, Form II eplerenone, solvated crystalline eplerenone and amorphous eplerenone. Such combinations are useful, for example, in the preparation of pharmaceutical compositions having differing dissolution profiles, including controlled release compositions.

In addition, depending upon the intended use of the solid state form of eplerenone, processing considerations may favor the selection of a specific solid state form of eplerenone or a specific combination of such solid state forms. Pure phase Form II, for example, generally is more easily prepared than pure phase Form I. A mixture of Form I and Form II, however, generally is more easily prepared than phase pure Form II and allows for the use of a low purity eplerenone starting material. Use of a solvated crystalline form instead of Form I or Form II in a composition eliminates a processing step, desolvation, for those processes that otherwise would proceed by desolvation of the solvated crystalline form. Alternatively, the desolvation step can be eliminated, for example, if Form II is directly crystallized from an appropriate solvent without the intervening preparation and desolvation of the intermediate solvated crystalline form. These processes are described in greater detail later in the application.

It has been discovered that reduction of the particle size of the solid state form of eplerenone to a D_{90} particle size (later defined herein) between about 10 nm to about 15 microns can improve the bioavailability of unformulated eplerenone and pharmaceutical compositions comprising that solid state form of eplerenone. Accordingly, the D_{90} particle size of the unformulated eplerenone or the eplerenone used as a starting material in the pharmaceutical composition generally is less than about 15 microns, preferably less than about 1 micron, more preferably less than about 800 nm, still more preferably less than about 600 nm, and still more preferably less than about 400 nm. In one embodiment, the D_{90} particle size is between about 10 nm to about 1 micron. In

another embodiment, the D_{90} particle size is between about 100 nm to about 800 nm. In another embodiment, the D_{90} particle size is between about 200 nm to about 600 nm. In another embodiment, the D_{90} particle size is between about 400 nm to about 800 nm.

Solid state forms of eplerenone having a particle size less than about 15 microns can be prepared in accordance with applicable particle size reduction techniques known in the art. Such techniques include, but are not limited to those described in U.S. Patents 5,145,684, 5,318,767, 5,384,124 and 5,747,001. U.S. Patents 5,145,684, 5,318,767, 5,384,124 and 5,747,001 are expressly incorporated by reference as if fully set forth at length. In accordance with the method of U.S. Patent 5,145,684, for example, particles of suitable size are prepared by dispersing the eplerenone in a liquid dispersion medium and wet-grinding the mixture in the presence of grinding media to reduce the particles to the desired size. If necessary or advantageous, the particles can be reduced in size in the presence of a surface modifier.

Definitions

The term "amorphous" as applied to eplerenone refers to a solid state wherein the eplerenone molecules are present in a disordered arrangement and do not form a distinguishable crystal lattice or unit cell. When subjected to X-ray powder diffraction, amorphous eplerenone does not produce any characteristic crystalline peaks.

Where reference is made in this application to the "boiling point" of a substance or solution, the term "boiling point" means the boiling point of the substance or solution under the applicable process conditions.

The term "crystalline form" as applied to eplerenone refers to a solid state form wherein the eplerenone molecules are arranged to form a distinguishable crystal lattice (i) comprising distinguishable unit cells, and (ii) yielding diffraction peaks when subjected to X-ray radiation.

The term "crystallization" as used throughout this application can refer to crystallization and/or recrystallization depending upon the applicable circumstances relating to the preparation of the eplerenone starting material.

The term "digestion" means a process in which a slurry of solid eplerenone in a solvent or mixture of solvents is heated at the boiling point of the solvent or mixture of solvents under the applicable process conditions.

The term "direct crystallization" as used herein refers to the crystallization of eplerenone directly from a suitable solvent without the formation and desolvation of an intermediate solvated crystalline solid state form of eplerenone.

The term "particle size" as used herein refers to particle size as measured by conventional particle size measuring techniques well known in the art, such as laser light scattering, sedimentation field flow fractionation, photon correlation spectroscopy, or disk centrifugation. The term "D_{90} particle size" means the particle size of at least 90% of the particles as measured by such conventional particle size measuring techniques.

The term "DSC" means differential scanning calorimetry.

The term "HPLC" means high pressure liquid chromatography.

The term "IR" means infra red.

The term "min" means minute.

The term "psi" means pounds per square inch absolute.

The term "purity" means the chemical purity of eplerenone according to conventional HPLC assay. As used herein, "low purity eplerenone" generally means eplerenone that contains an effective amount of a Form I growth promoter and/or a Form II growth inhibitor. As used herein, "high purity eplerenone" generally means eplerenone that does not contain, or contains less than an effective amount of, a Form I growth promoter and/or a Form II growth inhibitor.

The term "phase purity" means the solid state purity of eplerenone with regard to a particular crystalline or amorphous form of the eplerenone as determined by the infrared spectroscopy analytical methods described herein.

The term "XPRD" means X-ray powder diffraction.

The term "rpm" means revolutions per minute.

The term "TGA" means thermogravimetric analysis.

The term "T_m" means melting temperature.

Characterization of Crystalline Form

1. Molecular Conformation

Single crystal X-ray analysis indicates that the eplerenone molecular conformation differs between Form I and Form II, particularly with respect to the orientation of the ester group at the 7-position of the steroid ring. The orientation of the ester group can be defined by the C8-C7-C23-02 torsion angle.

In the Form I crystal lattice, the eplerenone molecule adopts a conformation in which the methoxy group of the ester is approximately aligned with the C-H bond at the 7-position

and the carbonyl group is approximately positioned over the center of the B-steroid ring. The C8-C7-C23-02 torsion angle is approximately -73.0° in this conformation. In this orientation, the carbonyl oxygen atom of the ester group (01) is in close contact with the oxygen atom of the 9,11-epoxide ring (04). The 01-04 distance is about 2.97 Å, which is just below the van der Waal's contact distance of 3.0 Å (assuming van der Waal's radii of 1.5Å for the oxygen).

In the Form II crystal lattice, the eplerenone molecule adopts a conformation in which the ester group is rotated approximately 150° relative to that of Form I and has a C8-C7-C23-02 torsion angle of approximately +76.9°. In this orientation, the methoxy group of the ester is directed toward the 4,5-alkene segment of the A-steroid ring. In this orientation, the distance between either oxygen atom of the ester group (01,02) and the oxygen atom of the 9,11-epoxide ring is increased relative to the distance determined for Form I. The 02-04 distance is approximately 3.04Å, falling just above the van der Waal's contact distance. The 01-04 distance is about 3.45Å.

The eplerenone molecule appears to adopt a conformation characteristic of Form II in the solvated crystalline forms analyzed by single crystal X-ray diffraction to date.

2. X-Ray Powder Diffraction

The various crystalline forms of eplerenone were analyzed with either a Siemens D5000 powder diffractometer or an Inel Multipurpose Diffractometer. For the Siemens D500 powder diffractometer, the raw data was measured for 2q values from 2 to 50, with steps of 0.020 and step periods of two seconds. For the Inel Multipurpose Diffractometer,

samples were placed in an aluminum sample holder and raw data was collected for 30 minutes at all two theta values simultaneously.

Tables 1A, 1B and 1C set out the significant parameters of the main peaks in terms of 2q values and intensities for the Form I (prepared by desolvation of the ethanol solvate obtained by digestion of low purity eplerenone), Form II (prepared by desolvation of the methyl ethyl ketone solvate obtained by recrystallization of high purity eplerenone), and methyl ethyl ketone solvate (prepared by room temperature slurry conversion of high purity eplerenone in methyl ethyl ketone) crystalline forms of eplerenone, respectively (X-ray radiation at a wavelength of 1.54056 Angstroms).

Minor shifts in peak positioning may be present in the diffraction patterns of Form I and Form II as a result of imperfections in the spacing of the crystal diffraction planes due to the route of manufacture of Form I and Form II (i.e. desolvation of a solvate). In addition, Form I is isolated from a solvate prepared by digestion of crude eplerenone. This method results in a lower overall chemical purity (approximately 90%) of the Form I. Finally, the solvated forms of eplerenone are expected to show some shifting in the positioning of the diffraction peaks due to the increased mobility of the solvent molecules within the solvent channels in the crystal lattice.

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TABLE 1A: FORM I DATA

Angle 2-theta	d-spacing Angstrom	Intensity	Intensity %
6.994	12.628	1188	7.2
8.291	10.655	2137	13
10.012	8.827	577	3.5
11.264	7.849	1854	11.3
12.04	7.344	7707	46.8
14.115	6.269	3121	19
14.438	6.13	15935	96.8
15.524	5.703	637	3.9
16.169	5.477	1349	8.2
16.699	5.306	1663	10.1
16.94	5.23	1692	10.3
17.147	5.167	2139	13
17.68	5.018	6883	41.8
17.91	4.949	16455	100
18.379	4.823	3106	18.9
18.658	4.752	1216	7.4
19.799	4.48	1499	9.1
20.236	4.385	383	2.3
21.707	4.091	1267	7.7
21.8	4.073	1280	7.7
21.959	4.044	1279	7.8
22.461	3.955	4264	25.9
23.191	3.832	1026	6.2
23.879	3.723	1000	6.1
24.599	3.616	1688	10.3
25.837	3.445	931	5.7
26.034	3.42	686	4.2
26.868	3.316	912	5.5
27.093	3.288	1322	8
27.782	3.209	1236	7.5
28.34	3.147	1845	11.2
28.861	3.091	957	5.8
29.886	2.9882	746	4.5
30.827	2.9166	992	6
31.106	2.8726	1205	7.3
33.216	2.6951	1287	7.8
33.716	2.656	802	4.9
34.434	2.6024	914	5.6

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TABLE 1B: FORM II DATA

Angle 2-Theta	d-spacing Angstrom	Intensity Cps	Intensity %
7.992	11.054	11596	26.6
10.044	8.799	12048	27.6
11.206	7.889	4929	11.3
12.441	7.109	1747	4
12.752	6.936	4340	9.9
13.257	6.673	2444	5.6
14.705	6.019	43646	100
15.46	5.727	2670	6.1
15.727	5.63	7982	18.3
16.016	5.529	3519	8.1
17.671	5.015	8897	20.4
17.9	4.951	2873	6.6
18.352	4.83	612	1.4
18.705	4.74	669	1.5
19.524	4.543	1126	2.6
20.103	4.413	3753	8.6
20.63	4.302	1451	3.3
21.067	4.214	876	2
21.675	4.097	2760	6.3
22.232	3.995	1951	4.5
22.652	3.922	1657	3.8
23.624	3.763	827	1.9
24.279	3.663	1242	2.8
25.021	3.556	5144	11.8
25.485	3.492	1702	3.9
25.707	3.463	2493	5.7
26.251	3.392	1371	3.1
26.85	3.318	1970	4.5
27.319	3.262	1029	2.4
27.931	3.192	440	1
27.969	3.187	440	1
28.937	3.083	1128	2.6
29.703	3.005	1211	2.8
30.173	2.9594	1506	3.5
30.584	2.9206	1602	3.7
30.885	2.8928	1550	3.6
31.217	2.8628	1068	2.4
31.605	2.8285	1038	2.4
32.059	2.7895	1211	2.8
32.64	2.7412	684	1.6
32.747	2.7324	758	1.7
33.48	2.6759	506	1.2
34.194	2.6201	1085	2.5
34.545	2.5943	915	2.1

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TABLE 1C: METHYL ETHYL KETONE DATA

Angle 2-Theta	d-spacing Angstrom	Intensity Cps	Intensity %
7.584	11.648	5629	32.6
7.753	11.393	15929	92.3
10.151	8.707	2877	16.7
11.31	7.817	701	4.1
12.646	6.994	1027	5.9
13.193	6.705	15188	88
13.556	6.526	14225	82.4
14.074	6.287	1966	11.4
14.748	6.002	2759	16
15.165	5.837	801	4.6
15.548	5.694	1896	11
17.031	5.202	7980	46.2
17.28	5.127	17287	100
17.706	5.005	6873	39.8
18.555	4.778	545	3.2
18.871	4.699	1112	6.4
19.766	4.488	1704	9.9
20.158	4.401	1396	8.1
20.725	4.282	2644	15.3
21.767	4.078	1127	6.6
22.06	4.026	451	2.6
22.864	3.886	1542	8.9
23.412	3.796	14185	82.2
23.75	3.743	1154	6.7
24.288	3.662	3063	17.7
25.253	3.524	1318	7.6
25.503	3.49	1736	10.1
25.761	3.455	1225	7.1
26.176	3.402	1348	7.8
26.548	3.355	1098	6.4
27.357	3.257	1944	11.3
27.605	3.229	2116	12.3
27.9	3.195	858	5
28.378	3.142	583	3.4
28.749	3.103	763	4.4
29.3	3.048	1162	6.8
29.679	3.008	2606	15.1
30.402	2.9377	2164	12.6
30.739	2.9063	648	3.8

Graphical examples of the x-ray diffraction patterns for Form I, Form II, and the methyl ethyl ketone solvate

crystalline forms of eplerenone are shown in Figs. 1-A, 1-B, and 1-C, respectively. Form I shows distinguishing peaks at 7.0 ± 0.2, 8.3 ± 0.2, and 12.0 ± 0.2 degrees two theta. Form II shows distinguishing peaks at 8.0 ± 0.2, 12.4 ± 0.2, 12.8 ± 0.2, and 13.3 ± 0.2 degrees two theta. The methyl ethyl ketone solvated crystalline form shows distinguishing peaks at 7.6 ± 0.2, 7.8 ± 0.2, and 13.6 ± 0.2 degrees two theta.

Examples of the x-ray diffraction patterns are shown in Figs. 1-D through 1-O for the following solvate crystalline forms of eplerenone: n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, acetone solvate, toluene solvate, isopropanol solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, methyl acetate solvate, and propyl acetate solvate, respectively.

3. Melting/Decomposition Temperature

The temperatures of melting and/or decomposition of non-solvated eplerenone crystalline forms were determined using a TA Instruments 2920 differential scanning calorimeter. Each sample (1-2 mg) was placed in either a sealed or unsealed aluminum pan and heated at 10°C/minute. Melting/decomposition ranges were defined from the extrapolated onset to the maximum of the melting/decomposition endotherm.

The melting of the non-solvated eplerenone crystals forms (Form I and Form II) was associated with chemical decomposition and loss of trapped solvent from the crystal lattice. The melting/decomposition temperature also was affected by the manipulation of the solid prior to analysis. For example, non-milled Form II (approximate D_{90} particle size of about 180-450 microns) prepared by direct crystallization

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from an appropriate solvent or from desolvation of a solvate obtained from crystallization of high purity eplerenone in an appropriate solvent or mixture of solvents generally had a melting range of about 237-242°C. Milled Form II (approximate D_{90} particle size of about 80-100 microns) (Form II prepared by crystallizing a solvate from a solution of high purity eplerenone in an appropriate solvent or mixture of solvents, desolvating the solvate to yield Form II, and milling the resulting Form II) generally had a lower and broader melting/decomposition range of about 223-234°C. Non-milled Form I (approximate D_{90} particle size of about 180-450 microns) prepared by desolvation of a solvate obtained by digestion of low purity eplerenone generally had a higher melting/decomposition range of about 247-251°C. Examples of the DSC thermograms of (a) non-milled Form II directly crystallized from methyl ethyl ketone, (b) non-milled Form II prepared by desolvation of a solvate obtained by crystallization of a high purity eplerenone from methyl ethyl ketone, (c) Form II prepared by milling a desolvated solvate obtained by crystallization of high purity eplerenone from methyl ethyl ketone, and (d) non-milled Form I prepared by desolvation of a solvate obtained by digestion of low purity eplerenone from methyl ethyl ketone are given in Figures 2-A, 2-B, 2-C and 2-D, respectively.

DSC thermograms of solvated forms of eplerenone were determined using a Perkin Elmer Pyris 1 differential scanning calorimeter. Each sample (1-10 mg) was placed in an unsealed aluminum pan and heated at 10°C/minute. One or more endothermal events at lower temperatures were associated with enthalpy changes that occurred as solvent was lost from the solvate crystal lattice. The highest temperature endotherm

or endotherms were associated with the melting/decomposition of Form II or Form I eplerenone. Examples of the DSC thermograms are shown in Figs. 2-E through 2-T for the following solvated crystalline forms of eplerenone: n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, chloroform solvate, acetone solvate, toluene solvate, isopropanol solvate, t-butyl acetate solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, methyl acetate solvate, propyl acetate solvate, n-butanol solvate, and n-octanol solvate, respectively.

4. Infrared Absorption Spectroscopy

Infrared absorption spectra of the non-solvated forms of eplerenone (Form I and Form II) were obtained with a Nicolet DRIFT (diffuse reflectance infrared fourier transform) Magna System 550 spectrophotometer. A Spectra-Tech Collector system and a microsample cup were used. Samples (5%) were analyzed in potassium bromide and scanned from 400-4000 cm^{-1}. Infrared absorption spectra of eplerenone in dilute chloroform solution (3%) or in the solvated crystal forms were obtained with a Bio-rad FTS-45 spectrophotometer. Chloroform solution samples were analyzed using a solution cell of 0.2 mm path length with sodium chloride salt plates. Solvate FTIR spectra were collected using an IBM micro-MIR (multiple internal reflectance) accessory. Samples were scanned from 400-4000 cm^{-1}. Examples of the infrared absorption spectra of (a) Form I, (b) Form II, (c) the methyl ethyl ketone solvate, and (d) eplerenone in chloroform solution are shown in Figures 3-A, 3-B, 3-C and 3-D, respectively.

Table 2 discloses illustrative absorption bands for

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eplerenone in the Form I, Form II, and methyl ethyl ketone solvate crystal forms. Illustrative absorption bands for eplerenone in chloroform solution are also disclosed for comparison. Differences between Form I and either Form II or the methyl ethyl ketone solvate were observed, for example, in the carbonyl region of the spectrum. Form I has an ester carbonyl stretch of approximately 1739 cm^{-1} while both Form II and the methyl ethyl ketone solvate have the corresponding stretch at approximately 1724 and 1722 cm^{-1}, respectively. The ester carbonyl stretch occurs at approximately 1727 cm^{-1} in the eplerenone in chloroform solution. The change in stretching frequency of the ester carbonyl between Form I and Form II reflects the change in orientation of the ester group between the two crystal forms. In addition, the stretch of the ester of the conjugated ketone in the A-steroid ring shifts from approximately 1664-1667 cm^{-1} in either Form I or the methyl ethyl ketone solvate to approximately 1655 cm^{-1} in Form II. The corresponding carbonyl stretch occurs at approximately 1665 cm^{-1} in dilute solution.

Another difference between Form I and Form II was seen in the C-H bending region. Form I has an absorption at approximately 1399 cm^{-1} which is not observed in Form II, the methyl ethyl ketone solvate, or the eplerenone in chloroform solution. The 1399 cm^{-1} stretch occurs in the region of CH_2 scissoring for the C2 and C21 methylene groups adjacent to carbonyl groups.

Table 2

Absorption Region	Form I (cm^{-1})	Form II (cm^{-1})	Methyl Ethyl Ketone Solvate (cm^{-1})	Eplerenone in Chloroform (cm^{-1})
ν C=O(lactone)	1773	1775	1767	1768
ν C=O(ester)	1739	1724	1722	1727
ν C=O(3keto)	1664	1655	1667	1665
ν C=C (3,4-olefin)	1619	1619	1622	1623
δ_{as}CH3,δCH2, δCH2(α to carbonyl)	1460, 1444, 1426	1467, 1438, 1422, 1399	1467, 1438, 1422	1464, 1438, 1422
δ_{s}CH3	1380	1381	-1380	1378

Examples of infrared absorption spectra are shown in Figs. 3-E through 3-S for the following solvated crystalline forms of eplerenone: n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, acetone solvate, toluene solvate, isopropanol solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, propyl acetate solvate, methyl acetate solvate, propylene glycol solvate, and t-butyl acetate solvate respectively.

5. Nuclear Magnetic Resonance

^{13}C NMR spectra were obtained at a field of 31.94 MHz. Examples of the ^{13}C NMR spectra of Form I and Form II eplerenone are shown in Figs. 4 and 5, respectively. The Form I eplerenone analyzed to obtain the data reflected in Fig. 4 was not phase pure and included a small amount of Form II eplerenone. Form I is most clearly distinguished by the carbon resonances at around 64.8 ppm, 24.7 ppm and 19.2 ppm.

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Form II is most clearly distinguished by the carbon resonances at around 67.1 ppm and 16.0 ppm.

6. Thermogravimetry

Thermogravimetric analysis was performed using a TA Instruments TGA 2950 thermogravimetric analyzer. Samples were placed in an unsealed aluminum pan under nitrogen purge. Starting temperature was 25°C with the temperature increased at a rate of about 10°C/minute.

Examples of thermogravimetry analysis profiles are shown in Figs. 6-A through 6-R for the following solvated crystalline forms of eplerenone: methyl ethyl ketone solvate, n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, chloroform solvate, acetone solvate, toluene solvate, isopropanol solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, methyl acetate solvate, propyl acetate solvate, propylene glycol solvate, n-butanol solvate, n-octanol solvate, and t-butyl acetate solvate, respectively.

7. Microscopy

Hot-stage microscopy was performed on single crystals of the methyl ethyl ketone solvate using a Linkam THMS 600 Hot Stage with Zeiss Universal Polarized Light Microscope. Under polarized light at room temperature the solvate crystal was birefringent and translucent indicating that the crystal lattice was highly ordered. As the temperature increased to about 60°C, noticeable defects began to emerge along the long crystal dimension. A scanning electron micrograph of Form II eplerenone obtained by desolvation of the methyl ethyl ketone solvate is shown in Fig. 7 and reveals surface defects,

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pores, cracks and fractures within the crystal lattice. A scanning electron micrograph of Form II eplerenone obtained by direct crystallization from ethyl acetate is shown in Fig. 8 and does not exhibit similar surface defects, pores, cracks and fractures within the crystal lattice.

8. Unit Cell Parameters

Tables 3A, 3B and 3C below summarize the unit cell parameters determined for Form I, Form II, and several solvated crystalline forms.

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TABLE 3A

Parameter	Form I	Form II	Methyl ethyl ketone Solvate
Crystal system	Ortho-rhombic	Monoclinic	Orthorhombic
Space group	$P2_12_12_1$	$P2_1$	$P2_12_12_1$
a	21.22 Å	8.78 Å	23.53 Å
b	15.40 Å	11.14 Å	8.16 Å
c	6.34 Å	11.06 Å	13.08 Å
α	90°	90°	90°
β	90°	93.52°	90°
γ	90°	90°	90°
z	4	2	4
Volume (Å)	2071.3	1081.8	2511.4
ρ (calculated)	1.329 g/cm³	1.275 g/cm³	1.287 g/cm³
R	0.0667	0.062	0.088

TABLE 3B

Parameter	Acetone Solvate	Toluene Solvate	Butyl Acetate Solvate[1]
Crystal system	Ortho-rhombic	Ortho-rhombic	Ortho-rhombic
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
a	23.31 Å	23.64 Å	23.07 Å
b	13.13 Å	13.46 Å	13.10 Å
c	8.28 Å	8.16 Å	8.24 Å
α	90°	90°	90°
β	90°	90°	90°
γ	90°	90°	90°
z	4	4	4
Volume (Å)	2533.7	2596.6	2490.0
ρ (calculated)	1.239 g/cm³	1.296 g/cm³	1.334 g/cm³
R	0.058	0.089	0.093

[1]The solvate molecules were not completely refined due to disorder of the solvent molecules in the channels.

TABLE 3C

Parameter	Isobutyl Acetate Solvate[1]	Isopropanol Solvate[1]	Ethanol Solvate[1]
Crystal system	Ortho-rhombic	Ortho-rhombic	Ortho-rhombic
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
a	23.19 Å	23.15 Å	23.51 Å
b	12.95 Å	12.73 Å	13.11 Å
c	8.25 Å	8.25 Å	8.27 Å
α	90°	90°	90°
β	90°	90°	90°
γ	90°	90°	90°
z	4	4	4
Volume (Å)	2476.4	2433.2	2548.6
ρ (calcu-lated)	1.337 g/cm³	1.296 g/cm³	1.234 g/cm³
R	0.098	0.152	0.067

[1]The solvate molecules were not refined completely due to disorder of the solvent molecules in the channels.

Additional information on selected solvated crystalline forms of eplerenone is reported in Table 4 below. The unit cell data reported in Table 3A above for the methyl ethyl ketone solvate also are representative of the unit cell parameters for many of these additional eplerenone crystalline solvates. Most of the eplerenone crystalline solvates tested are substantially isostructural to each other. While there may be some minor shifting in the X-ray powder diffraction peaks from one solvated crystalline form to the next due to the size of the incorporated solvent molecule, the overall diffraction patterns are substantially the same and the unit cell parameters and molecular positions are substantially identical for most of the solvates tested.

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TABLE 4

Solvent	Stoichiometry (Solvent: Eplerenone)	Isostructural to Methyl Ethyl ketone Solvate?	Desolvation Temperature[1] (°C)
Methyl Ethyl Ketone	1:1	N/A	89
2-Pentanone	---	---	---
Acetic Acid	1:2	Yes	203
Acetone	1:1	Yes	117
Butyl Acetate	1:2	Yes	108
Chloroform	---	Yes	125
Ethanol	1:1	Yes	166
Isobutanol	---	---	---
Isobutyl Acetate	1:2	Yes	112
Isopropanol	1:1	Yes	121
Methyl Acetate	1:1	Yes	103
Ethyl Propionate	1:1	Yes	122
n-Butanol	1:1	Yes	103
n-Octanol	---	Yes	116
n-Propanol	1:1	Yes	129
Propyl Acetate	1:1	Yes	130
Propylene Glycol	---	Yes	188
t-Butanol	---	---	---
Tetrahydrofuran	1:1	Yes	136
Toluene	1:1	Yes	83
t-Butyl Acetate	---	Yes	109

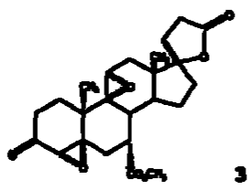
[1]Defined as the extrapolated desolvation temperature from the final solvent weight loss step as determined by thermogravimetric analysis at a heating rate of 10°C/minute under nitrogen purge. Desolvation temperatures, however, can be affected by the method of manufacture of the solvate. Different methods can produce different numbers of nucleation sites capable of initiating desolvation in the solvate at lower temperatures.

The unit cell of the solvate is composed of four eplerenone molecules. The stoichiometry of the eplerenone molecules and solvent molecules in the unit cell is also reported in Table 4 above for a number of solvates. The unit cell of Form I is composed of four eplerenone molecules. The unit cell of Form II is composed of two eplerenone molecules. The solvate unit cells are converted during desolvation into

Form I and/or Form II unit cells when the eplerenone molecules undergo translation and rotation to fill the spaces left by the solvent molecules. Table 4 also reports the desolvation temperatures for a number of different solvates.

9. Crystal Properties of Impurity Molecules

Selected impurities in eplerenone can induce the formation of Form I during the desolvation of the solvate. In particular, the effect of the following two impurity molecules was evaluated: 7-methyl hydrogen 4α,5α:9α,11α-diepoxy-17-hydroxy-3-oxo-17α-pregnane-7α,21-dicarboxylate, γ-lactone 3 (the "diepoxide"); and 7-methyl hydrogen 11α,12α-epoxy-17-hydroxy-3-oxo-17α-pregn-4-ene-7α,21-dicarboxylate, γ-lactone 4 (the "11,12-epoxide").



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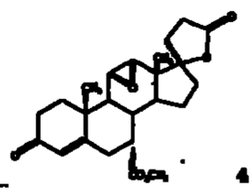

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The effect of these impurity molecules on the eplerenone crystalline form resulting from desolvation is described in greater detail in the examples of this application.

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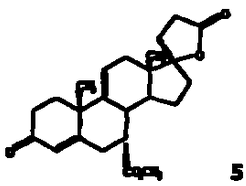
Given the similarity in single crystal structure of 7-methyl hydrogen 17-hydroxy-3-oxo-17α-pregna-4,9(11)-diene-7α,21-dicarboxylate, γ-lactone 5 (the "9,11-olefin") and Form I, it is hypothesized that the 9,11-olefin also can induce the formation of Form I during the desolvation of the solvate.



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A single crystal form was isolated for each impurity compound. Representative X-ray powder diffraction patterns for the crystal forms isolated for the diepoxide, 11,12-epoxide and 9,11-olefin are given in Figs. 9, 10 and 11, respectively. The X-ray powder diffraction pattern of each impurity molecule is similar to the X-ray powder diffraction pattern of Form I, suggesting that Form I and the three impurity compounds have similar single crystal structures.

Single crystals of each impurity compound also were isolated and subjected to X-ray structure determination to verify that these three compounds adopt single crystal structures similar to that of Form I. Single crystals of the diepoxide were isolated from methyl ethyl ketone. Single crystals of the 11,12-epoxide were isolated from isopropanol. Single crystals of the 9,11-olefin were isolated from n-butanol. Crystal structure data determined for the crystalline form of each impurity compound are given in Table 5. The resulting crystal system and cell parameters were substantially the same for the Form I, diepoxide, 11,12-epoxide, and 9,11-olefin crystalline forms.

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TABLE 5

Parameter	Form I	Diepoxide	11,12 Epoxide	9,11 olefin
Crystal system	Ortho-rhombic	Ortho-rhombic	Ortho-rhombic	Ortho-rhombic
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
a	21.22 Å	21.328 Å	20.90 Å	20.90 Å
b	15.40 Å	16.16 Å	15.55 Å	15.74 Å
c	6.34 Å	6.15 Å	6.38 Å	6.29 Å
α	90°	90°	90°	90°
β	90°	90°	90°	90°
γ	90°	90°	90°	90°
Z	4	4	4	4
Volume (Å)	2071.3	2119.0	2073.2	2069.3
ρ (calculated)	1.329 g/cm³	1.349 g/cm³	1.328 g/cm³	1.279 g/cm³
R	0.0667	0.0762	0.0865	0.0764

The four compounds reported in Table 5 crystallize into the same space group and have similar cell parameters (i.e., they are isostructural). It is hypothesized that the diepoxide, 11,12-epoxide and 9,11-olefin adopt a Form I conformation. The relative ease of isolation of a Form I packing (directly from solution) for each impurity compound, indicates that the Form I lattice is a stable packing mode for this series of structurally similar compounds.

Preparation of Eplerenone

The eplerenone starting material used to prepare the novel crystalline forms of the present invention can be prepared using the methods set forth in Ng et al., WO97/21720; and Ng et al., WO98/25948, particularly scheme 1 set forth in WO97/21720 and WO98/25948.

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Preparation of Crystalline Forms

1. Preparation of Solvated Crystalline Form

The solvated crystalline forms of eplerenone can be prepared by crystallization of eplerenone from a suitable solvent or a mixture of suitable solvents. A suitable solvent or mixture of suitable solvents generally comprises an organic solvent or a mixture of organic solvents that solubilizes the eplerenone together with any impurities at an elevated temperature, but upon cooling, preferentially crystallizes the solvate. The solubility of eplerenone in such solvents or mixtures of solvents generally is about 5 to about 200 mg/mL at room temperature. The solvent or mixtures of solvents preferably are selected from those solvents previously used in the process to prepare the eplerenone starting material, particularly those solvents that would be pharmaceutically acceptable if contained in the final pharmaceutical composition comprising the eplerenone crystalline form. For example, a solvent system comprising methylene chloride that yields a solvate comprising methylene chloride generally is not desirable.

Each solvent used preferably is a pharmaceutically acceptable solvent, particularly a Class 2 or Class 3 solvent as defined in "Impurities: Guideline For Residual Solvents", International Conference On Harmonisation Of Technical Requirements For Registration Of Pharmaceuticals For Human Use (Recommended for Adoption at Step 4 of the ICH Process on July 17, 1997 by the ICH Steering Committee). Still more preferably, the solvent or mixture of solvents is selected from the group consisting of methyl ethyl ketone, 1-propanol,

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2-pentanone, acetic acid, acetone, butyl acetate, chloroform, ethanol, isobutanol, isobutyl acetate, methyl acetate, ethyl propionate, n-butanol, n-octanol, isopropanol, propyl acetate, propylene glycol, t-butanol, tetrahydrofuran, toluene, methanol and t-butyl acetate. Still more preferably, the solvent is selected from the group consisting of methyl ethyl ketone and ethanol.

In another embodiment of this process, the solvent or mixture of solvents is selected from the group consisting of 1-propanol, 2-pentanone, acetic acid, acetone, butyl acetate, chloroform, isobutanol, isobutyl acetate, methyl acetate, ethyl propionate, n-butanol, n-octanol, propyl acetate, propylene glycol, t-butanol, tetrahydrofuran, toluene, methanol and t-butyl acetate.

In another embodiment of this process, the solvent or mixture of solvents is selected from the group consisting of 1-propanol, 2-pentanone, acetic acid, acetone, butyl acetate, chloroform, isobutanol, isobutyl acetate, methyl acetate, ethyl propionate, n-butanol, n-octanol, n-propanol, propyl acetate, propylene glycol, t-butanol, tetrahydrofuran, toluene, methanol and t-butyl acetate.

To prepare the solvated crystalline form of eplerenone, an amount of the eplerenone starting material is solubilized in a volume of the solvent and cooled until crystals form. The solvent temperature at which the eplerenone is added to the solvent generally will be selected based upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, this solvent temperature typically is at least about 25°C, preferably from about 30°C to the boiling point of the solvent, and more preferably from about 25°C below the

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boiling point of the solvent to the boiling point of the solvent.

Alternatively, hot solvent may be added to the eplerenone and the mixture can be cooled until crystals form. The solvent temperature at the time it is added to the eplerenone generally will be selected based upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, the solvent temperature typically is at least 25°C, preferably from about 50°C to the boiling point of the solvent, and more preferably from about 15°C below the boiling point of the solvent to the boiling point of the solvent.

The amount of the eplerenone starting material mixed with a given volume of solvent likewise will depend upon the solubility curve of the solvent or mixture of solvents. Typically, the amount of eplerenone added to the solvent will not completely solubilize in that volume of solvent at room temperature. For most of the solvents described herein, for example, the amount of eplerenone starting material mixed with a given volume of solvent usually is at least about 1.5 to about 4.0 times, preferably about 2.0 to about 3.5 times, and more preferably about 2.5 times, the amount of eplerenone that will solubilize in that volume of solvent at room temperature.

After the eplerenone starting material has completely solubilized in the solvent, the solution typically is cooled slowly to crystallize the solvated crystalline form of eplerenone. For most of the solvents described herein, for example, the solution is cooled at a rate slower than about 20°C/minute, preferably at a rate of about 10°C/minute or slower, more preferably at a rate of about 5°C/minute or

slower, and still more preferably at a rate of about 1°C/minute or slower.

The endpoint temperature at which the solvated crystalline form is harvested will depend upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, the endpoint temperature typically is less than about 25°C, preferably less than about 5°C, and more preferably less than about -5°C. Decreasing the endpoint temperature generally favors the formation of the solvated crystalline form.

Alternatively, other techniques may be used to prepare the solvate. Examples of such techniques include, but are not limited to, (i) dissolving the eplerenone starting material in one solvent and adding a co-solvent to aid in the crystallization of the solvate crystalline form, (ii) vapor diffusion growth of the solvate, (iii) isolation of the solvate by evaporation, such as rotary evaporation, and (iv) slurry conversion.

The crystals of the solvated crystalline form prepared as described above can be separated from the solvent by any suitable conventional means such as by filtration or centrifugation. Increased agitation of the solvent system during crystallization generally results in smaller crystal particle sizes.

2. Preparation of Form II From Solvate

Form II eplerenone can be prepared directly from the solvated crystalline form by desolvation. Desolvation can be accomplished by any suitable desolvation means such as, but not limited to, heating the solvate, reducing the ambient pressure surrounding the solvate, or combinations thereof.

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If the solvate is heated to remove the solvent, such as in an oven, the temperature of the solvate during this process typically does not exceed the enantiotropic transition temperature for Form I and Form II. This temperature preferably does not exceed about 150°C.

The desolvation pressure and time of desolvation are not narrowly critical. The desolvation pressure preferably is about one atmosphere or less. As the desolvation pressure is reduced, however, the temperature at which the desolvation can be carried out and/or the time of desolvation likewise is reduced. Particularly for solvates having higher desolvation temperatures, drying under vacuum will permit the use of lower drying temperatures. The time of desolvation need only be sufficient to allow for the desolvation, and thus the formation of Form II, to reach completion.

To ensure the preparation of a product that comprises substantially all Form II, the eplerenone starting material typically is a high purity eplerenone, preferably substantially pure eplerenone. The eplerenone starting material used to prepare Form II eplerenone generally is at least 90% pure, preferably at least 95% pure, and more preferably at least 99% pure. As discussed in greater detail elsewhere in this application, certain impurities in the eplerenone starting material can adversely affect the yield and Form II content of the product obtained from the process.

The crystallized eplerenone product prepared in this manner from a high purity eplerenone starting material generally comprises at least 10% Form II, preferably at least 50% Form II, more preferably at least 75% Form II, still more preferably at least 90% Form II, still more preferably at

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least about 95% Form II, and still more preferably substantially phase pure Form II.

3. Preparation of Form I From Solvate

A product comprising Form I can be prepared in substantially the same manner as set forth above for the preparation of Form II by (i) using a low purity eplerenone starting material instead of a high purity eplerenone starting material, (ii) seeding the solvent system with phase pure Form I crystals, or (iii) a combination of (i) and (ii).

A. Use Of Impurities As Growth Promoters and Inhibitors

The presence and amount of selected impurities in the eplerenone starting material, rather than the total amount of all impurities in the eplerenone starting material, affect the potential for Form I crystal formation during the desolvation of the solvate. The selected impurity generally is a Form I growth promoter or Form II growth inhibitor. It may be contained in the eplerenone starting material, contained in the solvent or mixture of solvents before the eplerenone starting material is added, and/or added to the solvent or mixture of solvents after the eplerenone starting material is added. Bonafede et al., "Selective Nucleation and Growth of an Organic Polymorph by Ledge-Directed Epitaxy on a Molecular Crystal Substate", J. Amer. Chem. Soc., Vol. 117, No. 30 (August 2, 1995) discusses the use of growth promoters and growth inhibitors in polymorph systems and is incorporated by reference herein. For the present invention, the impurity generally comprises a compound having a single crystal structure substantially identical to the single crystal structure of Form I. The impurity preferably is a compound

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having an X-ray powder diffraction pattern substantially identical to the X-ray powder diffraction pattern of Form I, and more preferably is selected from the group consisting of the diepoxide, the 11,12-epoxide, the 9,11-olefin and combinations thereof.

The amount of impurity needed to prepare Form I crystals typically can depend, in part, upon the solvent or mixture of solvents and the solubility of the impurity relative to eplerenone. In the crystallization of Form I from a methyl ethyl ketone solvent, for example, the weight ratio of diepoxide to low purity eplerenone starting material typically is at least about 1:100, preferably at least about 3:100, more preferably between about 3:100 and about 1:5, and still more preferably between about 3:100 and about 1:10. The 11,12-epoxide has a higher solubility in methyl ethyl ketone than the diepoxide and generally requires a larger amount of the 11,12-epoxide generally is necessary to prepare Form I crystals. Where the impurity comprises the 11,12-epoxide, the weight ratio of the diepoxide to the low purity eplerenone starting material typically is at least about 1:5, more preferably at least about 3:25, and still more preferably between about 3:25 and about 1:5. Where both the diexpoxide and the 11,12-epoxide impurities are used in the preparation of the Form I crystals, the weight ratio of each impurity to the eplerenone starting material may be lower than the corresponding ratio when only that impurity is used in the preparation of the Form I crystals.

A mixture of Form I and Form II is generally obtained when a solvate comprising the selected impurity is desolvated. The weight fraction of Form I in the product resulting from the initial desolvation of the solvate

typically is less than about 50%. Further treatment of this product by crystallization or digestion, as discussed below, generally will increase the weight fraction of Form II in the product.

B. Seeding

Form I crystals also can be prepared by seeding the solvent system with phase pure Form I crystals (or a Form I growth promoter and/or Form II growth inhibitor as previously discussed above) prior to crystallization of the eplerenone. The eplerenone starting material can be either a low purity eplerenone or a high purity eplerenone. When the resulting solvate prepared from either starting material is desolvated, the weight fraction of Form I in the product typically is at least about 70% and may be as great as about 100%.

The weight ratio of Form I seed crystals added to the solvent system to the eplerenone starting material added to the solvent system generally is at least about 0.75:100, preferably between about 0.75:100 to about 1:20, and more preferably between about 1:100 to about 1:50. The Form I seed crystals can be prepared by any of the methods discussed in this application for the preparation of Form I crystals, particularly the preparation of Form I crystals by digestion as discussed below.

The Form I seed crystals may be added at one time, in multiple additions or substantially continually over a period of time. The addition of the Form I seed crystals, however, generally is completed before the eplerenone begins to crystallize from solution, i.e., the seeding is completed before the cloud point (the lower end of the metastable zone) is reached. Seeding typically is performed when the solution

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temperature ranges from about 0.5°C above the cloud point to about 10°C above the cloud point, preferably within about 2°C to about 3°C above the cloud point. As the temperature above the cloud point at which the seeds are added increases, the amount of seeding needed for crystallization of Form I crystals generally increases.

The seeding preferably occurs not only above the cloud point, but within the metastable zone. Both the cloud point and the metastable zone are dependent on the eplerenone solubility and concentration in the solvent or mixture of solvents. For a 12 volume dilution of methyl ethyl ketone, for example, the high end of the metastable zone generally is between about 70°C to about 73°C and the lower end of the metastable zone (i.e., the cloud point) is between about 57°C and 63°C. For a concentration of 8 volumes of methyl ethyl ketone, the metastable zone is even narrower because the solution is supersaturated. At this concentration, the cloud point of the solution occurs at about 75°C to about 76°C. Because the boiling point of methyl ethyl ketone is about 80° C under ambient conditions, seeding for this solution typically occurs between about 76.5°C and the boiling point.

An illustrative non-limiting example of seeding with Form I is set forth below in Example 7.

The crystallized eplerenone product obtained using a Form I growth promoter or Form II growth inhibitor, and/or Form I seeding generally comprises at least 2% Form I, preferably at least 5% Form I, more preferably at least 7% Form I, and still more preferably at least about 10% Form I.

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The remaining crystallized eplerenone product generally is Form II.

C. Form I Prepared By Grinding Eplerenone

In yet another alternative, it has been discovered that a small amount of Form I can be prepared by suitable grinding eplerenone. Concentrations of Form I in ground eplerenone as high as about 3% have been observed.

4. Preparation of Form II From Solvate Prepared From Low Purity Eplerenone

As discussed above, crystallization of low purity eplerenone to form a solvate followed by desolvation of the solvate generally yields a product comprising both Form I and Form II. A product having a greater Form II content can be prepared from low purity eplerenone in substantially the same manner as set forth above for the preparation of Form I by seeding the solvent system with phase pure Form II crystals, or by using a Form II growth promoter and/or Form I growth inhibitor. The seeding protocol and the weight ratio of the amount of Form II seed crystals added to the solvent system to the amount of the eplerenone starting material added to the solvent system generally are similar to those ratios previously discussed above for the preparation of Form I eplerenone by seeding with phase pure Form I crystals.

The crystallized eplerenone product prepared in this manner generally comprises at least 10% Form II, preferably at least 50% Form II, more preferably at least 75% Form II, more preferably at least 90% Form II, still more preferably at least about 95% Form II, and still more preferably substantially phase pure Form II.

The seeding protocols described in this section and in the prior section relating to the preparation of Form I eplerenone also may allow for improved control of the particle size of the crystallized eplerenone.

5. Crystallization of Form II Directly From Solution

Form II eplerenone also can be prepared by the direct crystallization of eplerenone from a suitable solvent or mixture of solvents without the formation of an intermediate solvate and the accompanying need for desolvation. Typically, (i) the solvent has a molecular size that is incompatible with the available channel space in the solvate crystal lattice, (ii) the eplerenone and any impurities are soluble in the solvent at elevated temperatures, and (iii) upon cooling, results in the crystallization of the non-solvated Form II eplerenone. The solubility of eplerenone in the solvent or mixture of solvents generally is about 5 to about 200 mg/mL at room temperature. The solvent or mixture of solvents preferably comprises one or more solvents selected from the group consisting of methanol, ethyl acetate, isopropyl acetate, acetonitrile, nitrobenzene, water and ethyl benzene.

To crystallize Form II eplerenone directly from solution, an amount of the eplerenone starting material is solubilized in a volume of the solvent and cooled until crystals form. The solvent temperature at which the eplerenone is added to the solvent generally will be selected based upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, this solvent temperature typically is at least about 25°C, preferably from about 30°C to the boiling point of the

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solvent, and more preferably from about 25°C below the boiling point of the solvent to the boiling point of the solvent.

Alternatively, hot solvent may be added to the eplerenone and the mixture can be cooled until crystals form. The solvent temperature at the time it is added to the eplerenone generally will be selected based upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, the solvent temperature typically is at least 25°C, preferably from about 50°C to the boiling point of the solvent, and more preferably from about 15°C below the boiling point of the solvent to the boiling point of the solvent.

The amount of the eplerenone starting material mixed with a given volume of solvent likewise will depend upon the solubility curve of the solvent or mixture of solvents. Typically, the amount of eplerenone added to the solvent will not completely solubilize in that volume of solvent at room temperature. For most of the solvents described herein, for example, the amount of eplerenone starting material mixed with a given volume of solvent usually is at least about 1.5 to about 4.0 times, preferably about 2.0 to about 3.5 times, and more preferably about 2.5 times, the amount of eplerenone that will solubilize in that volume of solvent at room temperature.

To ensure the preparation of a product that comprises substantially phase pure Form II, the eplerenone starting material generally is a high purity eplerenone. The eplerenone starting material preferably is at least 65% pure, more preferably at least 90% pure, still more preferably at least 98% pure, and still more preferably at least 99% pure.

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After the eplerenone starting material has completely solubilized in the solvent, the solution typically is cooled slowly to crystallize the solvated crystalline form of eplerenone. For most of the solvents described herein, for example, the solution is cooled at a rate slower than about 1.0°C/minute, preferably at a rate of about 0.2°C/minute or slower, and more preferably at a rate between about 5°C/minute and about 0.1°C/minute.

The endpoint temperature at which the Form II crystals are harvested will depend upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, the endpoint temperature typically is less than about 25°C, preferably less than about 5°C, and more preferably less than about -5°C.

Alternatively, other techniques may be used to prepare the Form II crystals. Examples of such techniques include, but are not limited to, (i) dissolving the eplerenone starting material in one solvent and adding a co-solvent to aid in the crystallization of Form II eplerenone, (ii) vapor diffusion growth of Form II eplerenone, (iii) isolation of Form II eplerenone by evaporation, such as rotary evaporation, and (iv) slurry conversion.

The crystals of the solvated crystalline form prepared as described above can be separated from the solvent by any suitable conventional means such as by filtration or centrifugation.

In addition, Form II eplerenone also can be prepared by digesting (as described below) a slurry of high purity eplerenone in methyl ethyl ketone and filtering the digested eplerenone at the boiling point of the slurry.

6. Preparation of Form I Directly From Solution

It is hypothesized that if the crystallization is performed above the enantiotropic transition temperature (T_t) for Form I and Form II, particularly if Form I growth promoters or Form II growth inhibitors are present or the solvent is seeded with phase pure Form I crystals, Form I should crystallize directly from solution since Form I is more stable at these higher temperatures. The solvent system used preferably comprises a high boiling solvent such as nitrobenzene. Suitable Form I growth promoters would include, but would not be limited to, the diepoxide and the 11,12-olefin.

7. Digestion of Eplerenone With A Solvent

The solvated crystalline forms, Form I and Form II of eplerenone also can be prepared by digestion of an eplerenone starting material in a suitable solvent or mixture of solvents. In the digestion process, a slurry of eplerenone is heated at the boiling point of the solvent or mixture of solvents. For example, an amount of eplerenone starting material is combined with a volume of solvent or mixture of solvents, heated to reflux, and the distillate is removed while an additional amount of the solvent is added simultaneously with the removal of the distillate. Alternatively, the distillate can be condensed and recycled without the addition of more solvent during the digestion process. Typically, once the original volume of solvent has been removed or condensed and recycled, the slurry is cooled and solvated crystals form. The solvated crystals can be separated from the solvent by any suitable conventional means such as by filtration or centrifugation. Desolvation of the

solvate as previously described yields either Form I or Form II eplerenone depending upon the presence or absence of the selected impurities in the solvated crystals.

A suitable solvent or mixture of solvents generally comprises one or more of the solvents previously disclosed herein. The solvent may be selected, for example, from the group consisting of methyl ethyl ketone and ethanol.

The amount of eplerenone starting material added to the solvent used in the digestion process generally is sufficient to maintain a slurry (i.e., the eplerenone in the solvent or mixture of solvents is not completely solubilized) at the boiling point of the solvent or mixture of solvents. Illustrative values include, but are not limited to, about one gram of eplerenone per four mL methyl ethyl ketone and about one gram of eplerenone per eight mL ethanol.

The solution generally is cooled slowly once solvent turnover is complete to crystallize the solvated crystalline form of eplerenone. For the solvents tested, for example, the solution is cooled at a rate slower than about 20°C/minute, preferably about 10°C/minute or slower, more preferably about 5°C/minute or slower, and still more preferably about 1°C/minute or slower.

The endpoint temperature at which the solvated crystalline form is harvested will depend upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, the endpoint temperature typically is less than about 25°C, preferably less than about 5°C, and more preferably less than about -5°C.

If a product comprising primarily or exclusively Form II is desired, a high purity eplerenone starting material

typically is digested. The high purity eplerenone starting material preferably is at least 98% pure, more preferably at least 99% pure, and still more preferably at least 99.5% pure. The digested eplerenone product prepared in this manner generally comprises at least 10% Form II, preferably at least 50% Form II, more preferably at least 75% Form II, more preferably at least 90% Form II, still more preferably at least about 95% Form II, and still more preferably substantially phase pure Form II.

If a product comprising primarily or exclusively Form I is desired, a low purity eplerenone starting material typically is digested. The low purity eplerenone starting material generally contains only as much Form I growth promoter and/or Form II growth inhibitor as is needed to yield Form I. Preferably, the low purity eplerenone starting material is at least 65% pure, more preferably at least 75% pure, and still more preferably at least 80% pure. The digested eplerenone product prepared in this manner generally comprises at least 10% Form I, preferably at least 50% Form I, more preferably at least 75% Form I, more preferably at least 90% Form I, still more preferably at least about 95% Form I, and still more preferably substantially phase pure Form I.

8. Preparation of Amorphous Eplerenone

Amorphous eplerenone can be prepared in small quantities by suitable comminution of solid eplerenone, such as by crushing, grinding and/or micronizing. Phase pure amorphous eplerenone can be prepared, for example, by lyophilizing a solution of eplerenone, particularly an aqueous solution of

eplerenone. These processes are illustrated in Examples 13 and 14 below.

Additional Processing Considerations

1. Thermodynamic Stability Considerations

Form II is more thermodynamically stable than Form I at ambient temperatures. As described in Example 5 below, when an organic slurry containing equivalent amounts of Form I and Form II was allowed to stand overnight at room temperature and the residual solids were then collected and analyzed by X-ray powder diffraction, the analytical results indicated that the eplerenone had completely converted to Form II. The differential scanning calorimetry ("DSC") data (discussed previously) indicate that Form I is more thermodynamically stable than Form II at higher temperatures since it has the higher melting/decomposition temperature. Taken together, the slurry conversion and DSC data indicate that Form I and Form II are related enantiotropically, i.e., a change in the stability relationship between the two polymorphs occurs around a transition temperature (T_t), with Form II being the more stable at lower temperatures. Fig. 12 shows the Gibbs free energy-temperature relationship generally observed for enantiotropically related polymorphs such as Form I and Form II, wherein I and II refer to the crystal forms, T_t refers to the transition temperature, T_m refers to the melting points of Form I and Form II, and L represents the liquid or melt state.

Accordingly, processing temperatures preferably are maintained below the transition temperature during the preparation of compositions comprising Form II. For example, drying temperatures employed for desolvation typically are

less than about 150°C, preferably less than about 125°C, more preferably less than about 115°C, more preferably less than about 110°C, and still more preferably between about 80°C to about 110°C. In addition, cooling (such as by use of liquid nitrogen) may be necessary during particle size reduction process steps to maintain the temperature of the Form II crystals below the transition temperature.

2. Intrinsic Micronizing Considerations

The method used to prepare crystalline eplerenone may affect the properties of the resulting crystal. For example, Form II prepared by desolvation of a solvate exhibits a higher incidence of surface defects, pores, cracks and fractures within the crystal lattice than Form II prepared by direct crystallization from solution. This "intrinsic micronizing" of the desolvated crystal results in an increase in both the available surface area of the crystal and the dissolution rate of the crystal. Dissolution times, therefore, can be shortened by selection of Form II crystals prepared by desolvation, lengthened by selection of Form II crystals prepared by direct crystallization, or otherwise adjusted by selection of an appropriate combination of Form II crystals prepared by desolvation and Form II crystals prepared by direct crystallization.

This "intrinsic micronizing" also may effectively reduce or eliminate the need for crystal particle size reduction during processing steps where Form II crystals prepared by desolvation are used in the preparation of the pharmaceutical composition. One disadvantage to using such Form II crystals, however, is the need for a desolvation step that is

not required for Form II crystals prepared by direct crystallization.

Product-By-Process Solid State Forms

The present invention also comprises each of the solid state eplerenone forms prepared in accordance with the processes disclosed in this application. In particular, the invention comprises solvated eplerenone, Form I eplerenone, Form II eplerenone, and combinations thereof, prepared as set forth in this application.

Combinations of Solid State Forms

Also embraced within this invention are combinations comprising a first solid state form of eplerenone and a second solid state form of eplerenone, wherein the first and second solid state forms of eplerenone are selected from Form I, Form II, solvated eplerenone and amorphous eplerenone. In general, the weight ratio of said first solid state form to said second solid state form preferably is at least about 1:9, more preferably is at least about 1:1, more preferably at least about 2:1, more preferably at least about 5:1, and still more preferably at least about 9:1.

In one embodiment, for example, the first solid state form is Form I and the second solid state form is Form II.

In another embodiment, for example, the first solid state form is Form II and the second solid state form is Form I.

Pharmaceutical Compositions

Also embraced within this invention is a class of pharmaceutical compositions comprising a first solid state . . .

form of eplerenone and a second solid state form of eplerenone in association with one or more pharmaceutically-acceptable carriers and/or diluents and/or adjuvants (collectively referred to herein as "carrier" materials) and, if desired, other active ingredients. The first solid state form of eplerenone and the second solid state form of eplerenone are selected from Form I, Form II, solvated eplerenone and amorphous eplerenone. In general, the weight ratio of said first solid state form to said second solid state form preferably is at least about 1:9, more preferably is at least about 1:1, more preferably at least about 2:1, more preferably at least about 5:1, and still more preferably at least about 9:1.

In one embodiment of the invention, the entire amount of eplerenone contained in the composition is present as phase pure Form II.

In another embodiment of the invention, the entire amount of eplerenone contained in the composition is present as phase pure Form I.

In still another embodiment of the invention, the entire amount of eplerenone contained in the composition is present as phase pure solvated crystalline eplerenone.

In still another embodiment of the invention, the entire amount of eplerenone contained in the composition is present as amorphous eplerenone.

In still another embodiment of the invention, the composition comprises both Form I and Form II. The ratio of the amount of Form II to Form I in the composition generally is between about 1:20 to about 20:1. In other embodiments, for example, this ratio is between about 10:1 to about 1:10;

about 5:1 to about 1:5; about 2:1 to about 1:2; or is about 1:1.

In still another embodiment of the invention, the composition comprises a first solid state form of eplerenone and a second solid state form of eplerenone, wherein the first and second solid state forms of eplerenone are selected from Form I, Form II, solvated eplerenone and amorphous eplerenone. In general, the weight ratio of said first solid state form to said second solid state form preferably is at least about 1:9, preferably about 1:1, more preferably at least about 2:1, more preferably at least about 5:1, and still more preferably at least about 9:1.

In each of the above combination and pharmaceutical composition embodiments, the D_{90} particle size of the eplerenone used as a starting material in the pharmaceutical composition generally is between about 10 nm to about 15 microns, preferably less than about 1 micron, more preferably less than about 800 nm, still more preferably less than about 600 nm, and still more preferably less than about 400 nm. In one embodiment, the D_{90} particle size is between about 10 nm to about 1 micron. In another embodiment, the D_{90} particle size is between about 100 nm to about 800 nm. In another embodiment, the D_{90} particle size is between about 200 nm to about 600 nm. In another embodiment, the D_{90} particle size is between about 400 nm to about 800 nm.

These solid state forms of eplerenone of the present invention may be administered by any suitable route, preferably in the form of a pharmaceutical composition adapted to such a route, and in a dose effective for the treatment intended. The solid state forms of eplerenone and compositions thereof may, for example, be administered

orally, intravascularly, intraperitoneally, subcutaneously, intramuscularly or topically.

The amount of eplerenone that is administered and the dosage regimen for treating a condition or disorder with the solid state forms of eplerenone and/or compositions of this invention depends on a variety of factors, including the age, weight, sex and medical condition of the subject, the severity of the disease, the route and frequency of administration, and the particular compound employed, and thus may vary widely. The pharmaceutical compositions may contain eplerenone in the range of about 0.1 to 2000 mg, preferably in the range of about 0.5 to 500 mg and most preferably between about 1 and 200 mg. A daily dose of about 0.01 to 100 mg/kg body weight, preferably between about 0.5 and about 20 mg/kg body weight and most preferably between about 0.1 to 10 mg/kg body weight, may be appropriate. The daily dose can be administered in one to four doses per day.

For oral administration, the pharmaceutical composition may be in the form of, for example, a tablet, capsule, or suspension. The pharmaceutical composition is preferably made in the form of a dosage unit containing a particular amount of eplerenone. Examples of such dosage units are tablets or capsules. The eplerenone may also be administered by injection of a suspension wherein, for example, saline, dextrose or water may be used as a suitable carrier.

Other formulations may be in the form of a topical ointment or cream, or a suppository, containing eplerenone in a total amount of, for example, 0.075 to 30% w/w, preferably 0.2 to 20% w/w and most preferably 0.4 to 15% w/w. When formulated in an ointment, the eplerenone may be employed with either paraffinic or a water-miscible ointment base.

Alternatively, the eplerenone may be formulated in a cream with an oil-in-water cream base. If desired, the aqueous phase of the cream base may include, for example at least 30% w/w of a polyhydric alcohol such as propylene glycol, butane-1,3-diol, mannitol, sorbitol, glycerol, polyethylene glycol and mixtures thereof. The topical formulation may desirably include a compound which enhances absorption or penetration of the eplerenone through the skin or other affected areas. Examples of such dermal penetration enhancers include dimethylsulfoxide and related analogs.

The novel solid state forms of eplerenone also can be administered by a transdermal device. Preferably, topical administration will be accomplished using a patch either of the reservoir and porous membrane type or of a solid matrix variety. In either case, the eplerenone is delivered continuously from the reservoir or microcapsules through a membrane into the eplerenone permeable adhesive, which is in contact with the skin or mucosa of the recipient. If the eplerenone is absorbed through the skin, a controlled and predetermined flow of the eplerenone is administered to the recipient. In the case of microcapsules, the encapsulating agent may also function as the membrane.

The oily phase of the emulsions of this invention may be constituted from known ingredients in a known manner. While the phase may comprise merely an emulsifier, it may comprise a mixture of at least one emulsifier with a fat or an oil or with both a fat and an oil. Preferably, a hydrophilic emulsifier is included together with a lipophilic emulsifier which acts as a stabilizer. It is also preferred to include both an oil and a fat. Together, the emulsifier(s) with or without stabilizer(s) make-up the so-called emulsifying wax,

and the wax together with the oil and fat make up the so-called emulsifying ointment base which forms the oily dispersed phase of the cream formulations. Emulsifiers and emulsion stabilizers suitable for use in the formulation of the present invention include Tween 60, Span 80, cetostearyl alcohol, myristyl alcohol, glyceryl monostearate, and sodium lauryl sulfate, among others.

The choice of suitable oils or fats for the formulation is based on achieving the desired cosmetic properties, since the solubility of the eplerenone in most oils likely to be used in pharmaceutical emulsion formulations is very low. Thus, the cream should preferably be a non-greasy, non-staining and washable product with suitable consistency to avoid leakage from tubes or other containers. Straight or branched chain, mono- or dibasic alkyl esters such as di-isoadipate, isocetyl stearate, propylene glycol diester of coconut fatty acids, isopropyl myristate, decyl oleate, isopropyl palmitate, butyl stearate, 2-ethylhexyl palmitate or a blend of branched chain esters may be used. These may be used alone or in combination depending on the properties required. Alternatively, high melting point lipids such as white soft paraffin and/or liquid paraffin or other mineral oils can be used.

Formulations suitable for topical administration to the eye also include eye drops wherein the eplerenone is suspended in suitable carrier, especially an aqueous carrier for the eplerenone. The eplerenone is preferably present in such formulations in a concentration of 0.5 to 20%, advantageously 0.5 to 10% and particularly about 1.5% w/w.

For therapeutic purposes, the novel solid state forms of eplerenone ordinarily are combined with one or more adjuvants

appropriate to the indicated route of administration. If administered per os, the compound may be admixed with lactose, sucrose, starch powder, cellulose esters of alkanoic acids, cellulose alkyl esters, talc, stearic acid, magnesium stearate, magnesium oxide, sodium and calcium salts of phosphoric and sulfuric acids, gelatin, acacia gum, sodium alginate, polyvinylpyrrolidone, and/or polyvinyl alcohol, and then tableted or encapsulated for convenient administration. Such capsules or tablets may contain an immediate release formulation, or may contain a controlled-release formulation such as a dispersion of the eplerenone in hydroxypropylmethyl cellulose. Formulations for parenteral administration may be in the form of aqueous or non-aqueous isotonic sterile injection suspensions. These suspensions may be prepared from sterile powders or granules having one or more of the carriers or diluents mentioned for use in the formulations for oral administration. Other adjuvants and modes of administration are well and widely known in the pharmaceutical art.

Methods of Treatment or Prophylaxis

The present invention also comprises methods for the treatment and/or prophylaxis of an aldosterone-mediated condition and/or disorder such as hypertension in a subject, the methods comprising treating the subject having or susceptible to such condition or disorder with a therapeutically-effective amount of Form I, Form II, solvated crystalline form, or an amorphous form of eplerenone, or combinations thereof. These solid state forms of eplerenone would be useful for the treatment and/or prophylaxis of a condition and/or disorder in a subject where administration

of an aldosterone-antagonist is indicated including, but not limited to, the treatment of hyperaldosteronism such as hypertension, heart failure including cardiac insufficiency, cirrhosis of the liver, excess collagen/fibrosis, benign prostate hypertrophy and depression.

Besides being useful for human treatment, these solid state forms of eplerenone are also useful for veterinary treatment of companion animals, exotic animals and farm animals, including mammals, rodents, and the like. More preferred animals include horses, dogs, and cats.

These solid state forms of eplerenone also may be used (i) in combination therapies partially or completely in place of other conventional aldosterone receptor antagonists, or (ii) in combination therapies with other active drugs. The phrase "combination therapy" is intended to embrace the administration of each agent in a sequential manner in a regimen that will provide beneficial effects of the drug combination, and is intended as well to embrace co-administration of these agents in a substantially simultaneous manner, such as in a single capsule or injection having a fixed ratio of these active agents or in multiple, separate capsules or injections for each agent. Non-limiting examples of combination therapy include the treatment of cardiovascular diseases using a combination of an aldosterone receptor antagonist and an angiotensin II receptor antagonist as described in WO96/24373; the treatment of congestive heart failure using a combination of an aldosterone receptor antagonist and an angiotensin II antagonist as described in WO96/40257; and the treatment of heart failure using a combination of an aldosterone receptor antagonist, an ACE inhibitor and a diuretic as described in WO96/24372.

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5/4.

Working Examples

The following examples contain detailed descriptions of the methods of preparation of the various solid state forms of eplerenone described in this application. These detailed descriptions fall within the scope, and serve to exemplify the invention. These detailed descriptions are presented for illustrative purposes only and are not intended as a restriction on the scope of the invention. All parts are by weight and temperatures are in degrees Centigrade unless otherwise indicated. The eplerenone starting material used in each of the following examples was prepared in accordance with scheme 1 set forth in Ng et al., WO98/25948.

Example 1: Preparation of (a) methyl ethyl ketone solvate from high purity eplerenone starting material and (b) Form II crystalline eplerenone from resulting solvate

A. Preparation of Methyl Ethyl Ketone Solvate:

High purity eplerenone (437 mg; greater than 99% purity with less than 0.2% diepoxide and 11,12 epoxide present) was dissolved in 10 mL of methyl ethyl ketone by heating to boiling on a hot plate with magnetic stirring at 900 rpm. The resulting solution was allowed to cool to room temperature with continuous magnetic stirring. Once at room temperature, the solution was transferred to a 1°C bath with maintenance of the stirring for one hour. After one hour, the solid methyl ethyl ketone solvate was collected by vacuum filtration.

B. Preparation of Form II crystalline eplerenone:

The solid methyl ethyl ketone solvate prepared in Step A above was dried in an oven at 100°C for four hours at ambient

pressure. The dried solid was determined to be pure Form II by DSC and XPRD analysis.

Example 2: Preparation of additional solvates from high purity eplerenone starting material

Additional solvated crystalline forms were prepared by replacing methyl ethyl ketone with one of the following solvents: n-propanol, 2-pentanone, acetic acid, acetone, butyl acetate, chloroform, ethanol, isobutanol, isobutyl acetate, isopropanol, methyl acetate, ethyl propionate, n-butanol, n-octanol, propyl acetate, propylene glycol, t-butanol, tetrahydrofuran, and toluene and carrying out the crystallization substantially as described above in Step A of Example 1.

Example 3: Preparation of Methyl Ethyl Ketone Solvate by Vapor Diffusion Growth

Eplerenone (400 mg; greater than 99.9% purity) was dissolved in 20 mL of methyl ethyl ketone by warming on a hot plate to form a stock solution. An 8 mL amount of the stock solution was transferred to a first 20 mL scintillation vial and diluted to 10 mL with methyl ethyl ketone (80%). A 10 mL amount of the stock solution was transferred to a second 20 mL scintillation vial and diluted to 10 mL with methyl ethyl ketone (40%). The final 2 mL of the stock solution was diluted to 10 mL with methyl ethyl ketone (20%). The four vials containing the dilutions were transferred to a dessicator jar containing a small amount of hexane as an anti-solvent. The dessicator jar was sealed and the hexane vapor allowed to diffuse into the methyl ethyl ketone

solutions. Methyl ethyl ketone solvate crystals grew in the 80% dilution sample by the next day.

Example 4: Preparation of Methyl Ethyl Ketone Solvate by Rotary Evaporation

About 400 mg of eplerenone (greater than 99.9% purity) is weighed into a 250 mL round bottom flask. Solvent (150 mL) is added to the flask and, if necessary, the solution is heated gently until the solid is dissolved. The resulting clear solution is placed on a Buchi rotary evaporator with a bath temperature of about 85°C and the solvent is removed under vacuum. Solvent removal is stopped when approximately 10 mL of solvent remain in the round bottom flask. The resulting solids are analyzed by appropriate method (XPRD, DSC, TGA, microscopy, etc.) for determination of form.

Example 5: Slurry Conversion

Approximately 150 mg of Form II eplerenone and 150 mg of Form I eplerenone were added to 5 mL of ethyl acetate. The resulting slurry was allowed to stir at 300 rpm (magnetic stirring) overnight. The next day a sample of the solid was collected by filtration. Analysis of the sample by XPRD indicated that the sample was entirely composed of Form II eplerenone.

Example 6: Preparation of (a) solvate from low purity eplerenone starting material and (b) Form I crystalline eplerenone from resulting solvate

Samples containing varying amounts of the impurity 7-methyl hydrogen 4α,5α:9α,11α-diepoxy-17-hydroxy-3-oxo-17α-pregnane-7α,21-dicarboxylate, γ-lactone (the "diepoxide") or

the impurity 7-methyl hydrogen 11α,12α-epoxy-17-hydroxy-3-oxo-17α-pregn-4-ene-7α,21-dicarboxylate, γ-lactone (the "11,12-epoxide") were prepared by adding the desired amount of the impurity to a 7 mL scintillation vial together with an amount of eplerenone sufficient to provide a total sample mass of 100 mg. The weight percent of the diepoxide or 11,12-epoxide in each sample is given in Tables X-6A and X-6B, respectively. A micro-flea magnetic stirrer was added to each scintillation vial along with 1 mL of methyl ethyl ketone. The vials were loosely capped and the solid dissolved by heating to reflux on a hot plate with magnetic stirring. Once the solids were dissolved, the solutions were allowed to cool to room temperature on the hot plate. Magnetic stirring was maintained during the cooling period. After the solutions reached room temperature, the solids were collected by vacuum filtration and immediately analyzed by X-ray powder diffraction (XPRD). The solids were then placed in a 100°C oven and dried for one hour at ambient pressure. The dried solids were analyzed by XPRD for Form I content by monitoring the area of the Form I diffraction peak at about 12.1 degrees two theta. All XPRD diffraction patterns were recorded using an Inel Multipurpose Diffractometer.

TABLE X-6A

Weight Percent Diepoxide	Weight Eplerenone (mg)	Weight Diepoxide (mg)
0%	100.44	--
1%	99.08	1.24
2%	98.09	2.24
3%	97.08	3.04
5%	95.09	5.04

TABLE X-6B

Weight Percent 11,12-Epoxide	Weight Eplerenone (mg)	Weight 11,12-Epoxide (mg)
0%	101.38	0
1%	99.23	1.10
5%	94.97	5.36
10%	90.13	10.86

A. Diepoxide Results

Fig. 13 shows the X-ray powder diffraction patterns for the wet cake (methyl ethyl ketone solvate) obtained from the (a) 0%, (b) 1%, (c) 3%, and (d) 5% diepoxide-doped methyl

ethyl ketone crystallizations. The peak intensities have been normalized for ease of comparison. No peaks characteristic of Form I or the diepoxide are present in the diffraction patterns. The patterns are characteristic of the methyl ethyl ketone solvate of eplerenone.

Fig. 14 shows the X-ray powder diffraction patterns for the dried solids obtained from the (a) 0%, (b) 1%, (c) 3%, and (d) 5% diepoxide-doped methyl ethyl ketone crystallizations. The peak intensities have been normalized for ease of comparison. No Form I was detected for the dried samples corresponding to the methyl ethyl ketone crystallizations performed at doping levels of 0 and 1%. Form I was detected in the dried samples corresponding to the methyl ethyl ketone crystallizations performed at doping levels of 3 and 5%. The area for the Form I diffraction peak at about 12.1 degrees two theta and the estimated Form I content for each sample are given in Table X-6C below.

TABLE X-6C

Weight Percent of Diepoxide in Starting Eplerenone Mixture	Weight Percent of Diepoxide in Resulting Crystals (HPLC)	Form I Peak Area 12° Two Theta Peak	Estimated Weight Percent of Form I
0%	---	None Detected	None Detected
1%	0.29%	None Detected	None Detected
3%	0.58%	1168	10%
5%	1.05%	4175	30%

The results reported in Table X-6C confirm that the presence of the diepoxide affects the formation of Form I during the desolvation. These results indicate that the

diepoxide is effective in inducing the formation of Form I eplerenone when it is incorporated into and/or adsorbed onto the methyl ethyl ketone solvate crystals.

The 3% diepoxide doping experiment was repeated to analyze the impact of the route of preparation on the amount of Form I formed during the desolvation. In this experiment, the methyl ethyl ketone solvate obtained from the doped crystallization was divided into two portions. The first portion was left untreated while the second portion was lightly ground in a mortar and pestle to induce a higher level of crystal defects. The two portions were both dried at 100 °C for one hour at ambient pressure. The dried solids were analyzed by XPRD. The XPRD patterns are given in Fig. 15 for the dried solids from the methyl ethyl ketone crystallization with 3% doping of diepoxide (a) without grinding of the solvate prior to drying, and (b) with grinding of the solvate prior to drying. The XPRD patterns indicated a greater amount of Form I in the ground sample relative to the unground sample. These results suggest that the conditions under which the methyl ethyl ketone solvate is isolated and handled can affect the crystal form that results from the desolvation.

B. 11,12-Epoxide Results

Fig. 16 shows the X-ray powder diffraction patterns for the wet cake (methyl ethyl ketone solvate) obtained from the (a) 0%, (b) 1%, (c) 5%, and (d) 10% 11,12-epoxide-doped methyl ethyl ketone crystallizations. The peak intensities have been normalized for ease of comparison. No peaks characteristic of Form I or the 11,12-epoxide are present in the diffraction

patterns. The patterns are characteristic of the methyl ethyl ketone solvate of eplerenone.

Fig. 17 shows the X-ray powder diffraction patterns for the dried solids obtained from the (a) 0%, (b) 1%, (c) 5%, and (d) 10% 11,12-epoxide-doped methyl ethyl ketone crystallizations. The peak intensities have been normalized for ease of comparison. No Form I was detected for the dried samples corresponding to the methyl ethyl ketone crystallizations performed at doping levels of 0., 1% and 5%. Form I was detected in the dried samples corresponding to the methyl ethyl ketone crystallization performed at a doping level of 10%. The area for the Form I diffraction peak at 12.1 degrees two theta and estimated Form I content for each sample are given in Table X-6D.

TABLE X-6D

Weight Percent 11,12-Epoxide in Starting Eplerenone Mixture	Weight Percent 11,12-Epoxide in Resulting Crystals (HPLC)	Form I Peak Area 12° Two Theta Peak	Estimated Weight Percent of Form I
0%	Not Available	None Detected	None Detected
1%	Not Available	None Detected	None Detected
5%	Not Available	None Detected	None Detected
10%	Not Available	1541	10-15%

The results reported in Table X-6D confirm that the presence of the 11,12-epoxide impacts the formation of Form I during the desolvation. The percentage of impurity in the methyl ethyl ketone crystallization required to induce the formation of Form I eplerenone appears to be greater for the 11,12-epoxide than for the diepoxide.

Example 7: Effect of Crystallization and Drying on Final Crystal Form

The following four experiments analyzing the effect of crystallization and drying on the final crystal form were conducted: (i) methyl ethyl ketone crystallization of eplerenone (2^3+3 statistical design of experiment), (ii) crystallization of poor quality mother liquor residue, (iii) crystallization of high purity eplerenone with form I seeding, and (iv) crystallization of low purity eplerenone with form II seeding. Variables in the design of the experiments included cooling rate, starting material purity level, and end point temperature of crystallization. For purposes of this Example, high purity eplerenone was defined as ultra-pure milled eplerenone (HPLC analysis showed this material to be 100.8% pure) and low purity eplerenone was defined as 89% pure eplerenone. To prepare the low purity eplerenone, stripped-down mother liquors from the process for the preparation of eplerenone were analyzed and blended to yield a material that was 61.1% eplerenone, 12.8% diepoxide and 7.6% 11,12-epoxide. This material was then blended with a sufficient amount of high purity eplerenone to yield the 89% eplerenone.

A. Methyl Ethyl Ketone Crystallization

In the methyl ethyl ketone crystallization experiment, all runs were performed using 60 g of high purity eplerenone. High endpoint was defined as 45°C and low endpoint was defined as 5°C. High cooling rate was defined as 3°C/minute cooling and low cooling rate was defined as 0.1°C/minute cooling. Center points were 1.5°C/minute cooling, 94.5% pure eplerenone, and a 25°C endpoint.

After a background reading was taken with the FTIR, 250 mL of methyl ethyl ketone was charged to a 1L Mettler RC-1, MP10 reactor and stirred at 100 rpm. After several scans, eplerenone was charged to the reactor followed by an additional 470 mL of methyl ethyl ketone. Agitation was increased to 500 rpm to suspend solids and the batch temperature was increased to 80°C. The batch temperature was held at 80°C to ensure dissolution of the eplerenone. Black or white specks generally were visible in the resulting transparent solution. The batch temperature was then ramp cooled at the desired rate to the desired endpoint, where it was maintained for one hour before being pulled into a transfer flask and filtered. The vacuum was reactor, transfer flask and cake were then washed with 120 mL methyl ethyl ketone. Once the wash was pulled through the cake, the stopped. About 10 grams of each wet cake were dried in a vacuum oven under nominal conditions of 75°C with a light nitrogen bleed. For the "high, high, high" and "low, low, low" experiments described below, fluid bed drying was operated under high and low conditions. High fluid bed drying was defined as 100°C with a blower setting of "4" while low fluid bed drying was defined as 40°C with a blower setting of "1".

B. Crystallization of Poor Quality Mother Liquor Residue

In the crystallization of poor quality mother liquor residue experiment, 60 g of the 61.1% pure material and 720 mL methyl ethyl ketone were charged directly to a 1L Mettler RC-1, MP10 reactor. The 61.1% pure material was not blended with high purity eplerenone prior to being charged to the reactor. The resulting mixture was heated to 80°C and was an opaque

slurry at that temperature. The crystallization continued and the mixture was filtered at 45°C under fast cooling conditions.

C. Form I Seeding

In the Form I seeding experiment, 60 g of pure (100.8%) eplerenone and 720 mL of methyl ethyl ketone were charged to a 1L Mettler RC-1, MP10 reactor. The mixture was heated to 80°C and then cooled to 25°C at a rate of 1.5° C/minute. When the solution had cooled to 62°C, it was seeded with 3 g of phase pure Form I crystals to initiate crystallization. The Form I seed crystals were prepared by the digestion process described in Example 9 below.

D. Form II Seeding

In the Form II seeding experiment, 66.6 g of 89.3% eplerenone (prepared by mixing 48.3 g of 100% eplerenone with 18.3 g of 61.1% eplerenone) and 720 mL of methyl ethyl ketone were charged to a 1L Mettler RC-1, MP10 reactor. The mixture was heated to 80°C and then cooled to 25°C at a rate of 1.5° C/minute. When the solution had cooled to 63°C, it was seeded with 3 g of phase pure Form II crystals to initiate crystallization. The Form II seed crystals were prepared by the crystallization and desolvation process described in Example 1 above.

Results from the experiments are reported in Table X-7A. In the n+1 crystallization experiment, Form I was detected only in the experiments employing low purity eplerenone where the product contained the diepoxide. Elevated levels of the diepoxide in the final product were also observed with higher cooling rates.

The crystallization of poor quality mother liquor residue experiment yielded poor quality material that appeared to be a mixture of the diepoxide and Form I when analyzed by X-ray powder diffraction.

The Form I seeding experiment (where high purity eplerenone was seeded with Form I) yielded a product that was 77% Form I based on X-ray powder diffraction analysis, but entirely Form I based on DSC. The X-ray powder diffraction model, however, had not been tested for linearity beyond about 15% Form I. This experiment was the only one of the four experiments of this Example where Form I was created in the absence of the diepoxide.

The Form II seeding experiment (where low purity eplerenone was seeded with Form II) yielded a product that was entirely Form II.

The data obtained for the high fluid bed drying of eplerenone appeared to correspond to the data obtained for the vacuum oven drying. The low fluid bed dryings yielded results that differed from those of the vacuum oven dryings.

TABLE X-7A

Cooling Rate[1]	Cooling Endpoint[2]	Impurity Level[3]	Nucleation Temperature (°C)	Weight Percent 11,12-Epoxide[4]	Weight Percent Diepoxide[4]	Assay For Desolvated Crystal	Percent Yield	Weight Percent Form I (XPRD)
+	+	-	57.0	ND	ND	100.3	66.1	ND
+	-	-	54.9	ND	ND	100.3	98.1	ND
-	+	-	60.9	ND	ND	100.3		ND
-	-	-	63.4	ND	ND	100.5	79.3	ND
+	+	++	N/A	4.8	36.6	43.3	27	100[5]
+	+	+	52.2	0.49	0.88	98.3	62	29
+	-	+	53.3	0.56	1.0	98.1	87	9
0	0	0	59.0	0.18	0.36	99.4	75	5
-	+	+	63.3	0.20	0.44	99.4	36	31
-	-	+	61.4	0.18	0.40	99.5	87	ND
0	0	0	60.6	0.18	0.36	99.5	79.2	ND
0	0	0	55.9	0.38	0.80	98.6	80.5	<3%
0	0	100.8% eplerenone seeded with Form I		0.03	ND	100.4	82.2	77/100[6]
0	0	89.3% eplerenone seeded with Form II		0.33	0.50	97.5	80.2	ND

[1]Cooling Rate: (+) = 3°C/min.; (0) = 1.5 °C/min.; and (-) = 0.1 °C/min.
[2]Cooling Endpoint: (+) = 45°C; (0) = 25 °C; and (-) = 5°C.
[3]Impurity Level: : (+) = 89.3% purity eplerenone starting material; (++) = 61.1% purity eplerenone starting material; (0) = 100.8% purity eplerenone starting material; and (-) = 94.5% purity eplerenone starting material.
[4]Weight percent after drying solvate in a vacuum oven at 75°C.
[5]Appears to be mixture of Form I and diepoxide when analyzed by XPRD.
[6]Appears to be 77% Form I when analyzed by XPRD and 100% Form I when analyzed by DSC.

A. Material Purity

A cube plot of product purity, starting material purity, cooling rate and endpoint temperature based on the data reported in Table X-7A is shown in Fig. 18. The cube plot suggests that the use of a higher purity material at the start of crystallization will yield a higher purity product. The endpoint temperature of crystallization does not appear to greatly affect the product purity. The cooling rate, however, appears to have an effect with slightly less pure product resulting from a faster cooling rate. In fact, the level of diepoxide generally was higher with faster cooling rates.

Fig. 19 shows a half normal plot that was prepared using the results of cube plot to determine which variables, if any, had a statistically significant effect on the product purity. Starting material purity had the greatest statistically significant effect on product purity, although cooling rate and the interaction between cooling rate and starting material purity were also seen as statistically significant effects.

Fig. 20 is an interaction graph based on these results and showing the interaction between starting material purity and cooling rate on product purity. With the high purity eplerenone (100.8% eplerenone starting material) the cooling rate appears to have little or no effect on final purity. With the low purity eplerenone (89.3% eplerenone starting material), however, the product purity decreases as cooling rate increases. This result suggests that more impurities crystallize out in eplerenone crystallizations conducted at higher cooling rates.

B. Form I Content

A cube plot of Form I weight fraction, starting material product purity, cooling rate and endpoint temperature based on the data reported in Table X-7A is shown in Fig. 21. The cube plot suggests that the use of a higher purity eplerenone at the start of crystallization will yield a lower amount of Form I. The endpoint temperature of crystallization also appears to have an effect on the form of the final product. The cooling rate does not appear to greatly affect the formation of Form I although some Form I may result from faster cooling at the low endpoint temperature in the presence of impurities.

Fig. 22 shows a half normal plot that was prepared using the results of the cube plot to determine which variables, if any, had a statistically significant effect on the amount of Form I in the final material. Starting material purity, endpoint temperature of the crystallization and the interaction between the two variables were seen as statistically significant effects.

Fig. 23 is an interaction graph based on these results and showing the interaction between starting material purity and endpoint temperature on final Form I content. With the high purity eplerenone (100.8% eplerenone starting material), endpoint temperature appears to have little effect on Form I content. No Form I resulted in either case with pure eplerenone. With low purity eplerenone (89.3% eplerenone starting material), however, Form I was present in both cases, with significantly more Form I at higher endpoint temperatures.

Table X-7B reports the weight fraction of Form I measured in materials dried using either a fluid bed (LAB-LINE/P.R.L.

Hi-Speed Fluid Bed Dryer, Lab-Line Instruments, Inc.) or a vacuum oven (Baxter Scientific Products Vacuum Drying Oven, Model DP-32). Similar Form I content was observed for comparable materials dried in either the high fluid bed or the vacuum oven. A difference was observed, however, for comparable materials dried in the low fluid bed relative to the vacuum oven.

TABLE X-7B

Cooling Rate	End Point	Impurity Level	Drying Type	Weight Percent Form I
High	High	High	Vacuum Oven	29%
High	High	High	High Fluid Bed	25%
High	High	High	Low Fluid Bed	4.7%
Low	Low	Low	Vacuum Oven	ND
Low	Low	Low	High Fluid Bed	ND
Low	Low	Low	Low Fluid Bed	5.5%

Example 8: Crystallization of a Mixture of Form I and Form II From Methyl Ethyl Ketone To Prepare a Solvate, and (b) Desolvation of the Solvate to Prepare Form II

Form I eplerenone (10 g) was combined with 80 mL of methyl ethyl ketone. The mixture was heated to reflux (79°C) and stirred at this temperature for about 30 minutes. The resulting slurry was then cooled with a stepwise, holdpoint protocol by maintaining the slurry at 65°C, 50°C, 35°C and 25°C for about 90 minutes at each temperature. The slurry was filtered and rinsed with about 20 mL methyl ethyl ketone. The isolated solid was initially dried on the filter and then in a vacuum oven at 40-50°C.. The drying was completed in the vacuum oven

at 90-100°C. The desolvated solid was obtained with an 82% recovery. XPRD, MIR and DSC confirmed that the solid had a Form II crystalline structure.

Example 9: Digestion of Low Purity Eplerenone Starting Material With a Solvent to Prepare Form I

A. Digestion With Ethanol Solvent:

Low purity eplerenone (24.6 g; 64% by weight assay via HPLC) was combined with 126 mL of ethanol 3A. The slurry was heated to reflux and the distillate removed. An additional 126 mL of ethanol 3A was simultaneously added as 126 ml of solvent was removed via atmospheric distillation. Upon completion of the solvent turnover, the mixture was cooled to 25 °C and stirred for one hour. The solid was filtered and rinsed with ethanol 3A. The solid was air-dried to give the ethanol solvate. The solvate was further dried in a vacuum oven at 90-100°C for six hours to obtain 14.9 g of Form I eplerenone.

B. Digestion With Methyl Ethyl Ketone Solvent

In an alternative digestion process, 1 gram of low purity eplerenone (about 65% pure) was digested in 4 mL of methyl ethyl ketone for two hours. After the two hours, the mixture was allowed to cool to room temperature. Once cooled, the solid was collected by vacuum filtration and determined to be the methyl ethyl ketone solvate by XPRD analysis. The solid was dried at 100°C for 30 to 60 minutes. The dried solids were determined to be pure Form I by XPRD.

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Example 10: Digestion of High Purity Eplerenone Starting Material With a Solvent to Prepare Form II

A. Digestion With Ethanol Solvent:

High purity eplerenone (1 gram) was digested in 8 mL of ethanol for approximately two hours. The solution was then allowed to cool to room temperature and the solids were collected by vacuum filtration. Analysis of the solids by XPRD immediately after filtration indicated that the solids were a solvate (presumably the ethanol solvate). The solids were subsequently dried at 100°C at atmospheric pressure for 30 minutes. The dried solid was analyzed by XPRD and determined to be predominately Form II (no Form I detected).

B. Digestion with Methyl Ethyl Ketone Solvent:

High purity eplerenone (1 gram) was digested in 4 mL of methyl ethyl ketone for two hours. After the two hours, the solution was allowed to cool to room temperature and the solids collected by vacuum filtration. The solid was immediately analyzed by XPRD and determined to be a solvate of eplerenone (presumably the methyl ethyl ketone solvate). The solvate was subsequently dried at 100°C at ambient pressure for 30 to 60 minutes. The dried solids were analyzed by XPRD and determined to be primarily Form II with no diffraction peaks for Form I present.

Example 11: Crystallization of Form II Directly From Solution

Procedure A: Eplerenone (2.5 g) was dissolved in ethyl acetate by heating to 75°C. Once the eplerenone dissolved,

the solution was held at 75°C for 30 minutes to ensure complete dissolution. The solution was then cooled at 1 °C/min to 13°C. Once at 13°C, the slurry was allowed to stir for two hours at 750 rpm with an overhead stirrer. The crystals were collected by vacuum filtration and dried in a vacuum oven at 40°C for one hour. The XPRD pattern and DSC thermogram of the solid were characteristic of Form II eplerenone. Thermal gravimetric analysis (TGA) of the solid indicated no weight loss from the solid up to 200°C.

Procedure B: In an alternative procedure, 2 g of eplerenone was dissolved in 350 mL of 15/85% acetonitrile/water by heating on a hot plate with magnetic stirring. Once the eplerenone was dissolved, the solution was allowed to cool to room temperature overnight with magnetic stirring. The resulting solid was collected by vacuum filtration. The crystals were birefringent and had a triangular, plate-like crystal habit. The solid had an XPRD and DSC characteristic of Form II eplerenone. TGA indicated no weight loss up to 200°C.

Procedure C: In an alternative procedure, 640 mg of eplerenone was placed in a 50 mL flask with 20 mL of ethyl benzene. The resulting slurry was heated to 116°C and became a clear solution. The clear solution was cooled to 25°C over 30 minutes. Nucleation began at 84°C during the cooling period. The resulting solids were filtered from the solution and air-dried to give 530 mg of solids (83% recovery). Hot-stage microscopy and XPRD confirmed that the solids were Form II crystals.

Procedure D: In an alternative procedure, 1.55 g of eplerenone was added to 2.0 mL of nitrobenzene and heated to 200°C. The resulting slurry was stirred overnight at 200°C. The solution was allowed to cool to room temperature (natural air convection) the following day and the solid was isolated. The solid was determined to be Form II eplerenone by XPRD and polarized light microscopy.

Procedure E: In an alternative procedure, 5.0 g of eplerenone (purity greater than 99%) was added to 82 g of methanol (104 mL). Under stirring action (210 rpm), the solution was heated to 60°C and held at that temperature for 20 minutes to ensure complete dissolution. The solution was then cooled to -5°C at a rate of 0.16°C/minute under stirring. The crystals were collected by filtration and dried in a vacuum oven at 40°C for 20 hours. The dried solids were determined to be pure Form II eplerenone by DSC and XPRD analysis.

Procedure F: In an alternative procedure, 6.0 g of eplerenone (ethanol solvate containing 9% ethanol and having a corrected purity of 95.2%) was added to 82 g of methanol (104 mL). Under stirring action (210 rpm), the solution was heated to 60°C and held at that temperature for 20 minutes to ensure complete dissolution. The solution was then cooled to 50°C at a rate of 0.14°C/minute and then held at that temperature for about 2.5 hours. The solution was then cooled to -5°C at a rate of 0.13°C/minute under stirring. The crystals were collected by filtration and dried in a vacuum

oven at 40°C for 16 hours. The dried solids were determined to be pure Form II eplerenone by DSC and XPRD analysis.

Example 12: Crystallization of Form I Directly From Solution

150.5 mg of the diepoxide and 2.85 g of eplerenone were added to 1.5 mL of nitrobenzene. The mixture was magnetically stirred at 200°C for several hours. The slurry was then allowed to cool to room temperature by natural air convection. The sample was dried and analyzed by polarized light microscopy and XPRD. The XPRD indicated that the sample was a mixture of Form I and Form II. The crystals were translucent by microscopy, indicating that desolvation (and conversion to either Form I or Form II) did not occur.

Example 13: Preparation of Amorphous Eplerenone By Comminution

Approximately one-half of a steel Wig-L-Bug container was filled with about 60 g of eplerenone (greater than 99.9% purity). A steel ball and cap were placed on the sample container and agitated for 30 seconds by the Wig-L-Bug apparatus. The eplerenone was scraped off the surface of the Wig-L-Bug container and the container agitated for an additional 30 seconds. The resulting solid was analyzed by XPRD and DSC and determined to be a mixture of amorphous eplerenone and Form II crystalline eplerenone.

Example 14: Preparation of Amorphous By Lyophilization

Approximately 100 mg of crude eplerenone was weighed into a beaker containing 400 mL of water. The solution was heated slightly for five minutes, and then sonicated and heated with stirring for an additional five minutes.

Approximately 350 mL of the eplerenone solution was filtered into a 1000 mL round bottom flask containing 50 mL of HPLC water. The solution was flashed frozen in a dry ice/acetone bath over a time period of one to two minutes. The flask was attached to a Labconco Freezone 4.5 freeze dryer and dried overnight. The solids in the flask were transferred to a small brown bottle. A small aliquot was observed under polarized light microscopy at 10X, 1.25X optivar in cargille oil (1.404) and observed to be at least 95% amorphous eplerenone. Figures 24 and 25 show the XPRD pattern and DSC thermogram obtained for the amorphous eplerenone. The peak observed at 39 degrees two theta in Figure 24 is attributable to the aluminum sample container.

Example 15: Measurement of Form II Solubility

The aqueous solubility of Form II eplerenone was measured at pH 7 (100 mM phosphate buffer) at 5, 25, and 40°C. Approximately 30 mg of Form II eplerenone was mixed with approximately 10 mL of buffer to form a slurry of eplerenone at both 5 and 25 °C. Approximately 40 mg of Form II eplerenone was mixed with approximately 10 mL of buffer to form a slurry of eplerenone at 40 °C. Samples were prepared in duplicate for each condition. The slurries were allowed to equilibrate in appropriate temperature water shaker baths and the solutions were analyzed for eplerenone content by ultraviolet visible analysis (245 nm) at the 1, 5, 12, 19, 27, and 36 day time intervals. Data from each condition was appropriately averaged to determine the solubility of eplerenone at each condition and is reported in Table X-15. The residual solids from each time point were analyzed by DSC and TGA at the end of the 36 day equilibration and were

determined to be Form II.

TABLE X-15

Temperature (°C)	Form II Solubility
5	0.24 mg/mL
25	0.29 mg/mL
40	0.39 mg/mL

Example 16: Measurement of Intrinsic Dissolution Rates

Intrinsic dissolution rates were measured for the following four eplerenone polymorph samples: (i) Form II eplerenone prepared by direct crystallization from acetonitrile using water as an anti-solvent in the same manner as Example 12, Procedure B, (ii) Form I eplerenone prepared by digestion in ethanol in the same manner as Example 10, Procedure A, (iii) a mixture of Form I (5 weight percent) and Form II (95 weight percent), and (iv) Form II eplerenone that was micronized to provide the following particle size distribution: 10% of the particles under 9 microns, 50% of the particles under 22 microns, and 90% of the particles under 41 microns.

Eplerenone (150 mg) was weighed and placed into a VanKel intrinsic dissolution cavity. The powder was compressed at 1200 psi using a Carver press. The sample was then mounted onto the intrinsic dissolution apparatus. The dissolution media used was 1% SDS in HPLC grade water. All experiments were conducted at 37°C for two hours. Before the start of the experiment, 500 mL of the dissolution medium was equilibrated at 37°C for 30 minutes in the dissolution bathing chamber. An initial sample was taken from each dissolution vessel which served as time zero for the experiment. The eplerenone

tablets were then lowered into the dissolution medium. Samples were drawn at determined intervals for the determination of rate of dissolution. Care was taken to avoid air bubbles forming at the surface of the tablet. The samples were analyzed by UV absorbance detection at 243 nm. Intrinsic dissolution rates were calculated from the slope of the linear portion of the concentration versus time profiles corrected for volume and normalized for the surface area of the dissolution tablet (0.5 cm^2).

Fig. 26 reports the intrinsic dissolution rates measured for the four samples. These studies appear to indicate that Form I eplerenone has faster intrinsic dissolution rate than Form II eplerenone. X-Ray powder diffraction measurements comparing compressed against uncompressed eplerenone confirmed that the polymorphs did not interconvert upon compression or during the course of the dissolution studies.

Example 17: Eplerenone Polymorph Composition

Tablets containing 25 mg, 50 mg, 100 mg and 200 mg doses of Form II eplerenone are prepared and have the following composition:

Ingredient	Weight % of Tablet
Form II Eplerenone	29.41
Form I Eplerenone	Not Detected
Lactose Monohydrate (#310, NF)	42.00
Microcrystalline Cellulose (NF, Avicel PH101)	18.09
Croscarmellose Sodium (NF, Ac-Di-Sol)	5.00
Hydroxypropyl Methylcellulose (#2910, USP, Pharmacoat 603)	3.00
Sodium Lauryl Sulfate (NF)	1.00
Talc (USP)	1.00
Magnesium Stearate (NF)	0.5
Total	100.00

Example 18: Eplerenone Polymorph Composition

Capsules (hard gelatin capsule, #0) are prepared containing a 100 mg dose of eplerenone and have the following composition:

Ingredient	Amount (mg)
Form II Eplerenone	90.0
Form I Eplerenone	10.0
Lactose, Hydrous, NF	231.4
Microcrystalline Cellulose, NF	45.4
Talc, USP	10.0
Croscarmellose Sodium, NF	8.0
Sodium Lauryl Sulfate, NF	2.0
Colloidal Silicon Dioxide, NF	2.0
Magnesium Stearate, NF	1.2
Total Capsule Fill Weight	400.0

Example 19: Eplerenone Polymorph Composition

Capsules (hard gelatin capsule, size #0) are prepared containing a 200 mg dose of eplerenone and have the following composition:

Ingredient	Amount (mg)
Form II Eplerenone	190.0
Form I Eplerenone	10.0
Lactose, Hydrous, NF	147.8
Microcrystalline Cellulose, NF	29.0
Talc, USP	10.0
Croscarmellose Sodium, NF	8.0
Sodium Lauryl Sulfate, NF	2.0
Colloidal Silicon Dioxide, NF	2.0
Magnesium Stearate, NF	1.2
Total Capsule Fill Weight	400.0

Example 20: Preparation of Milled Eplerenone

Dried methyl ethyl ketone solvate is first delumped by passing the solvate through a 20 mesh screen on a Fitzmill. The delumped solid is then pin milled using an Alpine Hosakawa stud disk pin mill operating under liquid nitrogen cooling at a feed rate of approximately 250 kilograms/hour. Pin milling produces milled eplerenone with a D_{90} size of approximately 65-100 microns.

Example 21: Effect of Eplerenone Particle Size (Dog Study)

The effect of the particle size of Form II eplerenone on eplerenone plasma concentrations and relative bioavailability was studied in a dog model. Four healthy female beagle dogs

weighing between 8 to 12 kg were intragastrically administered one immediate release capsule containing the formulation described in Table X-21A below followed by about 10 mL of water.

Table X-21A

INGREDIENT	WEIGHT % OF TABLET	Amount (mg)
Eplerenone	50.00	200.00
Lactose, Fast-Flo, Hydrous	36.95	147.80
Microcrystalline Cellulose (Avicel PH102)	7.25	29.00
Sodium Lauryl Sulfate	0.50	2.00
Croscarmellose Sodium	2.00	8.00
Talc	2.50	10.00
Colloidal Silicon Dioxide	0.50	2.00
Magnesium Stearate	0.30	1.20
Total	100.00	400.00
Capsules, Size #0, White Opaque	1 Capsule	

The dogs were fasted for 15 to 20 hours prior to administration of the capsule and were not fed again until at least 4 hours after dose administration. Blood samples (approximately 3 mL) were collected by venipuncture in chilled tubes containing heparin at 0, 0.5, 1, 2, 3, 4, 6, 8 and 24 hours after dose administration. The blood samples were immediately placed on ice. Separation of plasma from the blood samples was complete after about 15 minutes of centrifugation. The resulting plasma samples were frozen at about -20°C and stored until analyzed. Analysis was performed using an LC/MS/MS procedure.

This experiment was conducted using the same four dogs

for three formulations that were identical except for the particle size of the eplerenone starting material. The three formulations tested used eplerenone starting materials having a D_{90} particle size (that is, at least 90% of the particles were) less than about 212 microns, less than about 86 microns, and less than about 36 microns, respectively. A minimum of a five day wash-out period was allowed between administration of each of the formulations. Reducing the D_{90} particle size of the eplerenone starting material from about 212 microns to above 86 microns increased relative bioavailability by almost 100%. The mean results are reported in Tables X-21B and X-21C below.

Table X-21B

Time (hours)	Blood Serum Eplerenone Concentration (μg/mL)		
	D_{90} = 212 microns	D_{90} = 86 microns	D_{90} = 36 microns
0	0	0	0
0.5	1.83	3.65	1.99
1.0	2.40	6.18	5.86
2.0	3.77	6.89	6.77
3.0	2.85	5.70	6.60
4.0	2.61	4.39	5.56
6.0	1.63	3.11	3.31
8.0	1.10	1.90	2.09
24.0	0.0252	0.032	0.0706

Table X-21C

Pharmacokinetic Parameter	Pharmacokinetic Parameter Value		
	D_{90} = 212 microns	D_{90} = 86 microns	D_{90} = 36 microns
C_{max} (μg/mL)	3.98	7.02	7.39
T_{max} (hours)	1.50	1.75	2.25
AUC ((μg/mL)hr)	26.6	49.2	53.1
Relative Bioavailability (%)	53.25	100	107.9

Example 22: Effect of Eplerenone Particle Size (Human Study)

The effect of the particle size of a Form II eplerenone starting material used in a pharmaceutical composition on eplerenone plasma concentrations and relative bioavailability was studied in a human model. Subjects received single doses of Form II eplerenone medication on Days 1, 8, 15, 22 and 29 according to a randomization schedule. All medication was administered with 180 mL of water at 0800 hours. Blood samples for eplerenone pharmacokinetic analyses were collected at -0.5 (predose), 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 16, 24, 36 and 48 hours postdose. Urine samples were collected and pooled between the hours of 0-24 and 24-48 hour postdose.

Plasma and urinary concentrations of eplerenone were determined using a validated high performance liquid chromatography method with MS/MS Detection. Pharmacokinetic data obtained is reported in Table X-23A. The particle size distributions of the eplerenone used in the preparation of the medications was determined in the dry powder state using laser light scattering.

Table X-23A

Pharmacokinetic Parameter	Pharmacokinetic Parameter Value		
	100 mg Capsule A (D_{90}=40 microns)	100 mg Tablet (D_{90}=82 microns)	100 mg Capsule B (D_{90}=96 microns)
C_{max} (ng/mL)	1747	1704	1669
T_{max} (hours)	1.8	1.8	1.3
AUC ((ng/mL)hr)	11349	11945	11981

The specific composition of each medication is reported in Table X-23B below.

Table X-23B

INGREDIENT	WEIGHT % OF TABLET/CAPSULE		
	Capsule A	Tablet A	Capsule B
Form II Eplerenone	25	30	25
Lactose Monohydrate	–	42	57.86 (fast flo lactose)
Lactose, Hydrous NF	57.8	–	–
Microcrystalline Cellulose (Avicel PH101)	11.4	17.5 (7.5% intragranular plus 10% extragranular)	11.34 (Avicel PH 102)
Croscarmellose Sodium (Ac-Di-Sol)	2	5	2
Hydroxypropyl Methylcellulose (Pharmacoat 603)	–	3	–
Sodium Lauryl Sulfate	0.5	1	0.5
Talc	2.5	1	2.5
Magnesium Stearate	0.3	0.5	0.3
Colloidal Silicon Dioxide	0.5	–	0.5
Total	100	100	100

All mentioned references are expressly incorporated by reference herein as if fully set forth at length. In addition, U.S. Provisional Application Serial No. 60/111,646 filed December 9, 1998, and the corresponding nonprovisional application, U.S. Application Serial No. 09/_____ filed December 8, 1999, are expressly incorporated by reference herein as if fully set forth at length.

When introducing elements of the present invention or the preferred embodiment(s) thereof, the articles "a", "an", "the" and "said" are intended to mean that there are one or more of the elements. The terms "comprising", "including" and "having" are intended to be inclusive and mean that there may be additional elements other than the listed elements.

Although this invention has been described with respect to specific embodiments, the details of these embodiments are not to be construed as limitations.

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WHAT IS CLAIMED IS

1. Particles comprising a crystalline form of eplerenone, wherein said crystalline form comprises a solvent, said crystalline form not does not comprise methylene chloride, ethyl ether or isopropanol, and at least 90% of said particles have a particle size less than about 15 microns.

2. Particles of Claim 1 wherein said crystalline form does not comprise methylene chloride, ethyl ether, isopropanol, methyl ethyl ketone or ethanol.

3. Particles of Claim 1 wherein said crystalline form comprises a solvent selected from the group consisting of methylethyl ketone, 2-pentanone, acetic acid, acetone, butyl acetate, chloroform, ethanol, isobutanol, isobutyl acetate, methyl acetate, ethyl propionate, n-butanol, n-octanol, n-propanol, propyl acetate, propylene glycol, t-butanol, tetrahydrofuran, toluene and t-butyl acetate.

4. Particles of Claim 1 wherein said crystalline form comprises a solvent selected from the group consisting of 2-pentanone, acetic acid, acetone, butyl acetate, chloroform, ethanol, isobutanol, isobutyl acetate, methyl acetate, ethyl propionate, n-butanol, n-octanol, n-propanol, propyl acetate, propylene glycol, t-butanol, tetrahydrofuran, toluene and t-butyl acetate.

5. Particles of Claim 1 wherein said crystalline form comprises ethanol.

6. Particles of Claim 1 wherein said crystalline form comprises methylethyl ketone.

7. Particles of Claim 6 wherein said crystalline form has an X-ray powder diffraction pattern with a peak at 13.6 ± 0.2 degrees two theta.

8. Particles of Claim 6 wherein said crystalline form has an X-ray powder diffraction pattern with peaks at 7.6 ± 0.2 and 13.6 ± 0.2 degrees two theta.

9. Particles of Claim 6 wherein said crystalline form has an X-ray powder diffraction pattern substantially as shown in Figure 1-C.

10. Particles of Claim 6 wherein said crystalline form has an orthorhombic crystal system.

11. Particles of Claim 6 wherein said crystalline form has a $P2_12_12_1$ space group.

12. Particles of Claim 6 wherein said crystalline form has unit cell a, b and c values of about 23.53 Å, about 8.16 Å, and about 13.08 Å, respectively.

13. Particles of Claim 6 wherein said crystalline form has unit cell α, β and γ values that are each about 90°.

14. Particles of Claim 1 wherein at least 90% of said particles have a particle size less than about 1 micron.

15. The particles of Claim 14 wherein at least 90% of said particles have a particle size less than about 800 nm.

16. The particles of Claim 14 wherein at least 90% of said particles have a particle size between about 10 nm to about 1 micron.

17. The particles of Claim 14 wherein at least 90% of said particles have a particle size between about 100 nm to about 800 nm.

18. The particles of Claim 14 wherein at least 90% of said particles have a particle size between about 200 nm to about 800 nm.

19. The particles of Claim 14 wherein at least 90% of said particles have a particle size between about 400 nm to about 800 nm.

20. Eplerenone comprising the particles of Claim 1, wherein said eplerenone has a phase purity of at least about 50% solvated crystalline eplerenone.

21. The eplerenone of Claim 20 wherein said eplerenone has a phase purity of at least about 90% solvated crystalline eplerenone.

22. The eplerenone of Claim 20 wherein said eplerenone is substantially phase pure solvated crystalline eplerenone.

23. A pharmaceutical composition comprising a therapeutically-effective amount of the particles of any one of Claims 1 to 22 in association with at least one pharmaceutically-acceptable carrier, adjuvant or diluent.

24. A composition of Claim 23 wherein the only eplerenone crystalline form present in the composition is a form of any one of Claims 1 to 6.

25. A method of treating or preventing an aldosterone-mediated condition or disorder in a subject, said method comprising administering to a subject having or susceptible to said condition or disorder a therapeutically-effective amount of the particles of any one of Claims 1 to 22.

26. The method of Claim 25 for use in treatment of hyperaldosteronism.

27. The method of Claim 25 for use in treatment of hypertension.

28. The method of Claim 25 for use in treatment of heart failure.

29. The method of Claim 25 for use in treatment of cirrhosis of the liver.

30. The method of Claim 25 for use in treatment of a condition selected from the group consisting of excess collagen/fibrosis, benign prostate hypertrophy and depression.

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584

31. The particles of Claim 1 wherein said particles further comprise a compound selected from the group consisting of 7-methyl hydrogen 4α,5α:9α,11α-diepoxy-17-hydroxy-3-oxo-17α-pregnane-7α,21-dicarboxylate, γ-lactone; 7-methyl hydrogen 11α,12α-epoxy-17-hydroxy-3-oxo-17α-pregn-4-ene-7α,21-dicarboxylate, γ-lactone; and 7-methyl hydrogen 17-hydroxy-3-oxo-17α-pregna-4,9(11)-diene-7α,21-dicarboxylate, γ-lactone.

32. The particles of Claim 1 wherein said particles further comprise 7-methyl hydrogen 4α,5α:9α,11α-diepoxy-17-hydroxy-3-oxo-17α-pregnane-7α,21-dicarboxylate, γ-lactone.

09/08/2001 12:09 6513756139 BUETSOW

ABSTRACT OF THE DISCLOSURE

Novel nanoparticles comprising a solvated crystalline form of eplerenone exclusive of methylene chloride, ethyl ether and isopropanol are described. These eplerenone nanoparticles have unique chemical and physical properties relative to other solid state forms of eplerenone and are characterized by their x-ray powder diffraction pattern, infrared spectrum, nuclear magnetic resonance spectrum, differential scanning calorimetric thermogram, and other physical characterizations.

Figure

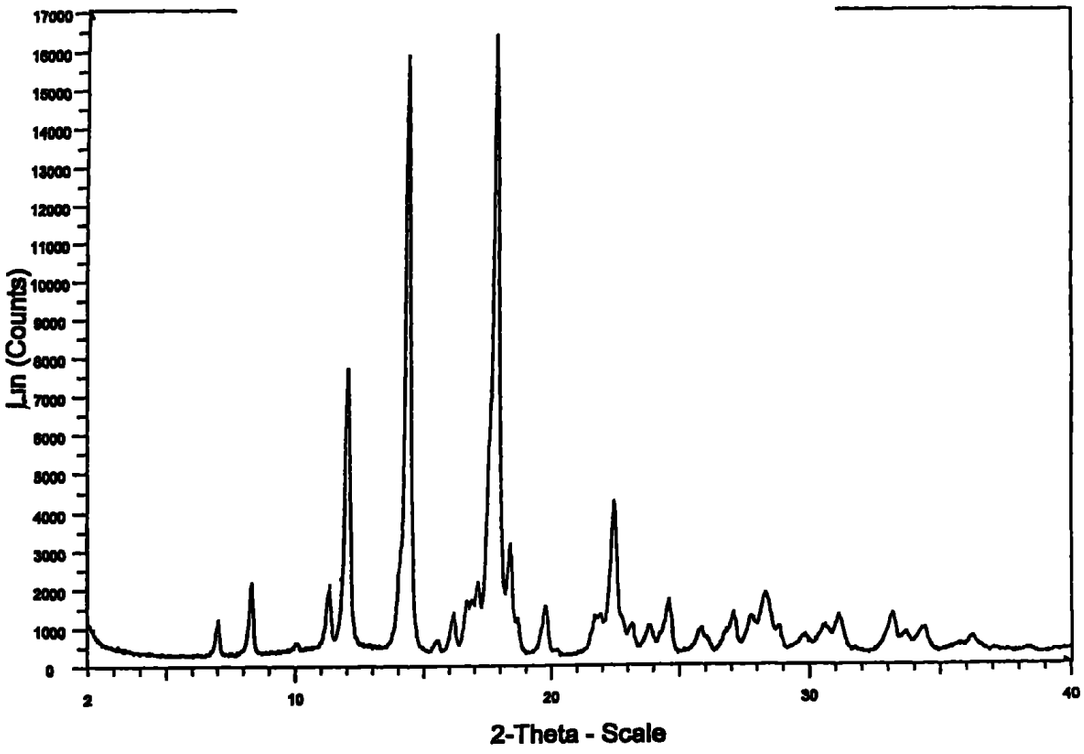

Lin (Counts)
17000
16000
15000
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
2
10
20
30
40
2-Theta - Scale

Figure 3

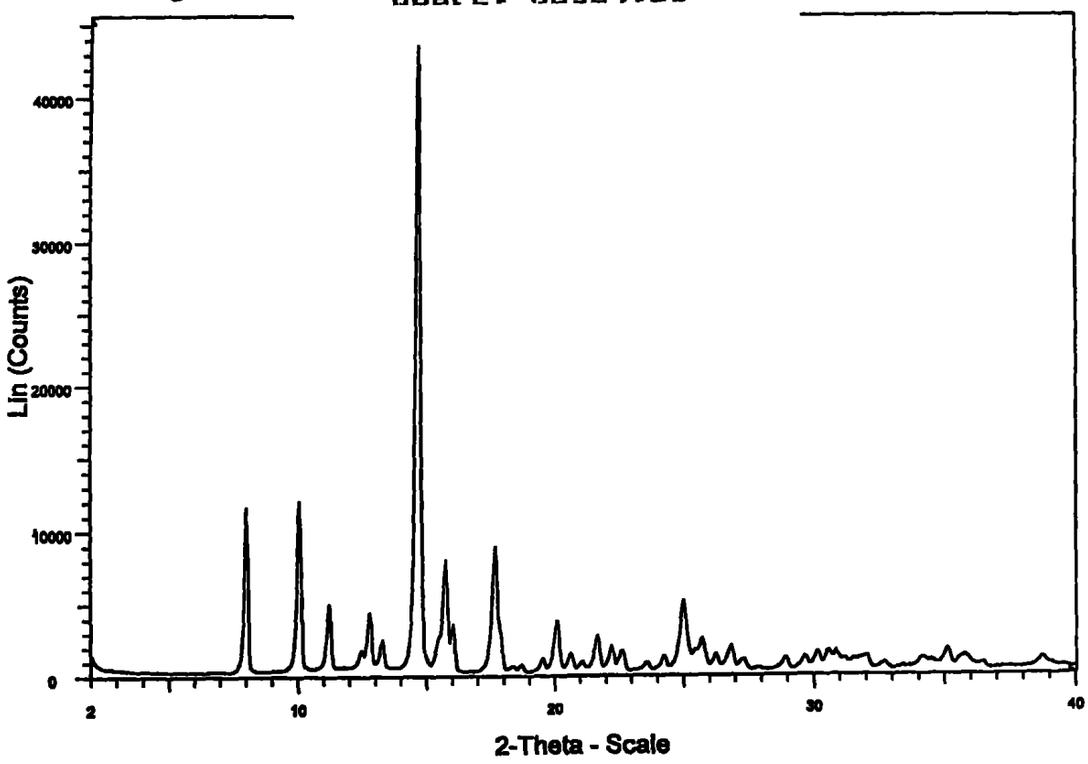

40000
30000
20000
10000
0
Lin (Counts)
2
10
20
30
40
2-Theta - Scale

Figure 6C

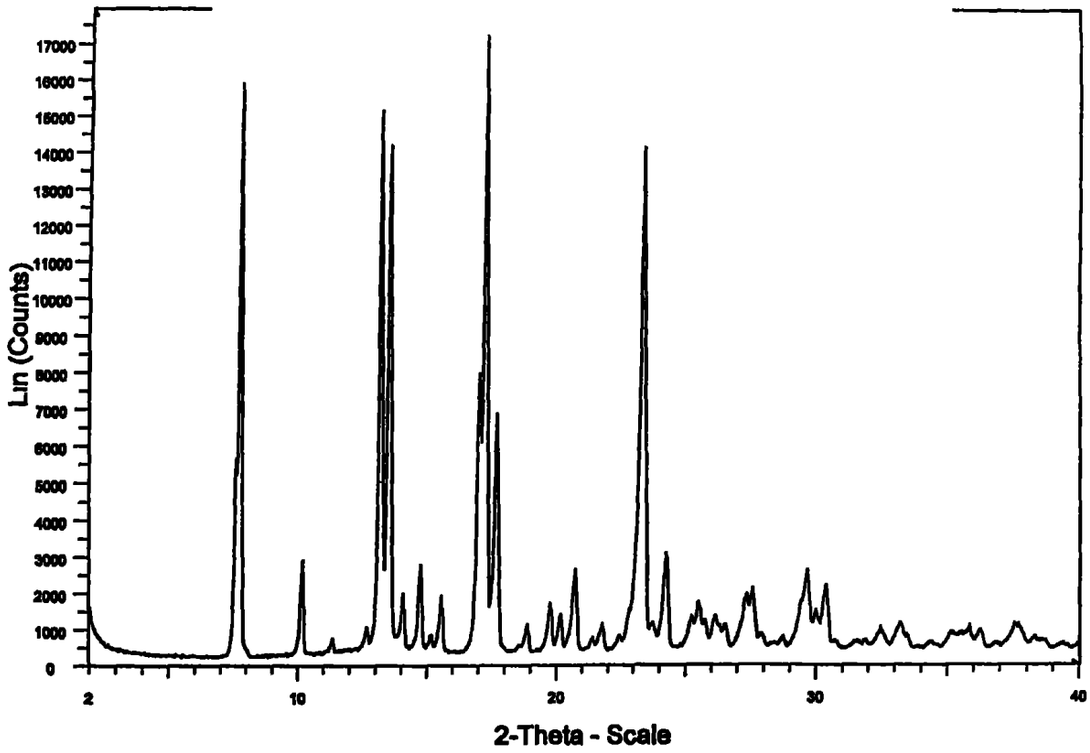
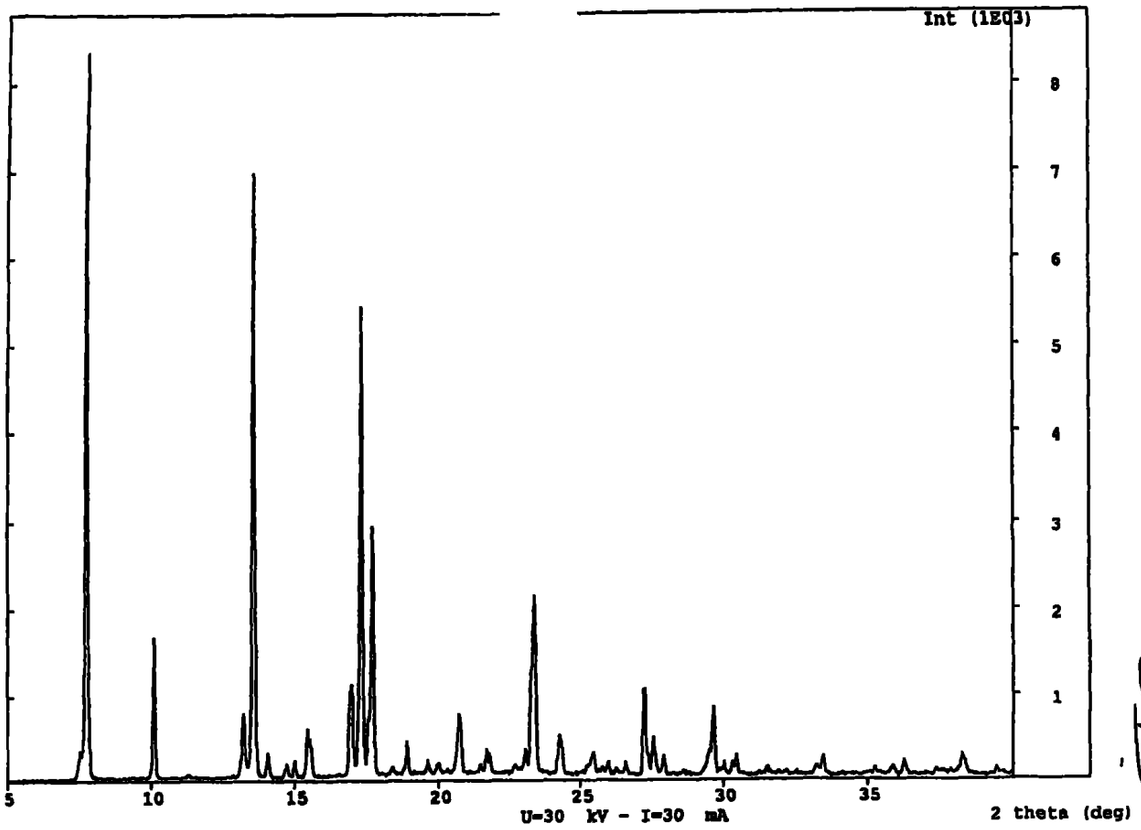

Lin (Counts)
17000
16000
15000
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
2
10
20
30
40
2-Theta - Scale

Figure 1-D

n-propyl alcohol solvate

INEL



590

Figure 1-E

INEL

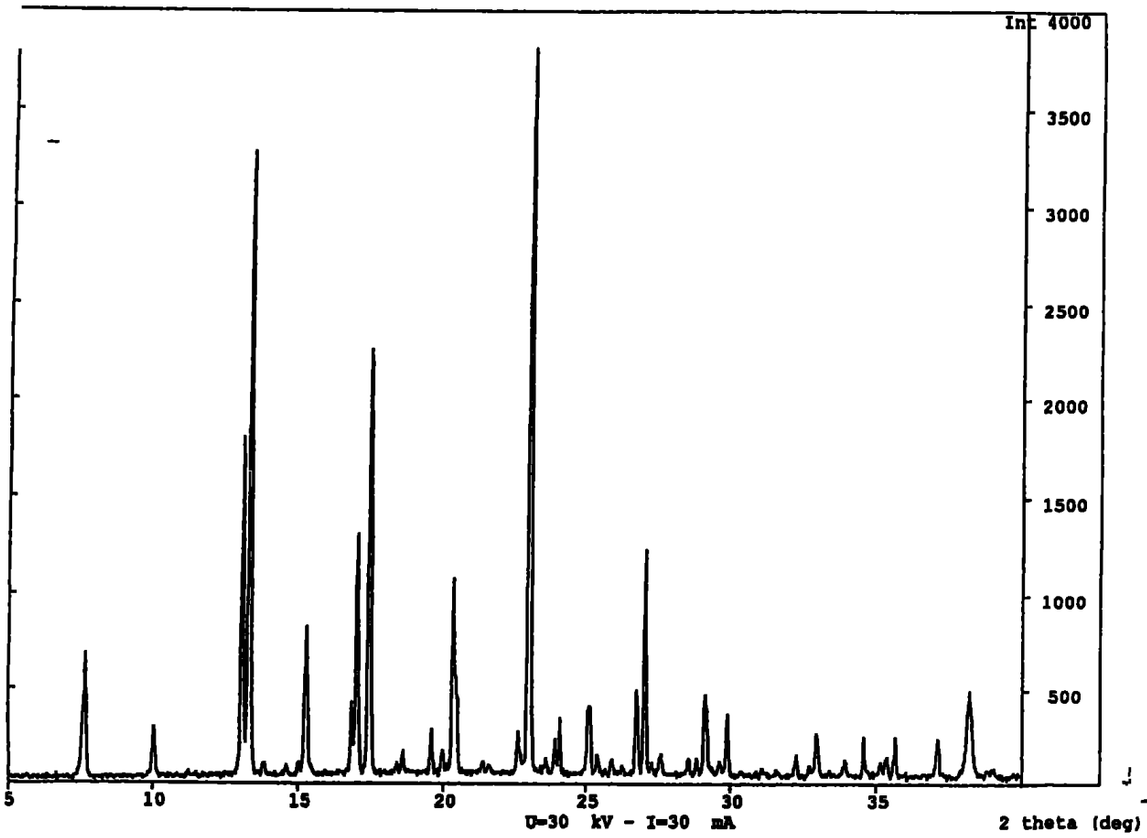


Figure 1-F

Ethyl Propionate solvate

INEL

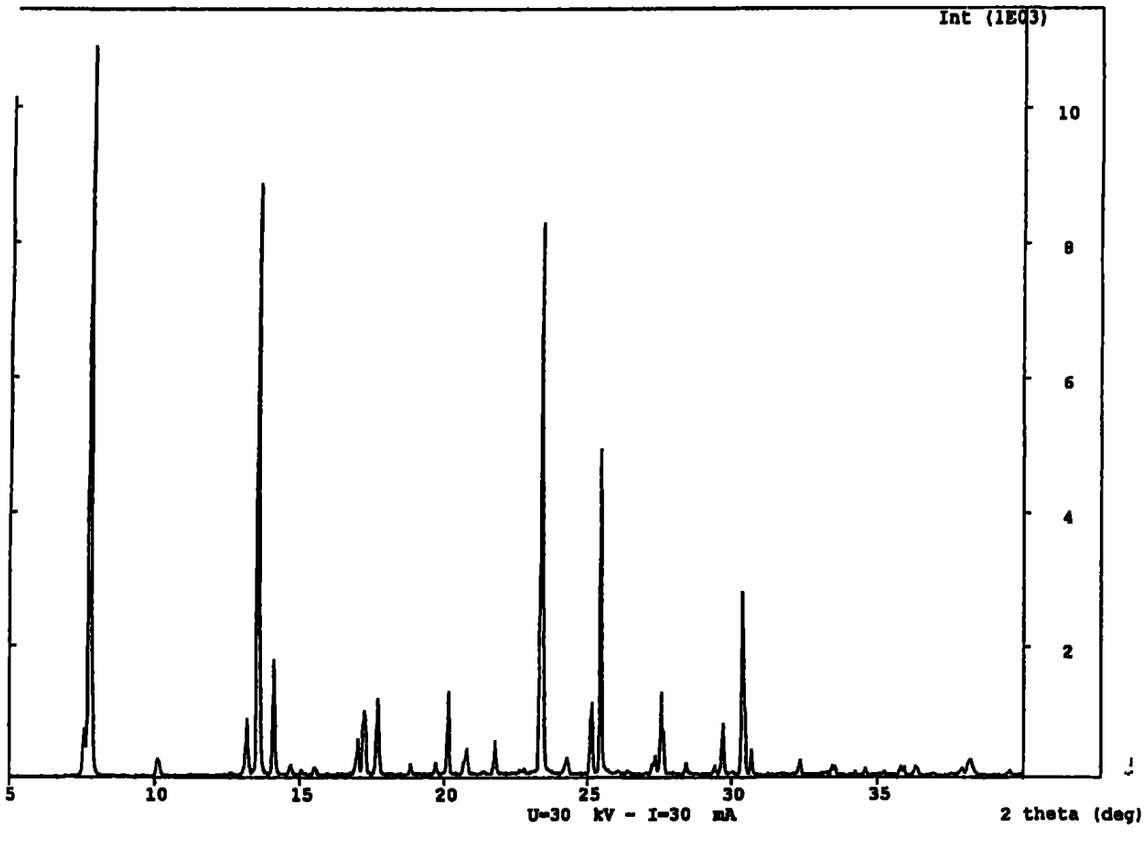


Figure 6

Acetic Acid solvate

INEL

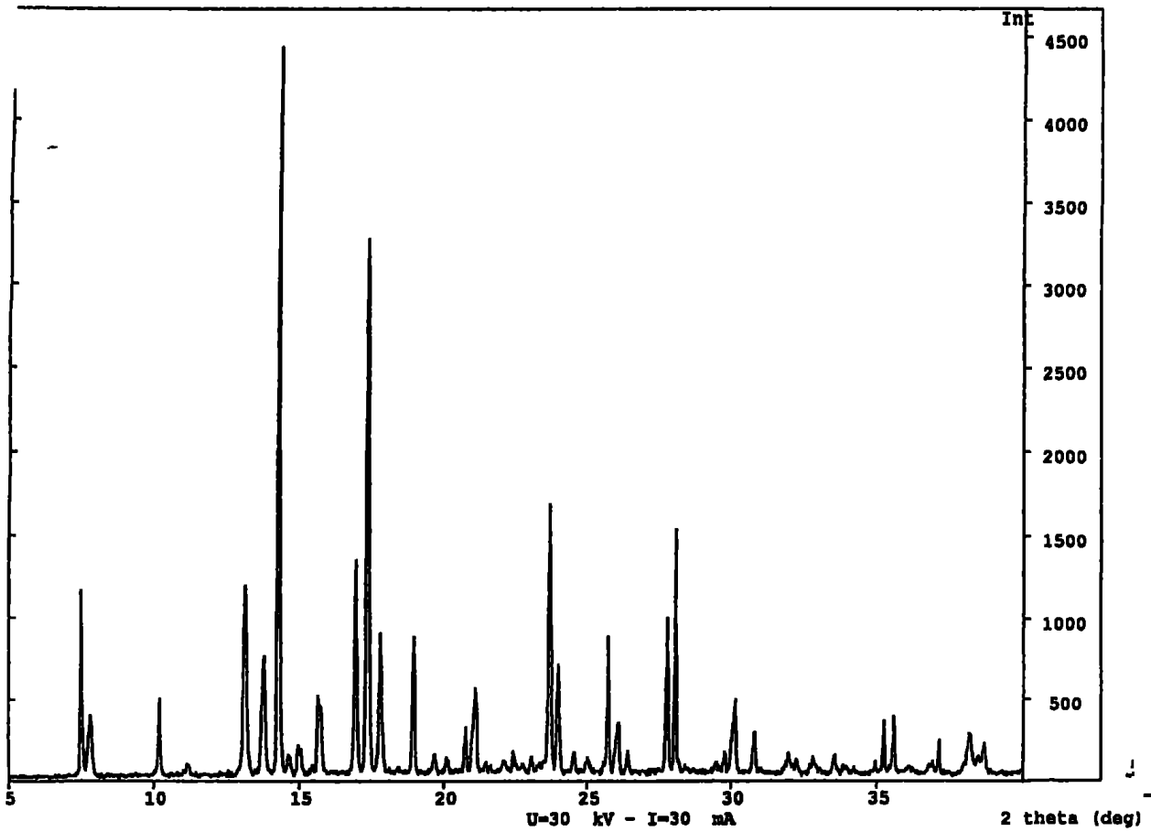


Figure [illegible]

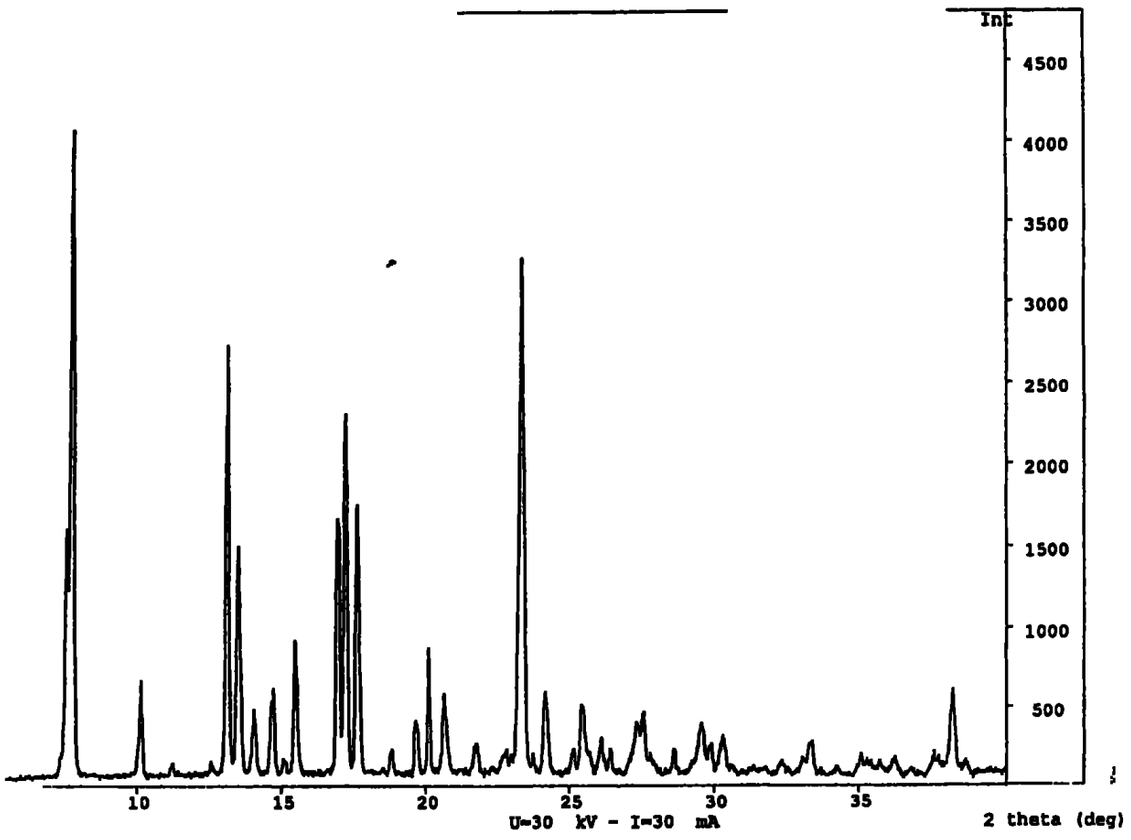

INEL
Int
4500
4000
3500
3000
2500
2000
1500
1000
500
10
15
20
25
30
35
U=30 kV - I=30 mA
2 theta (deg)

Figure 4.1

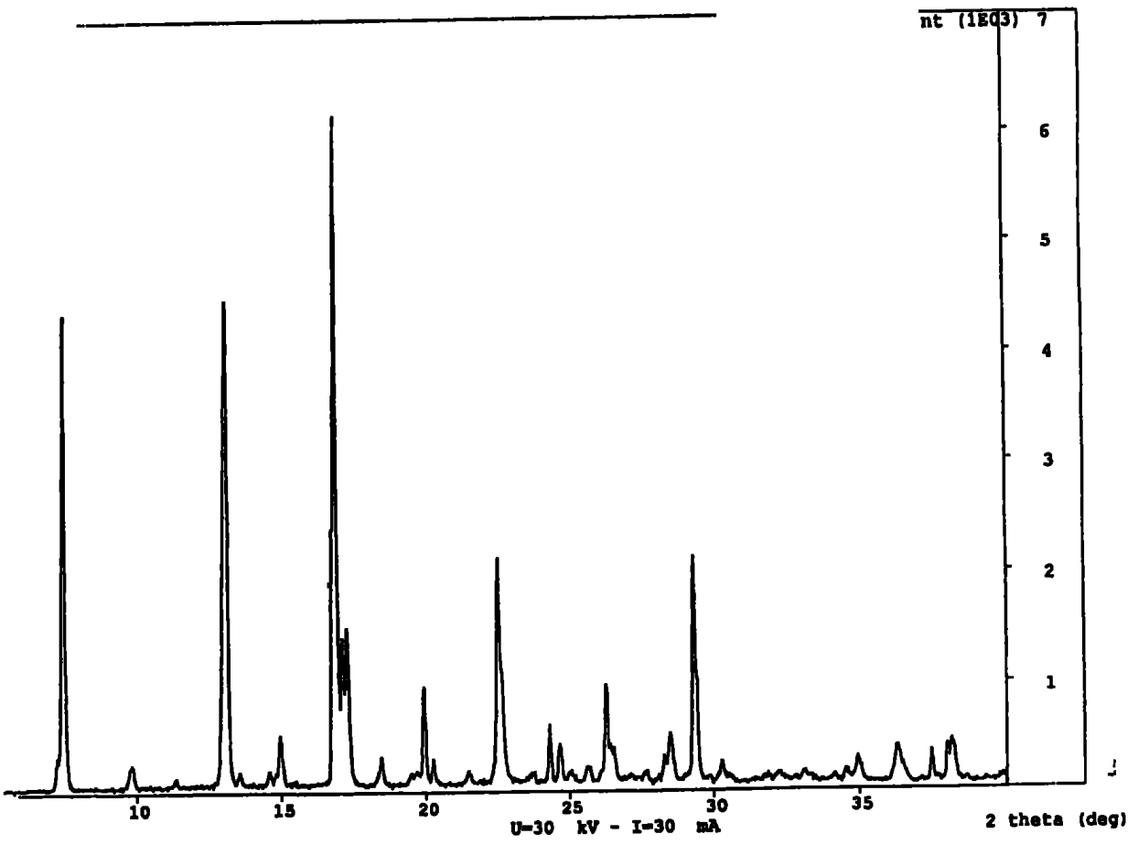
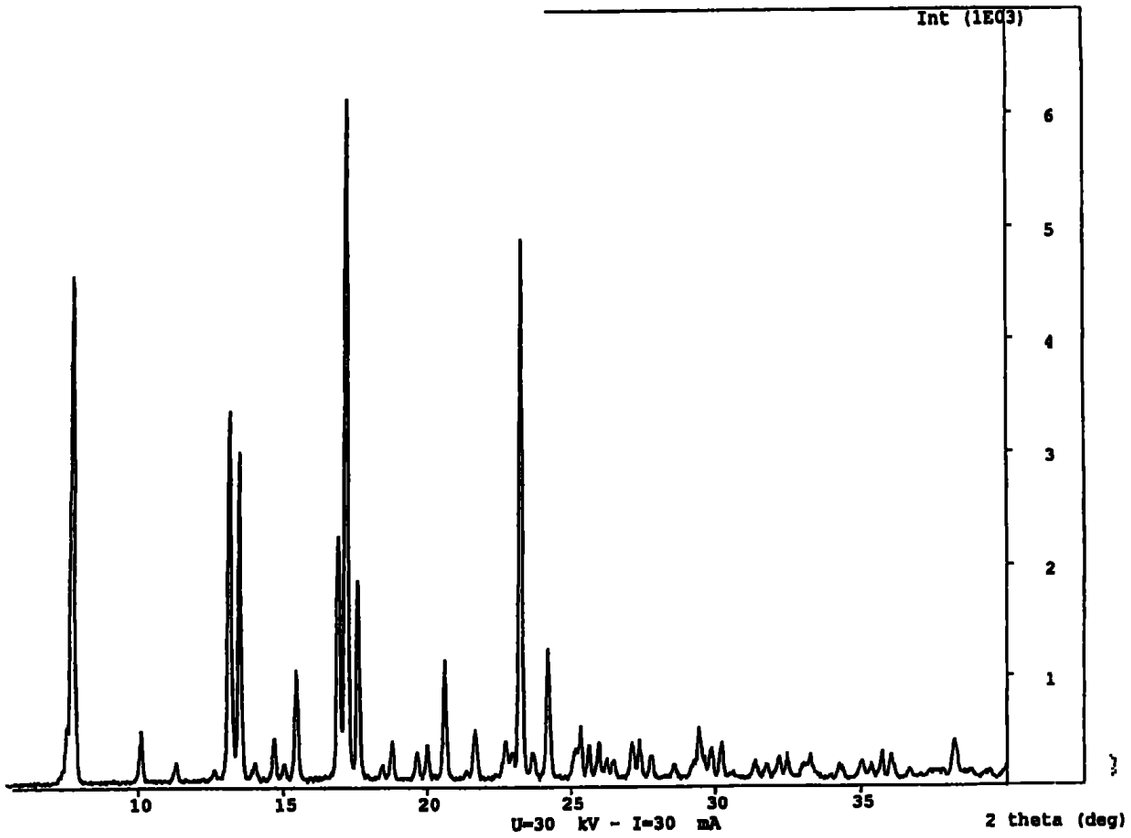


Figure [illegible]

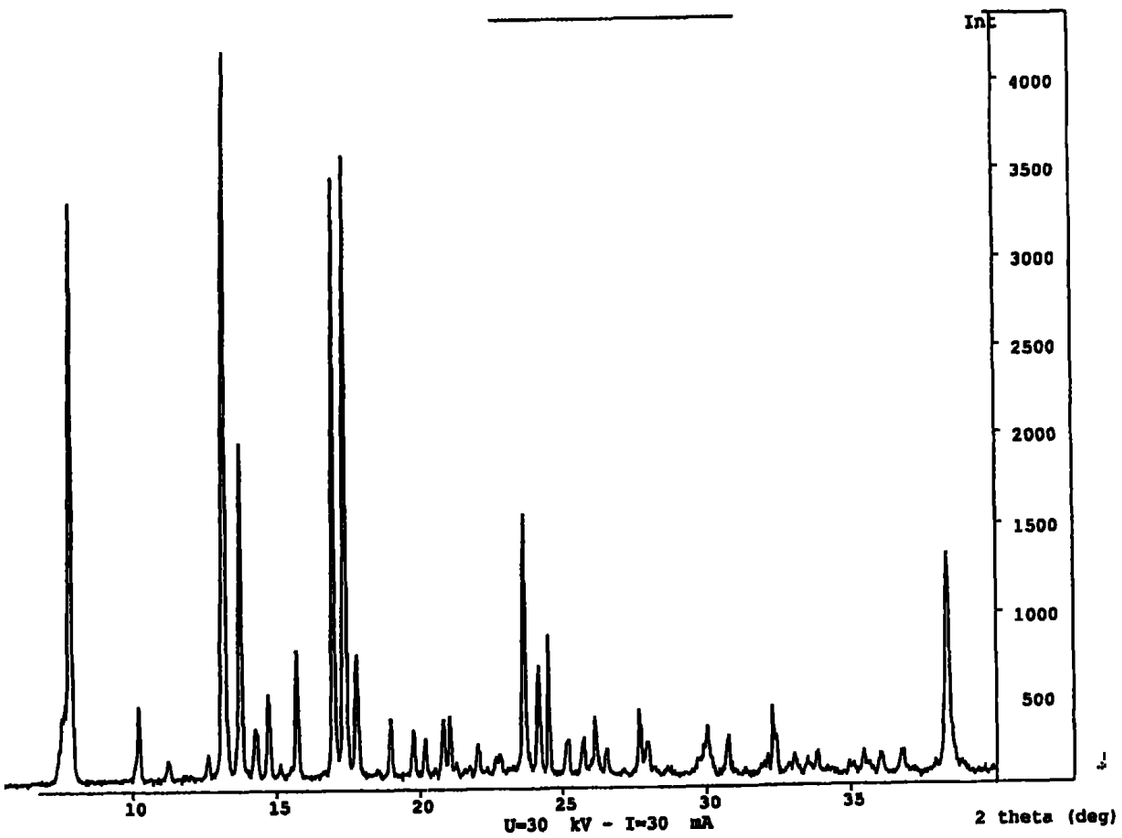
[illegible] INEL



604

Figure [illegible]

[illegible] ethanol solvate [illegible]



602

Figure 1-L

[illegible] solvate

INEL

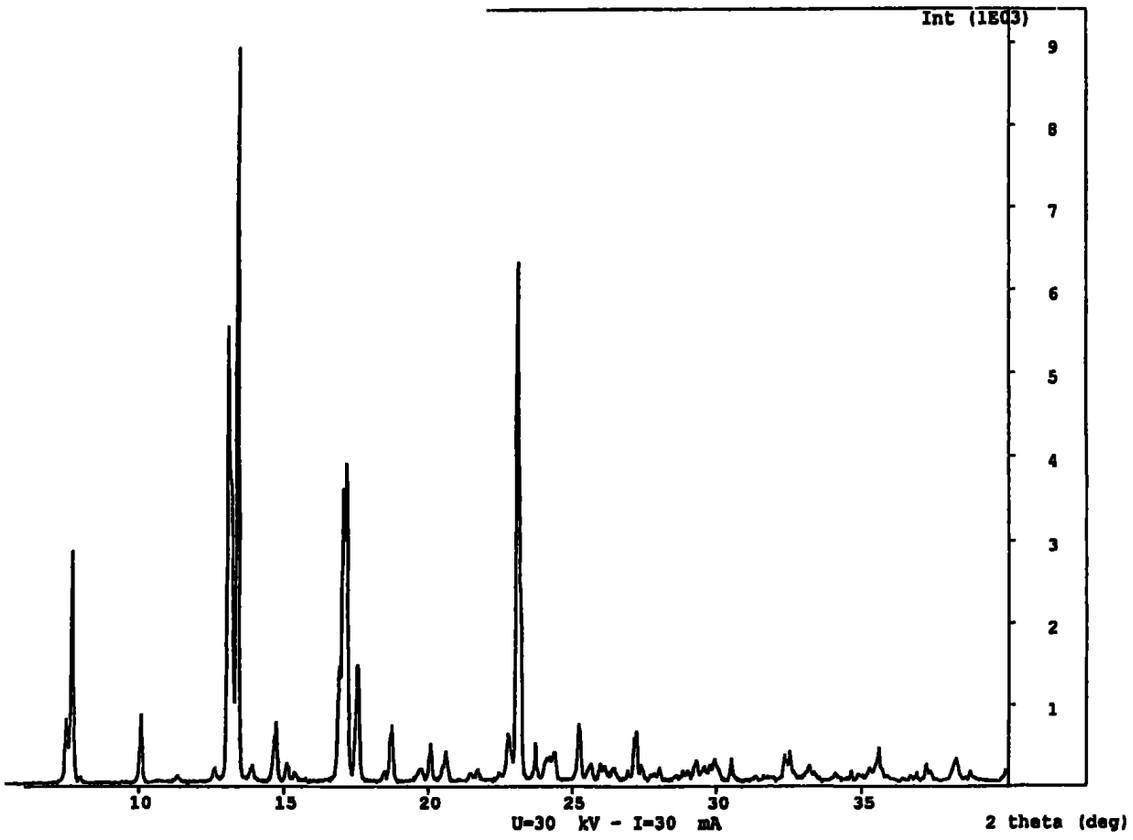


Figure 1-M

168027 [illegible] Butyl acetate Solvate

INEL

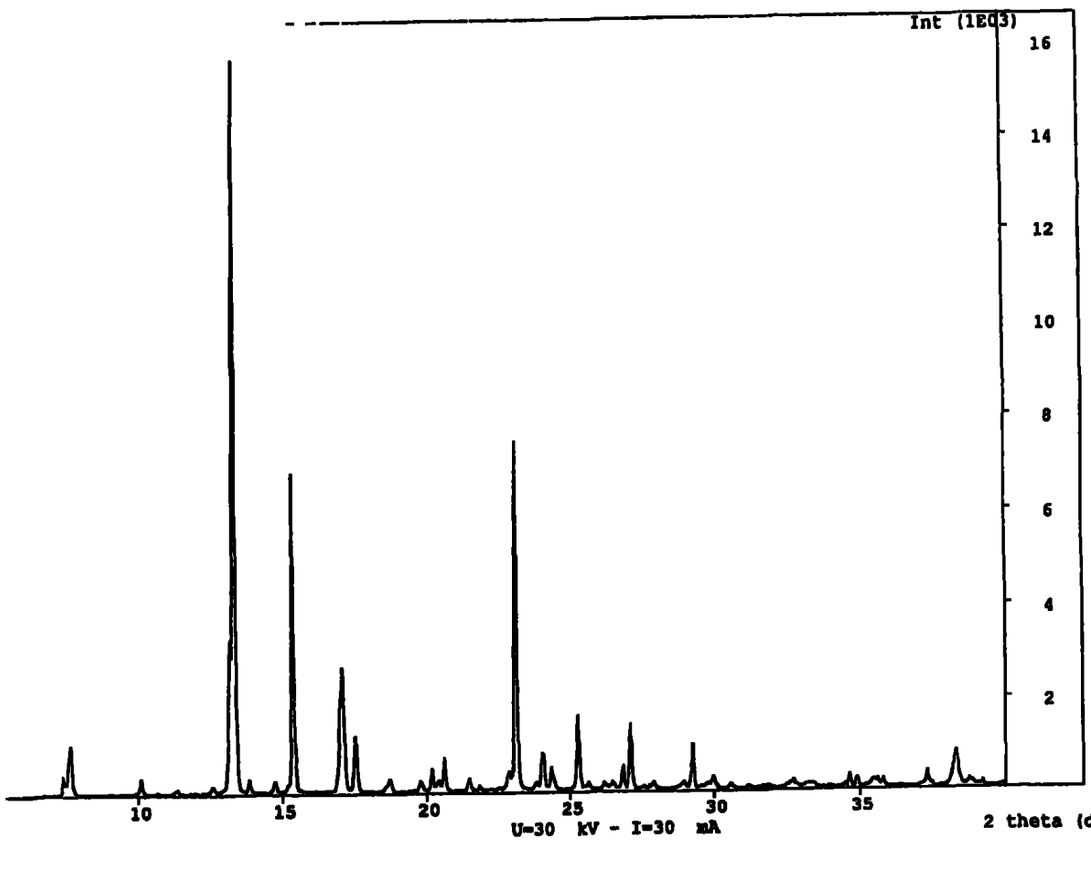


Figure 1-N

GG802T[illegible]olvate

INEL

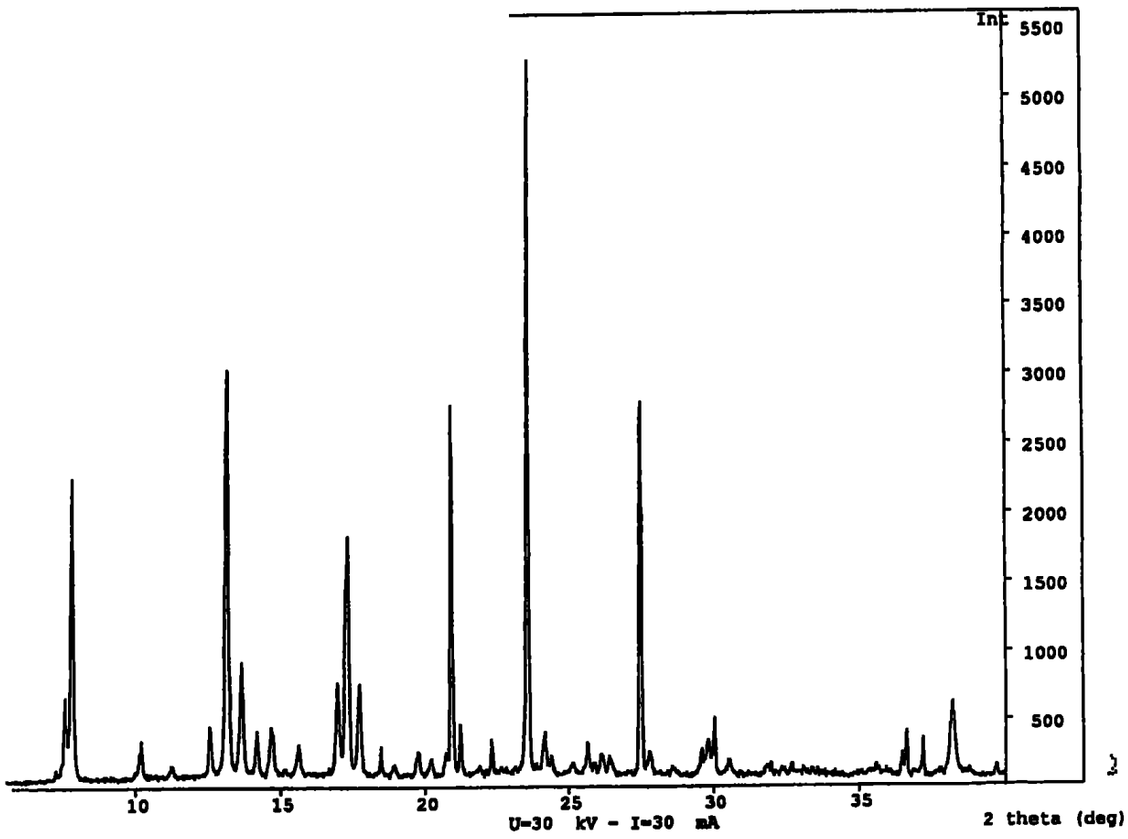


Figure 1-O

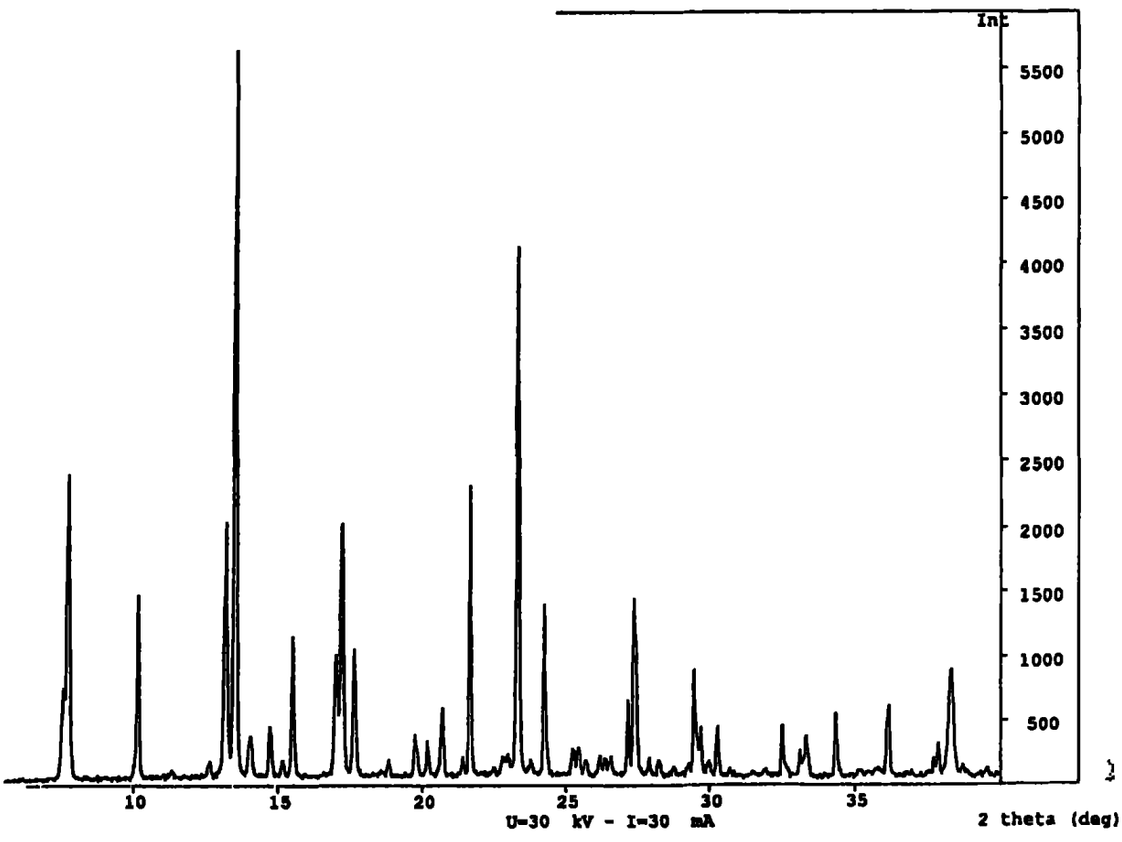

INEL
Int
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
10
15
20
25
30
35
U=30 kV - I=30 mA
2 theta (deg)

Figure 2 D

GG8021' 8096STOS

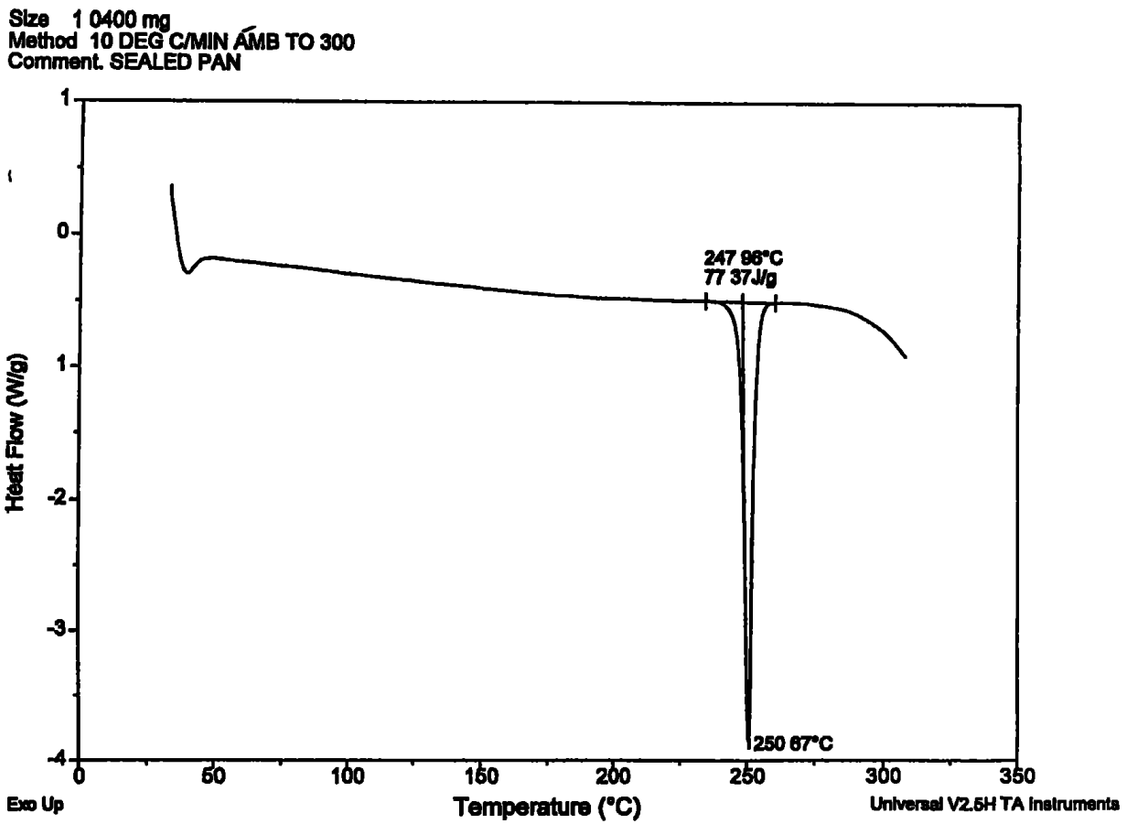
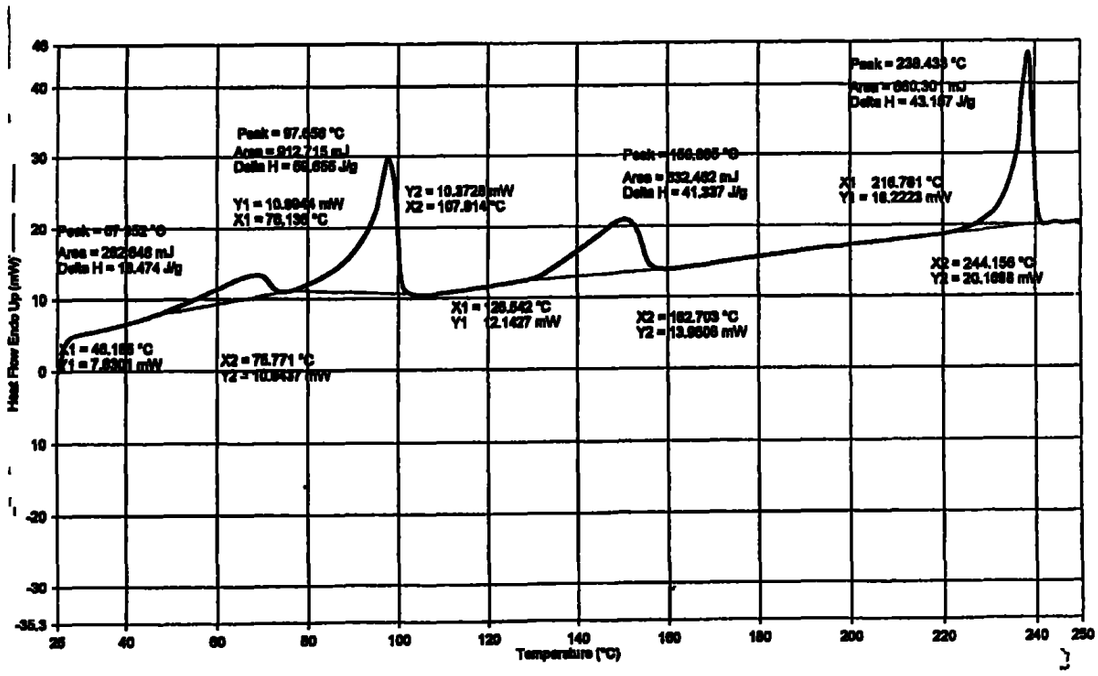


Figure 2-E

602

Sample Weight: 16.300 mg
Comment: N-Propyl Alcohol activate 1
unsealed pan

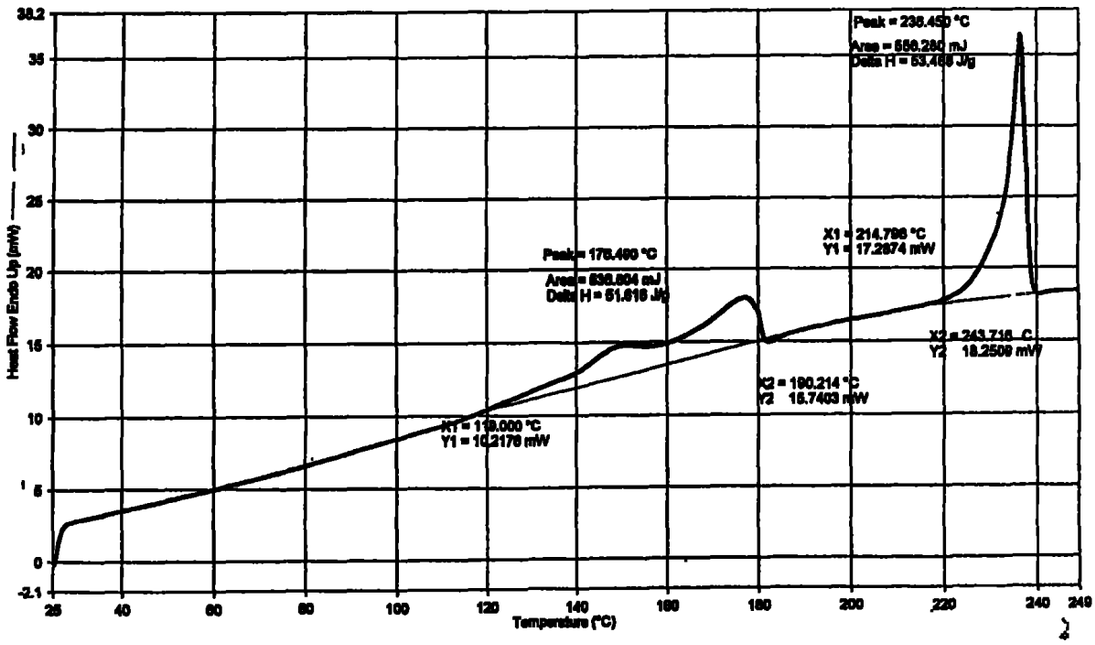


608

Figure 2F

603

Sample Weight:	10.400 mg
Comment:	THF solvate 1
	unsealed pan

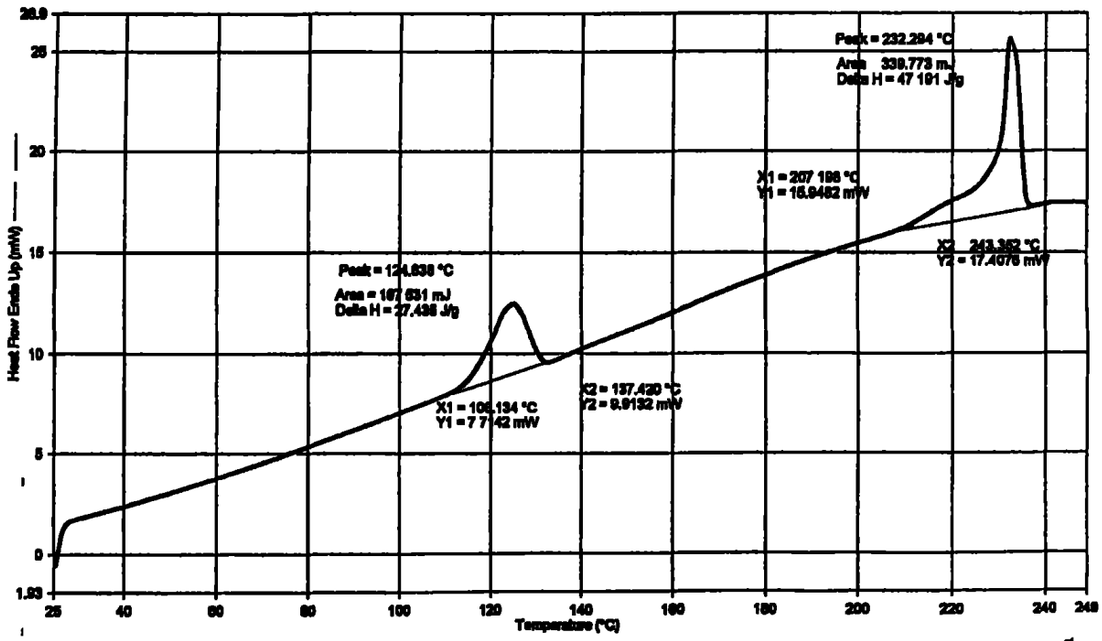


609

Figure 2C

budget augustus

Sample Weight:	7.200 mg
Comment:	Lot 9890230-F3b
	Ethyl propionate solvate
	Uncorrected data



640

Figure [illegible]

GDB021-B0406105

Sample Weight: 8.000 mg
Comment:
Acetic ACid solvate 1
unwashed pan

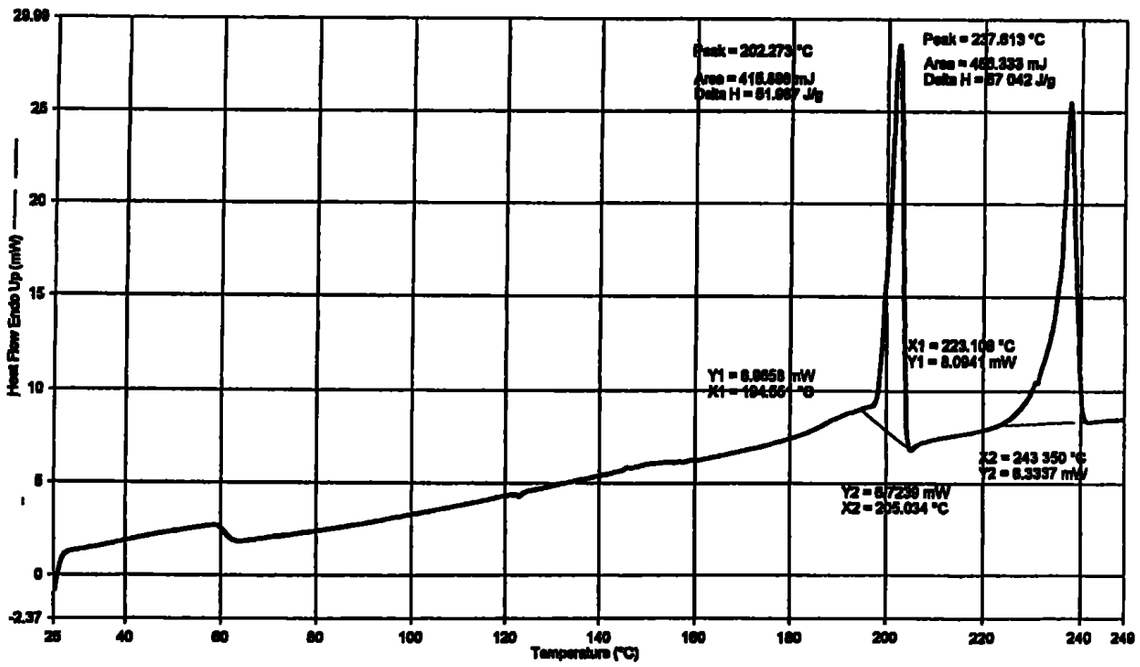


Figure 2-I

Sample Weight: 12.700 mg
Comment: Chloroform solvate 1
unsealed pan

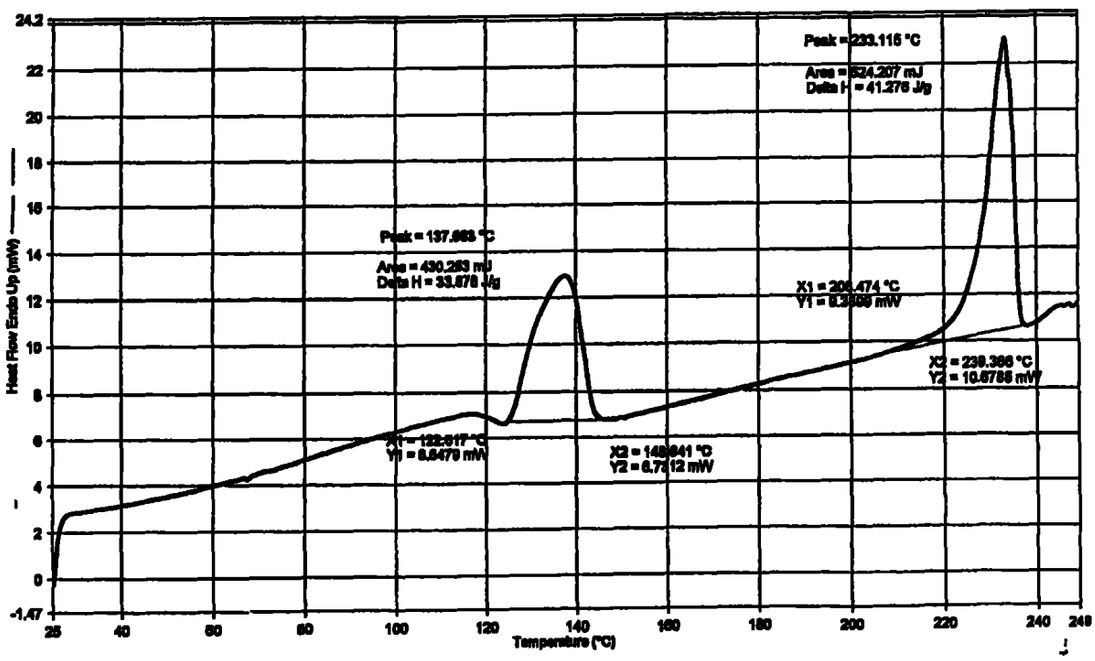


Figure 2-J

Sample Weight: 6.704 mg
Comment: Acetone Solvate

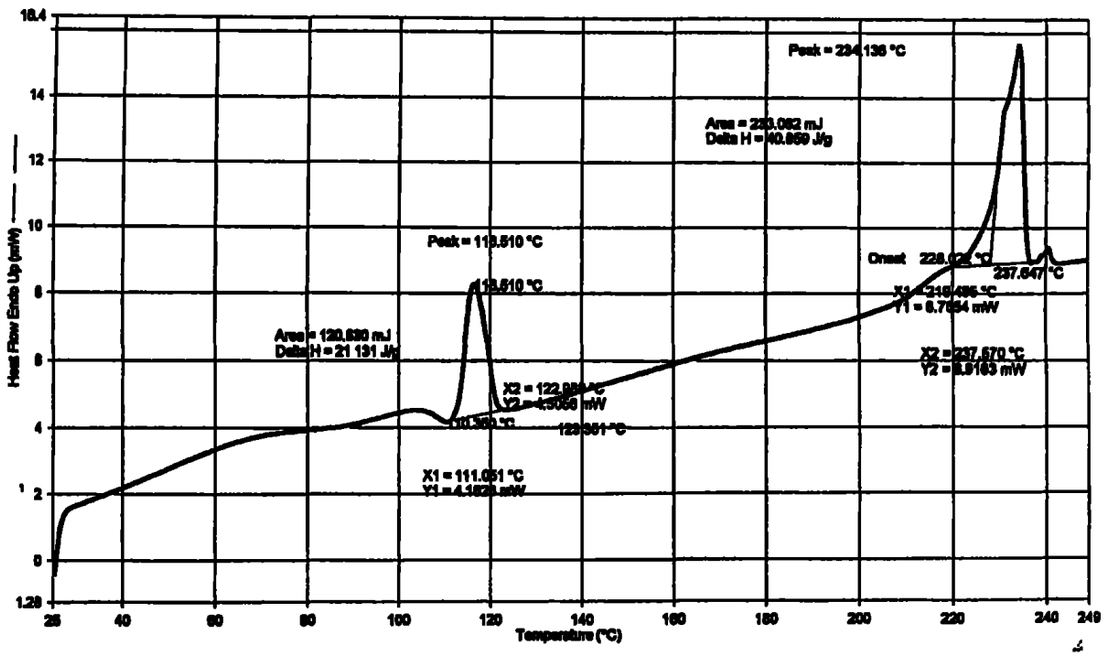
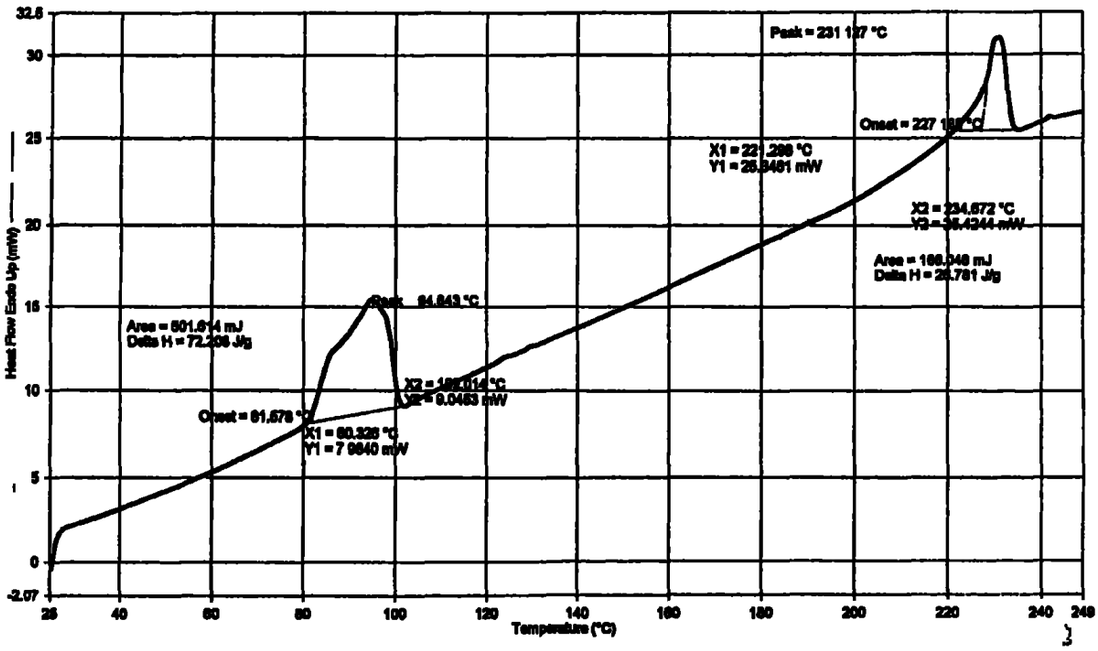


Figure 2-K

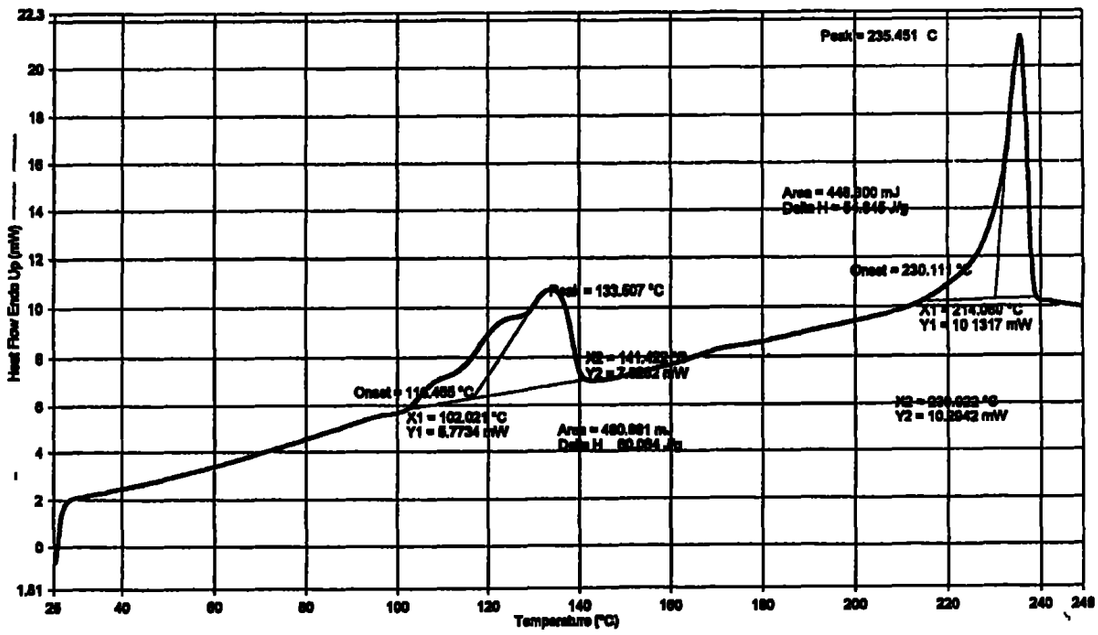
Sample Weight: 8.947 mg
Comment: Toluene Solvate



614

Figure 6L

Sample Weight:	5.174 mg
Comment:	Isopropanol Solvate



645

Figure 2 H

Sample Weight: 6.122 mg
Comment: ethanol solvate
10 °C/min sealed pan

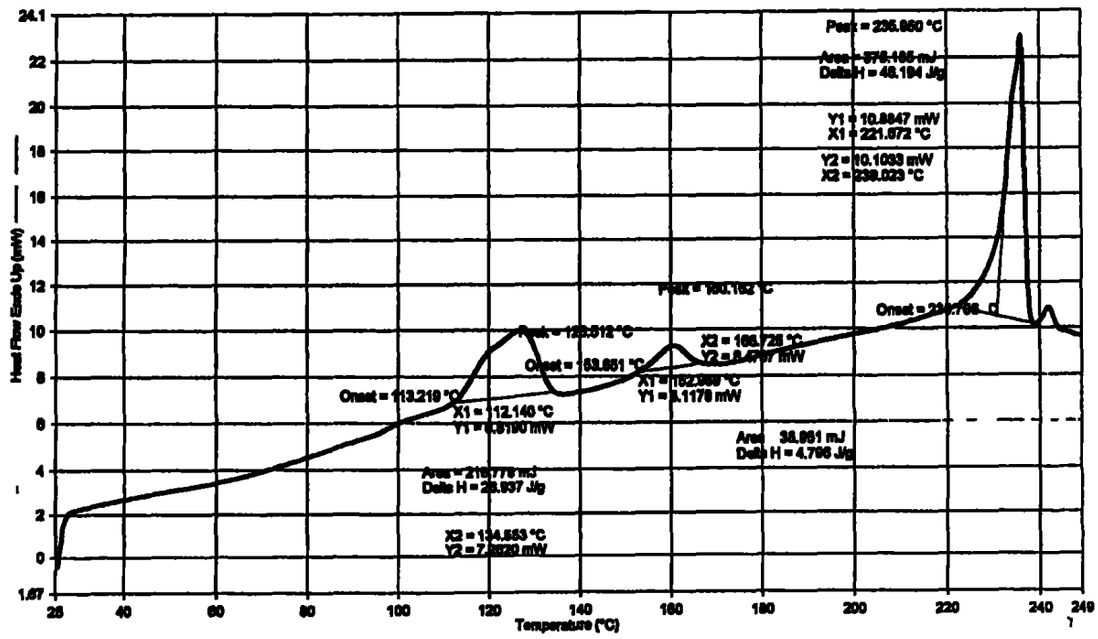
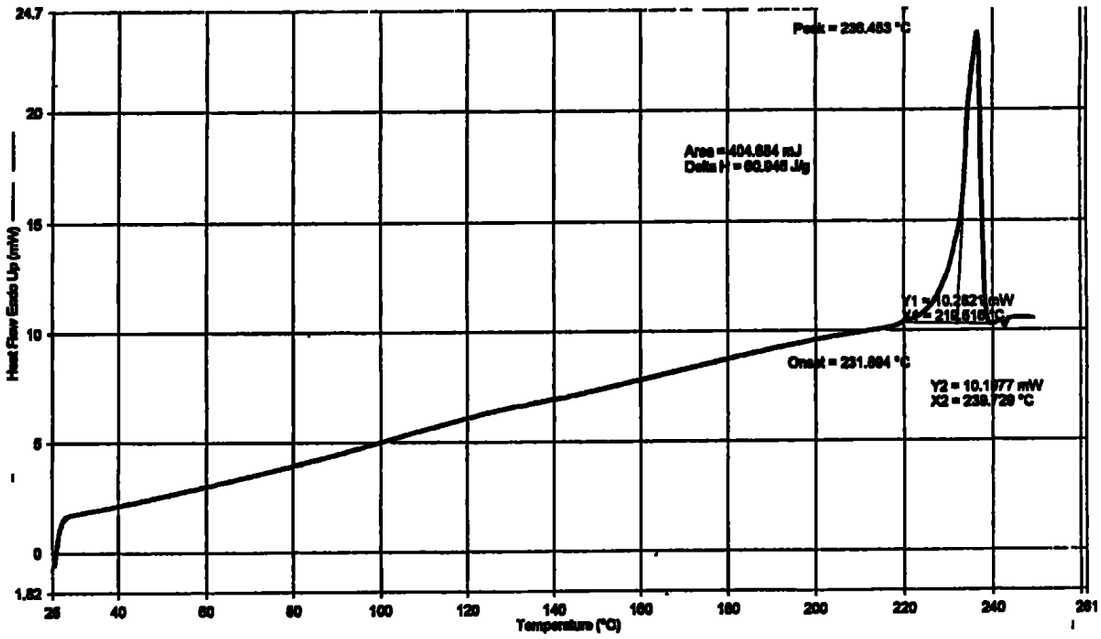


Figure 2-N

[redacted]

Sample Weight: 6.640 mg
Comment: t-butyl acetate solvate
10 °C/min sealed pan



617

Figure 10

Sample Weight: 9.800 mg
Comment: Isobutyl acetate solvate
10 °C/min sealed pan

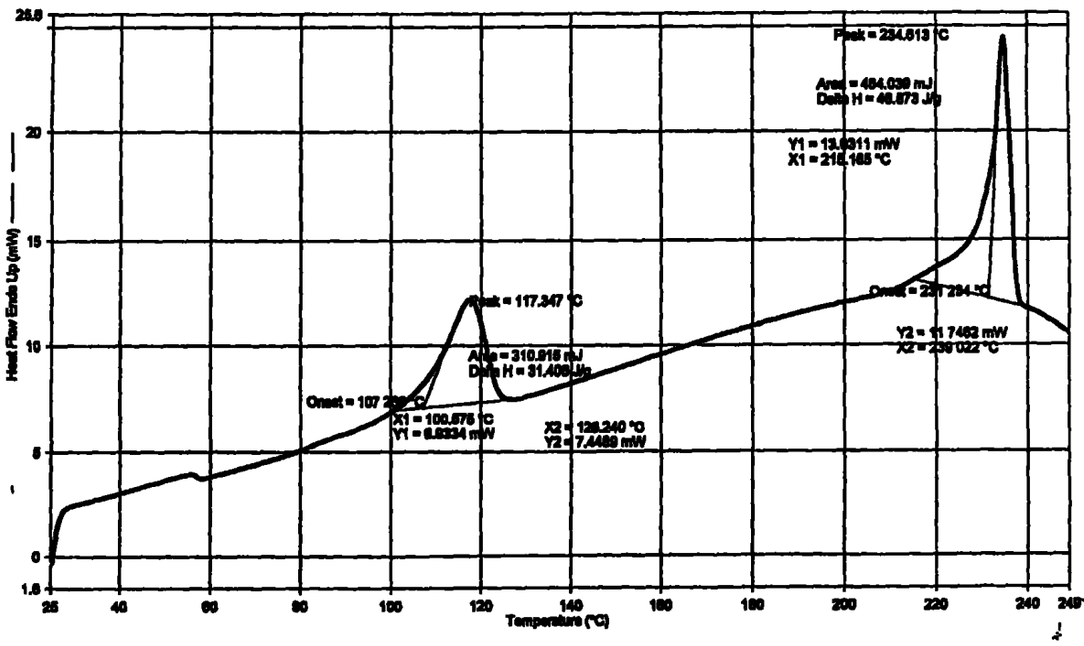
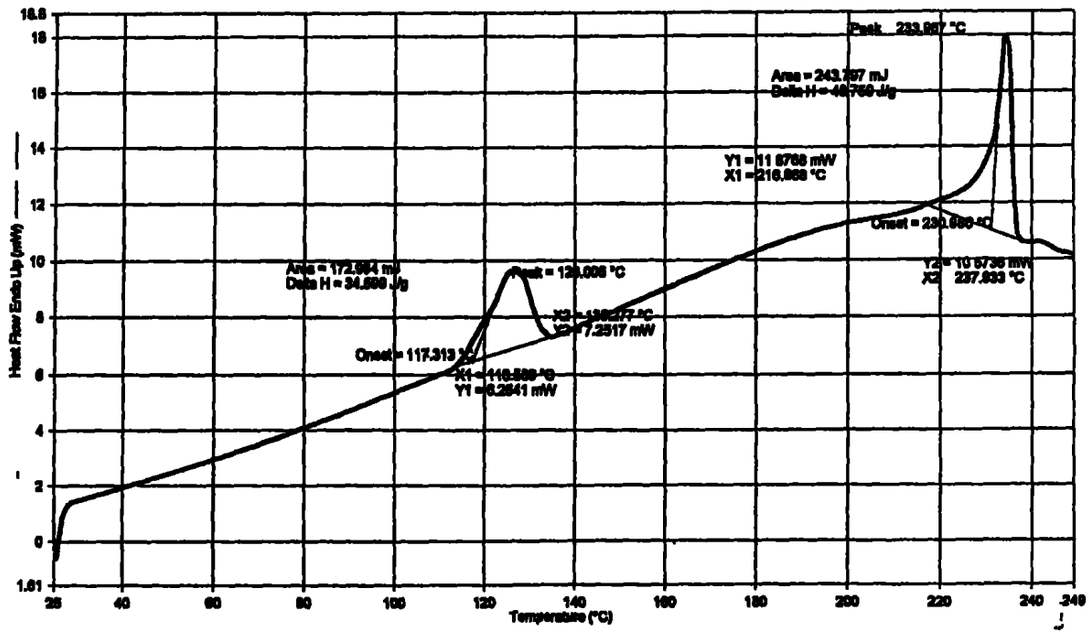


Figure P

Sample Weight: 8.0000 mg
Comment: butyl acetate solvate
10 °C/min sealed pan



649

Figure 10

Sample Weight: 5.538 mg
Comment:
methyl acetate solvate 1
10 °C/min sealed pan

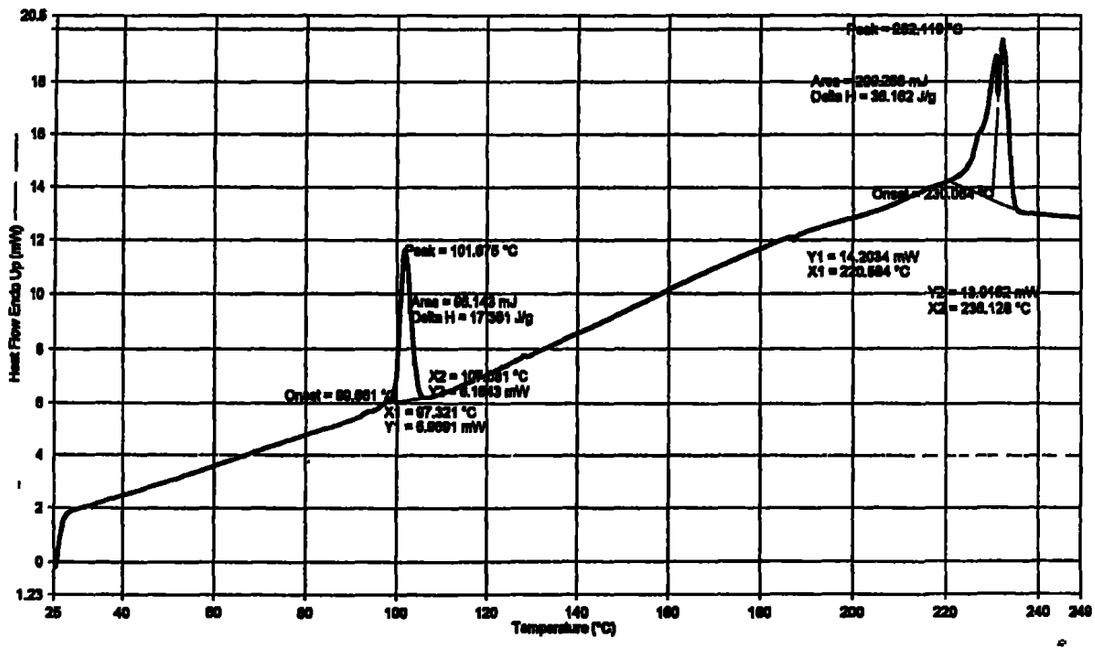


Figure 2-R

Sample Weight: 6.074 mg
Comment:
propyl acetate solvate 1
10 °C/min sealed pan

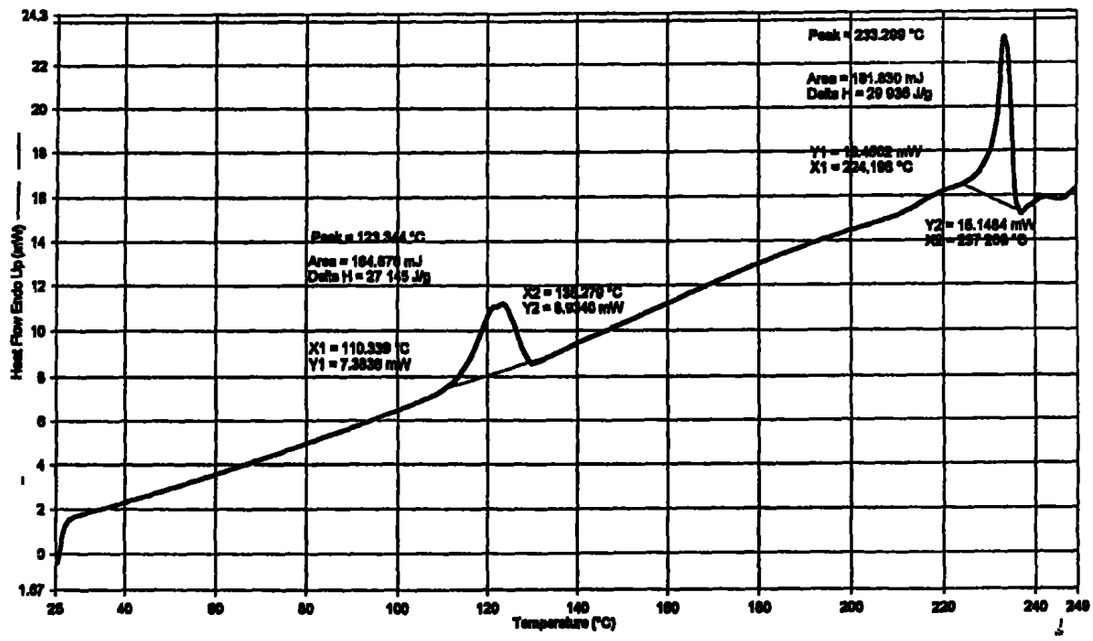


Figure 2-S

n-butanol solvate

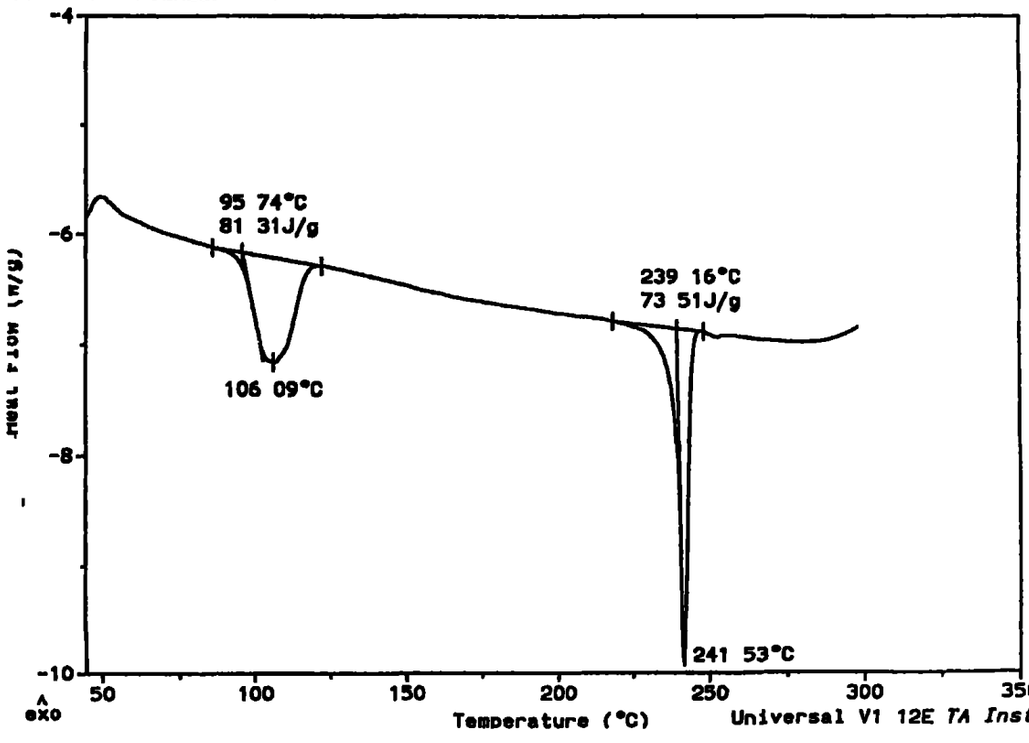
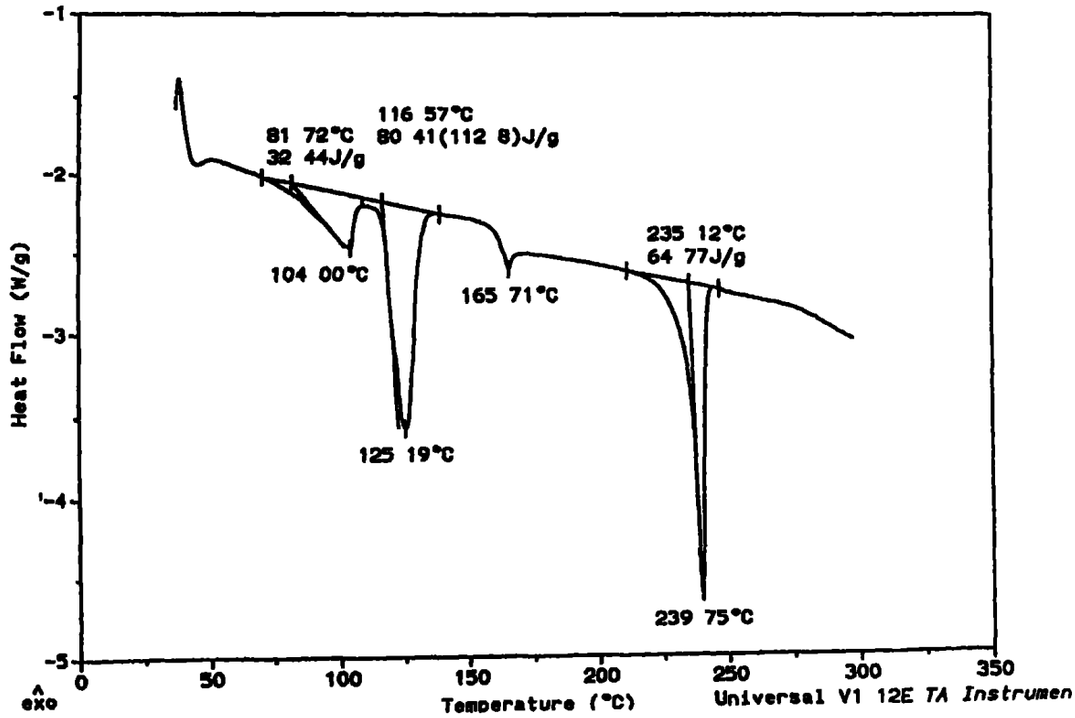


Figure 7

n-octanol solvate

Size 0 5220 mg
Method 10 DEG C/MIN AMB TO 300
Comment UNSEALED PAN



623

Figure 2A

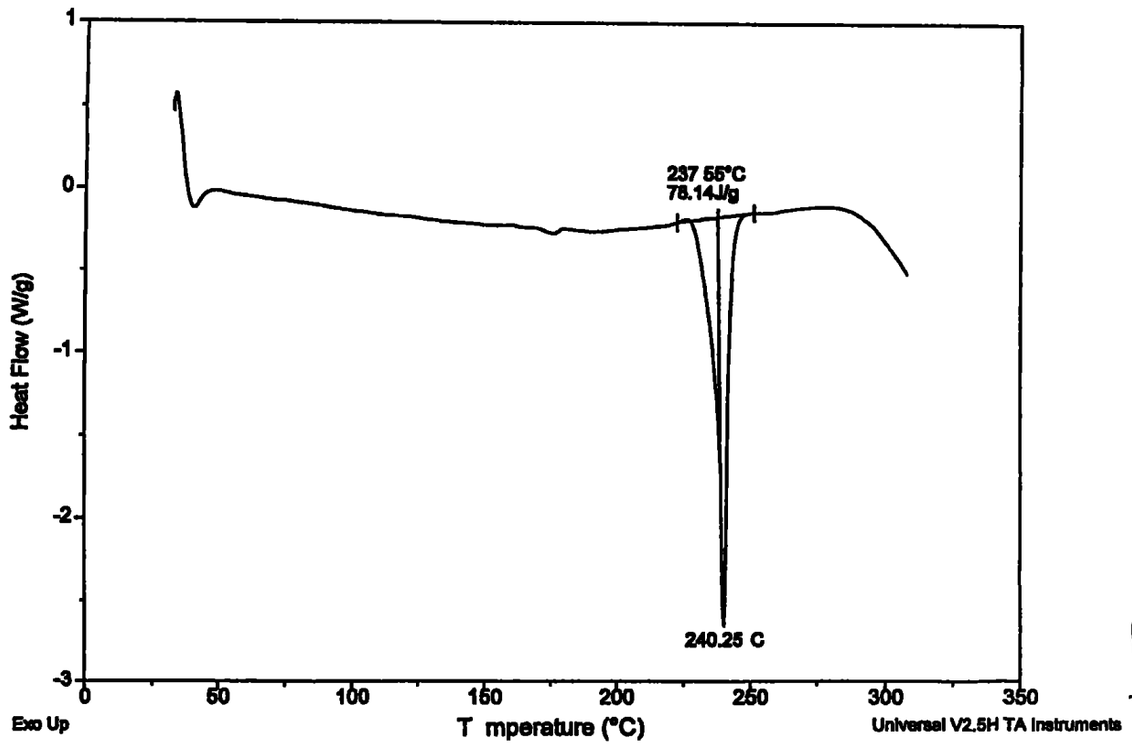

Size. 0 6360 mg
Method 10 DEG C/MIN AMB TO 300
Comment: SEALED PAN
1
0
-1
-2
-3
Heat Flow (W/g)
237.55°C
78.14J/g
240.25 C
0
50
100
150
200
250
300
350
Exo Up
T mperature (°C)
Universal V2.5H TA Instruments

Figure 2-8

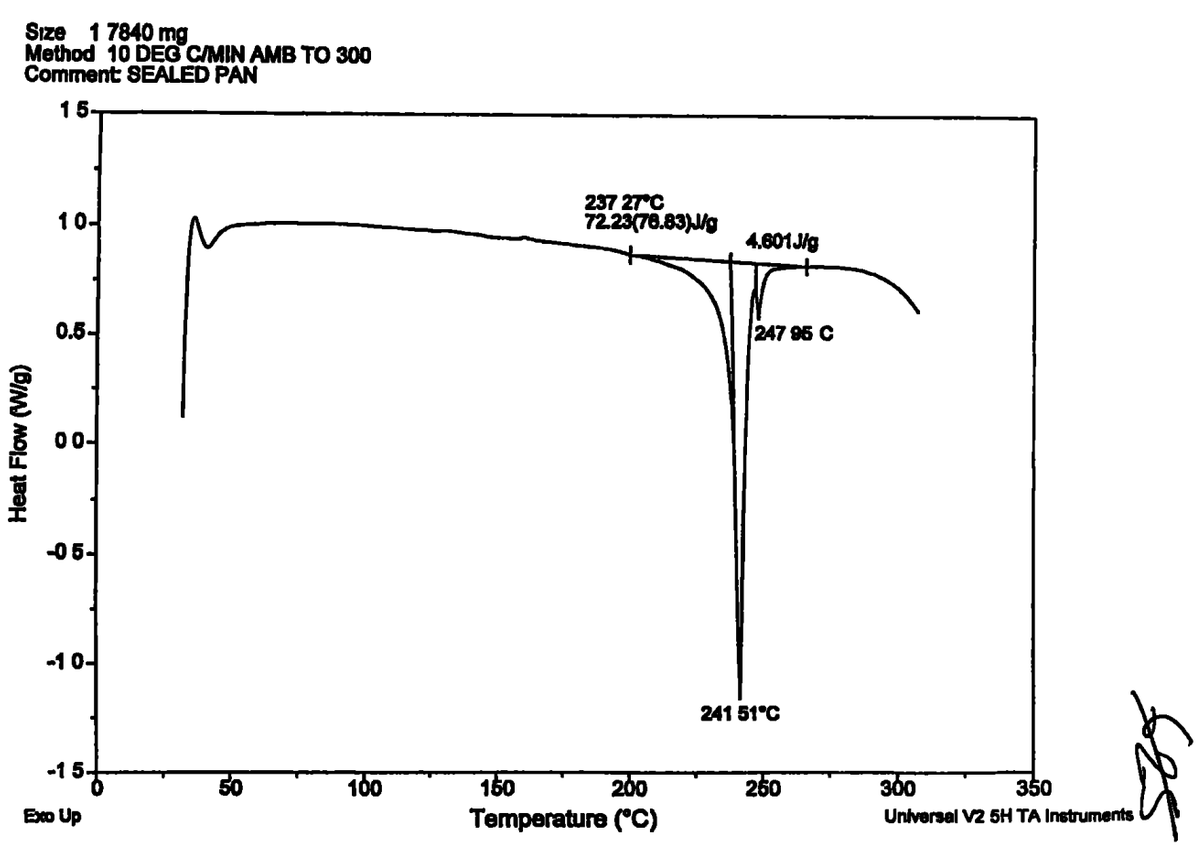
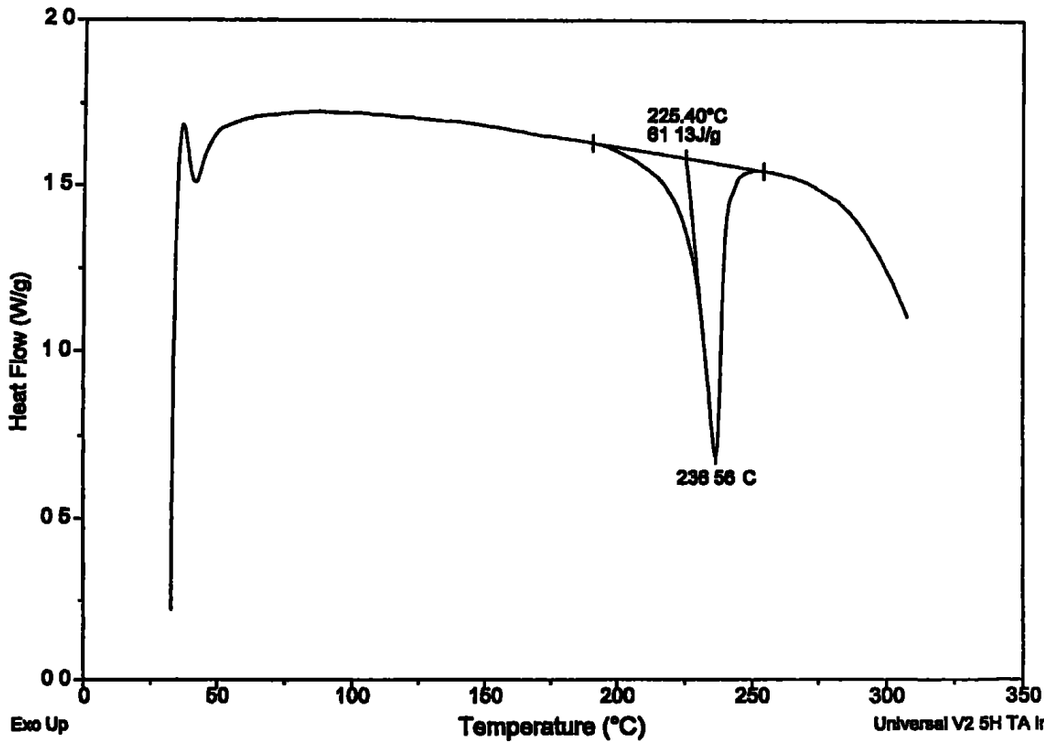

Size 1 7840 mg
Method 10 DEG C/MIN AMB TO 300
Comment: SEALED PAN
237 27°C
72.23(76.83)J/g
4.601J/g
247 95 C
241 51°C
Heat Flow (W/g)
Exo Up
Temperature (°C)
Universal V2 5H TA Instruments

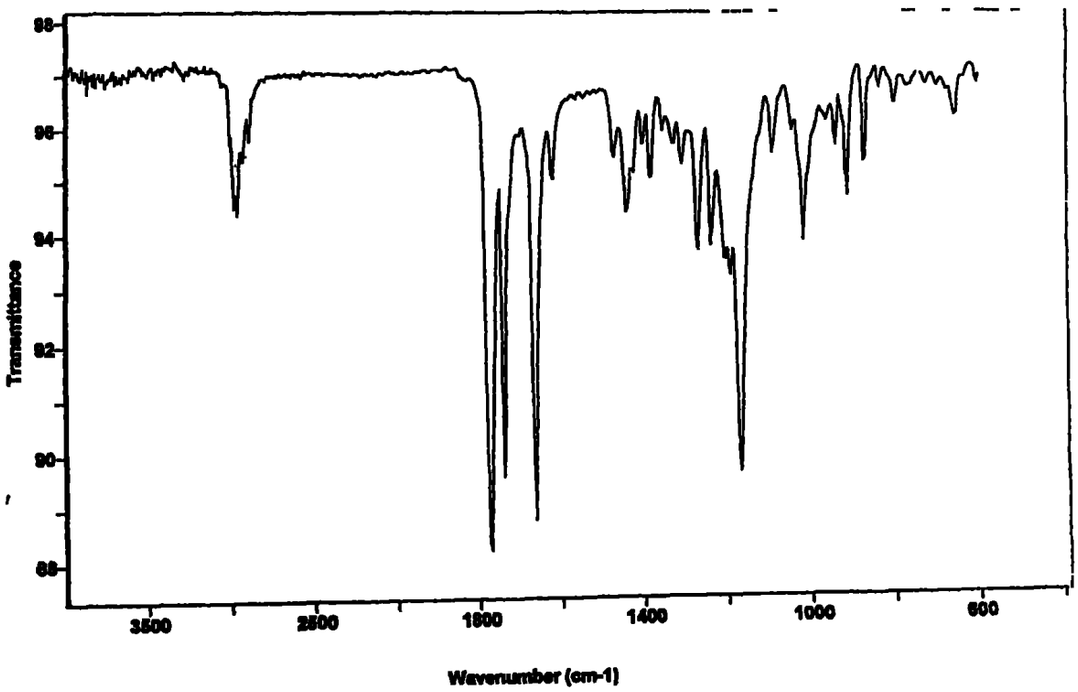
Figure 2-C

SG802T 8096STO9

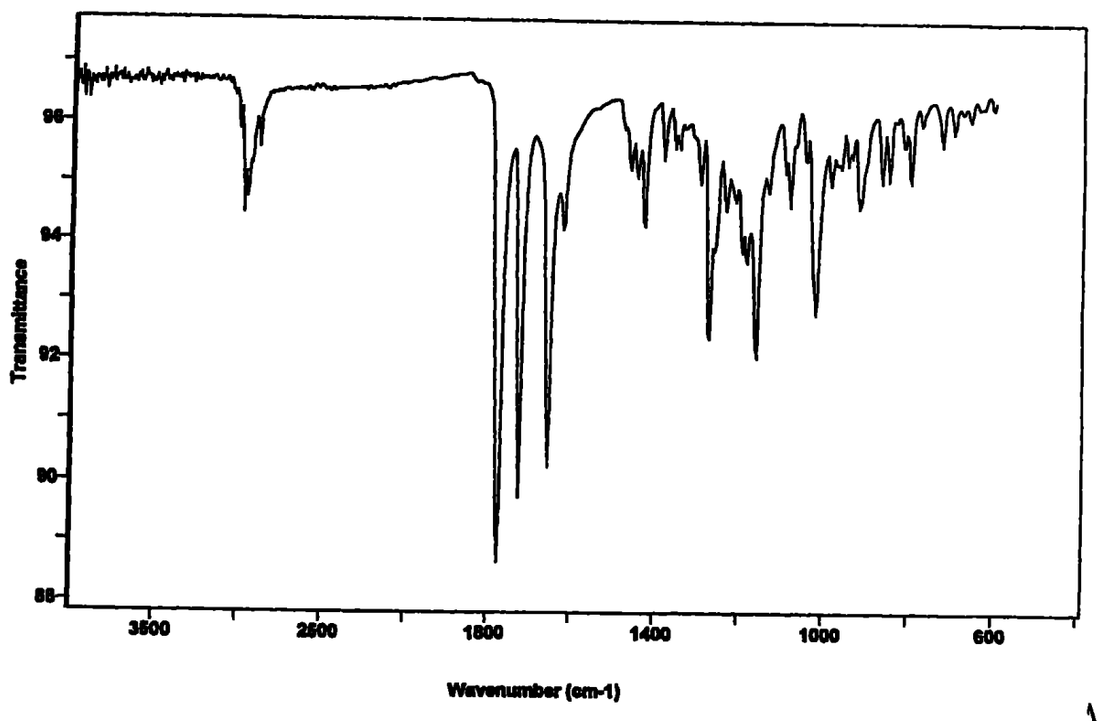
Size 1 4230 mg
Method 10 DEG C/MIN AMB TO 300
Comment. SEALED PAN



Figure


Transmittance
98
96
94
92
90
88
3500
2500
1800
1400
1000
600
Wavenumber (cm-1)

Figure


Transmittance
98
94
92
90
88
3500
2500
1800
1400
1000
600
Wavenumber (cm-1)

Figure

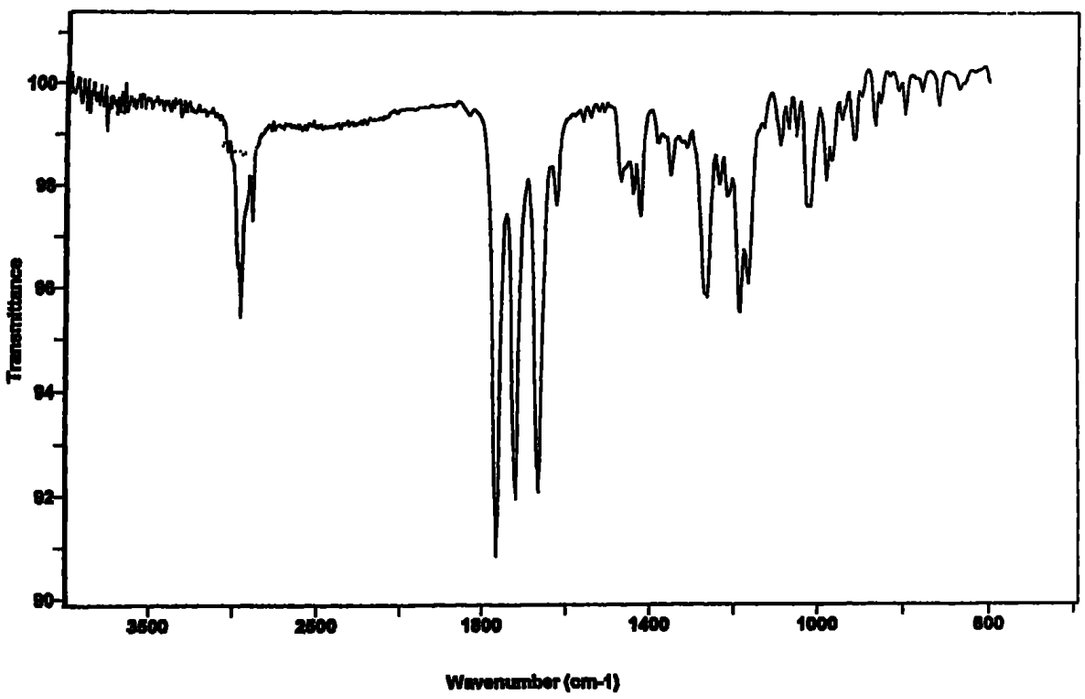

100
98
96
94
92
90
Transmittance
3500
2500
1800
1400
1000
800
Wavenumber (cm-1)

Figure 10D

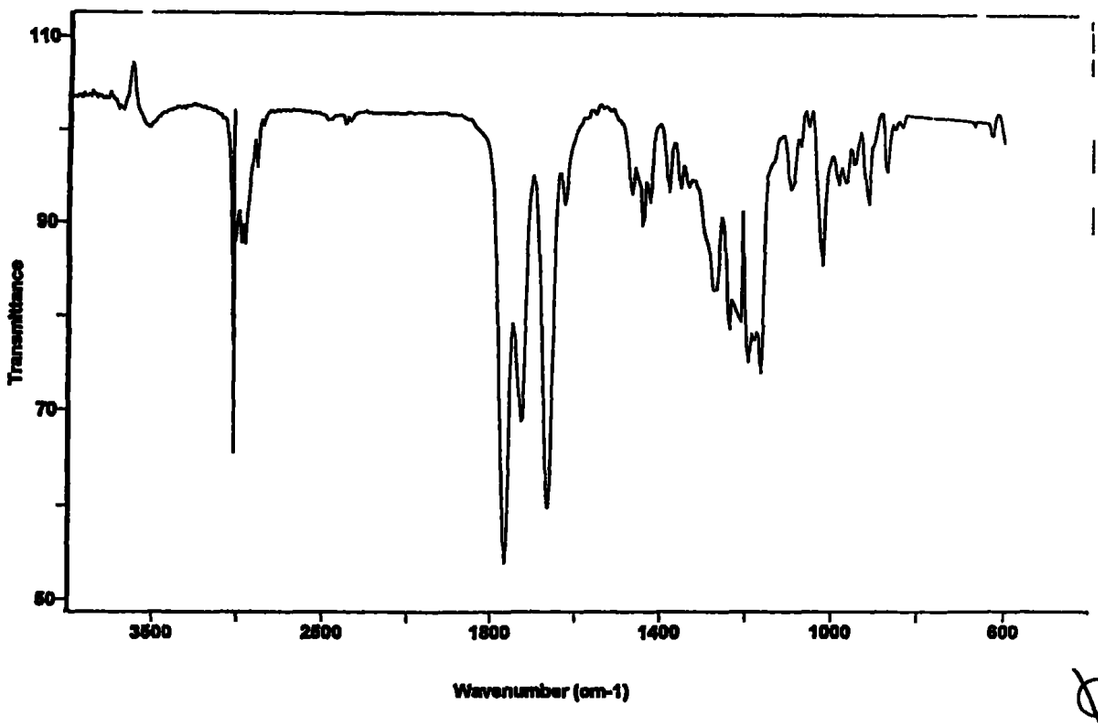

110
90
70
50
Transmittance
3500
2500
1800
1400
1000
600
Wavenumber (cm-1)

Figure E

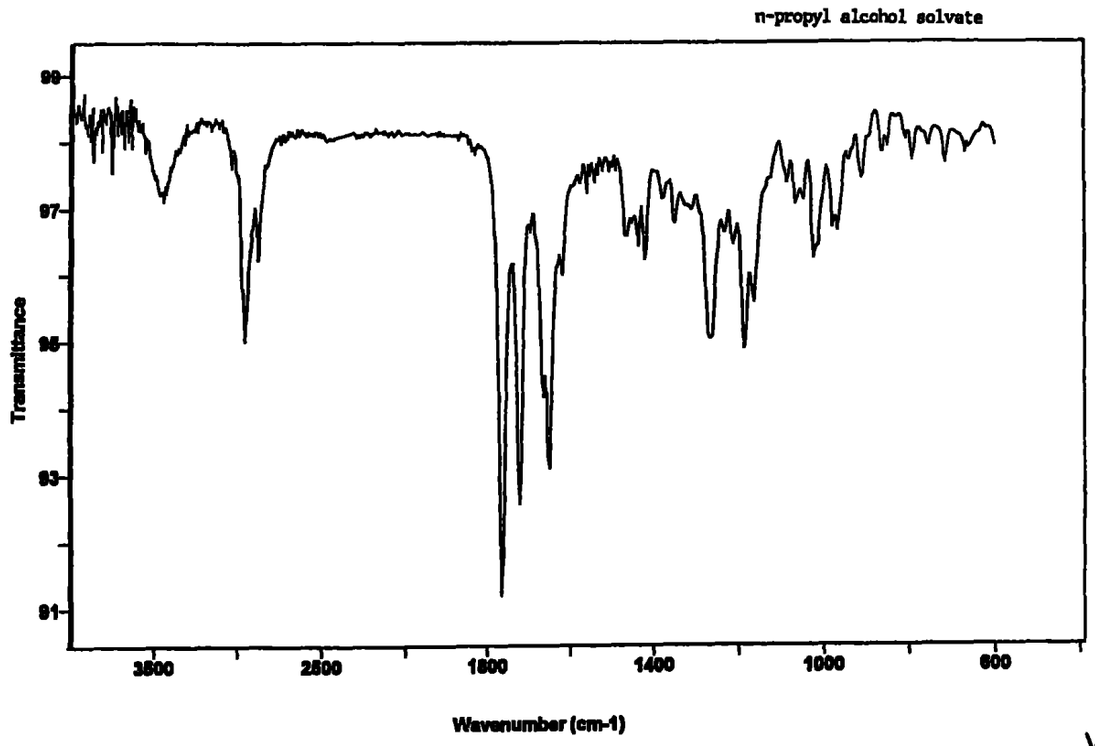

n-propyl alcohol solvate
99
97
95
93
91
Transmittance
3800
2800
1800
1400
1000
600
Wavenumber (cm-1)

Figure F

tetrahydrofuran solvate

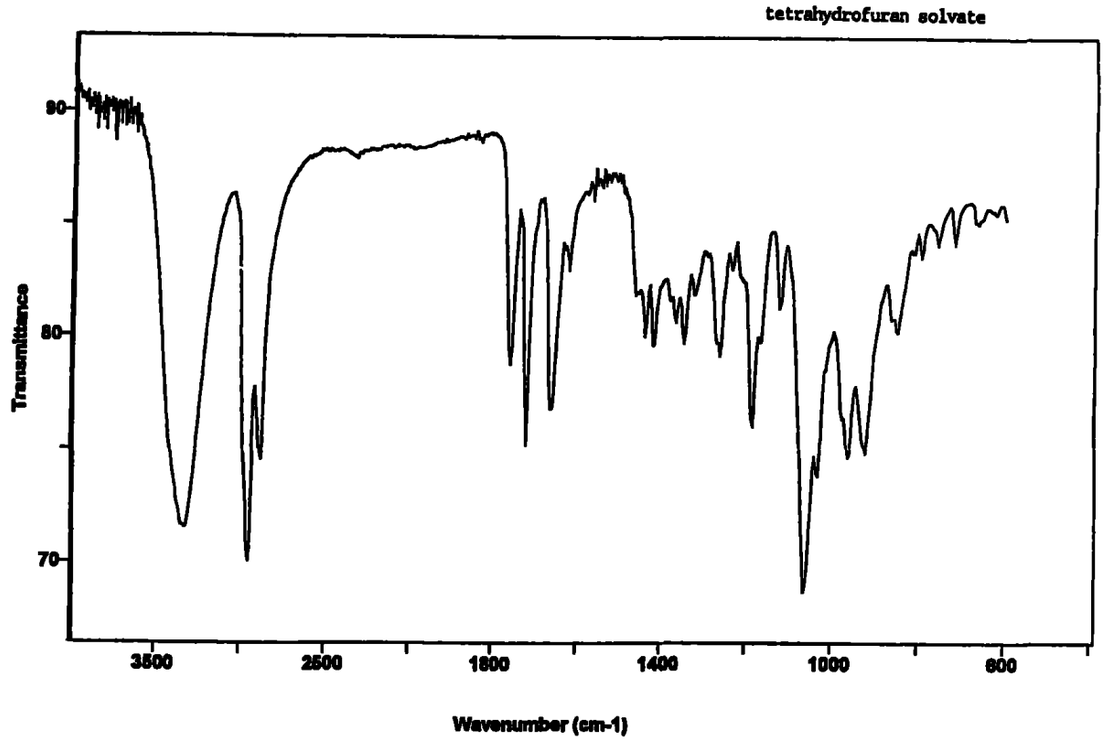


Figure 6G

GG8027 8096908

ethyl propionate solvate

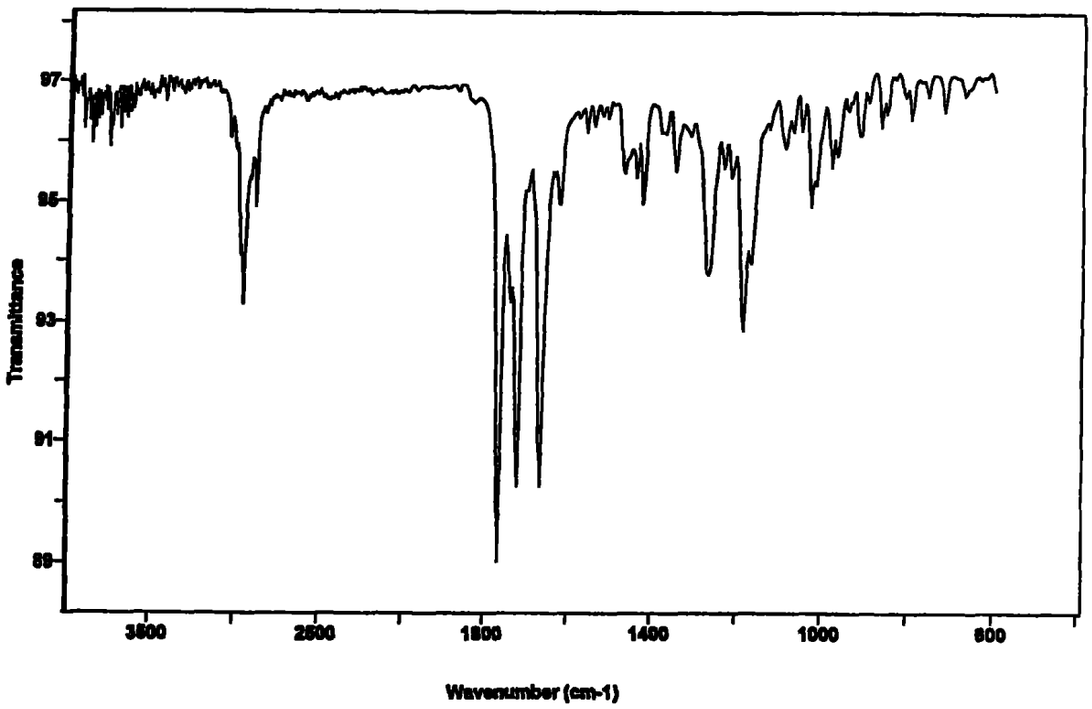


Figure [illegible]

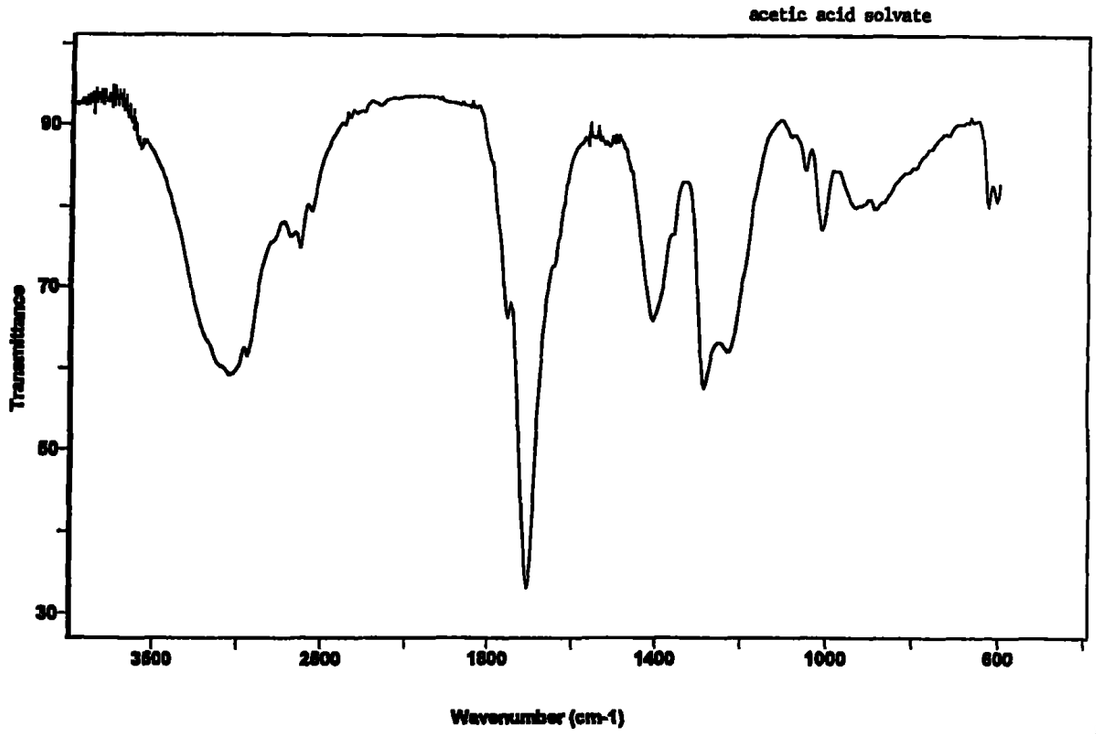

acetic acid solvate
90
70
50
30
Transmittance
3500
2500
1500
1400
1000
600
Wavenumber (cm-1)

Figure [redacted]

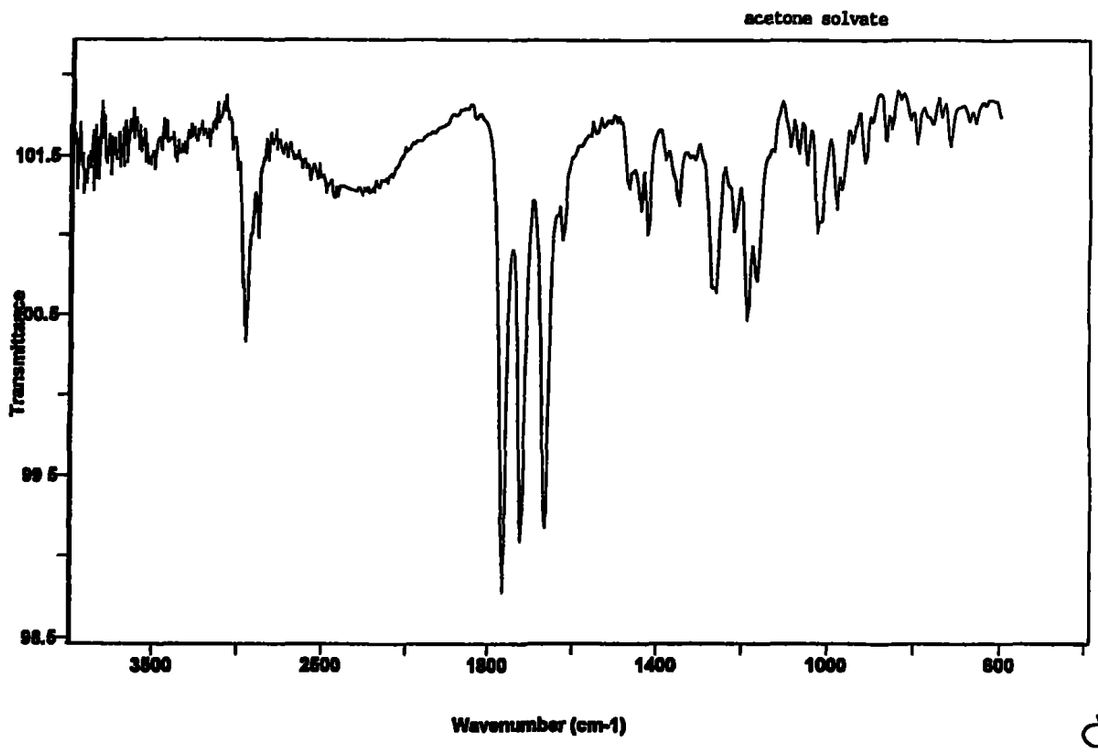


Figure 2J

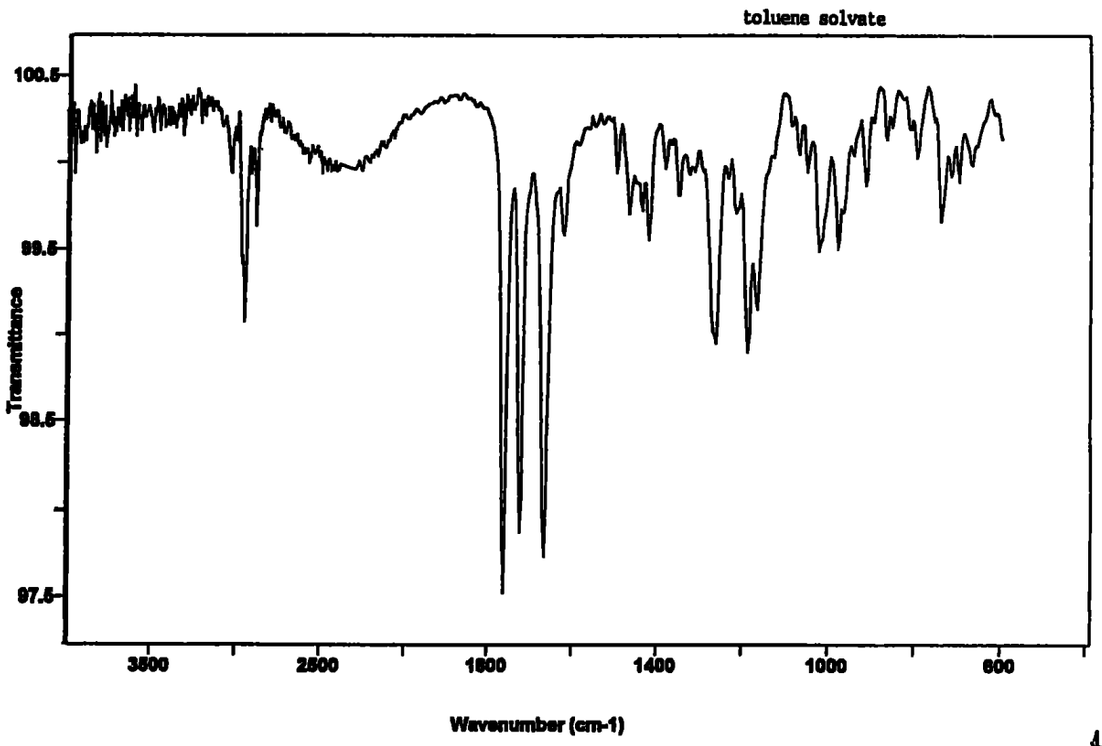
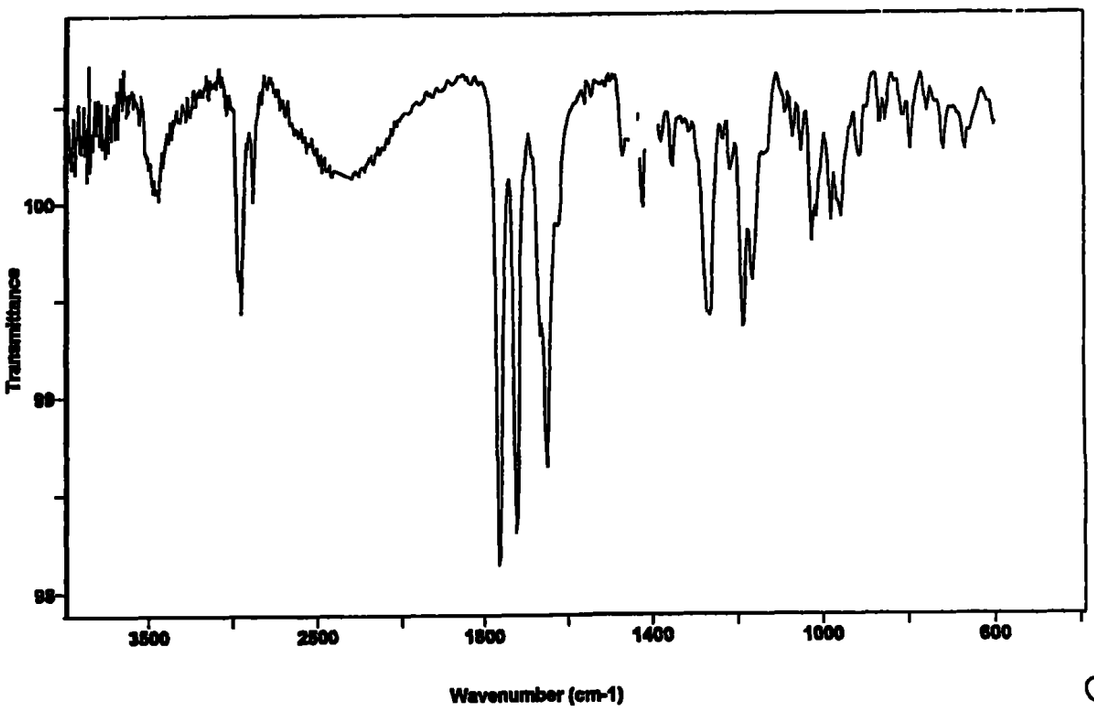

toluene solvate
100.5
99.5
98.5
97.5
Transmittance
3500
2500
1800
1400
1000
600
Wavenumber (cm-1)

Figure [illegible]K

GG80ZT 809G9TOS

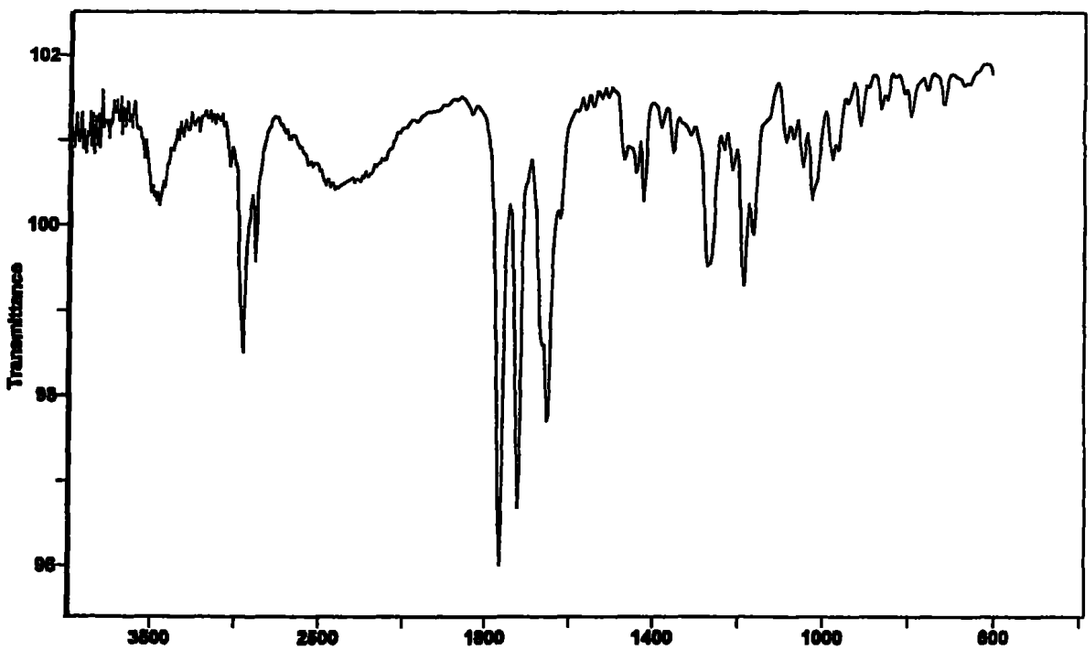
isopropanol solvate



634

Figure 1L

ethanol solvate



File # 1 = P4829A02

X-Zoom CURSOR

Figure 3M

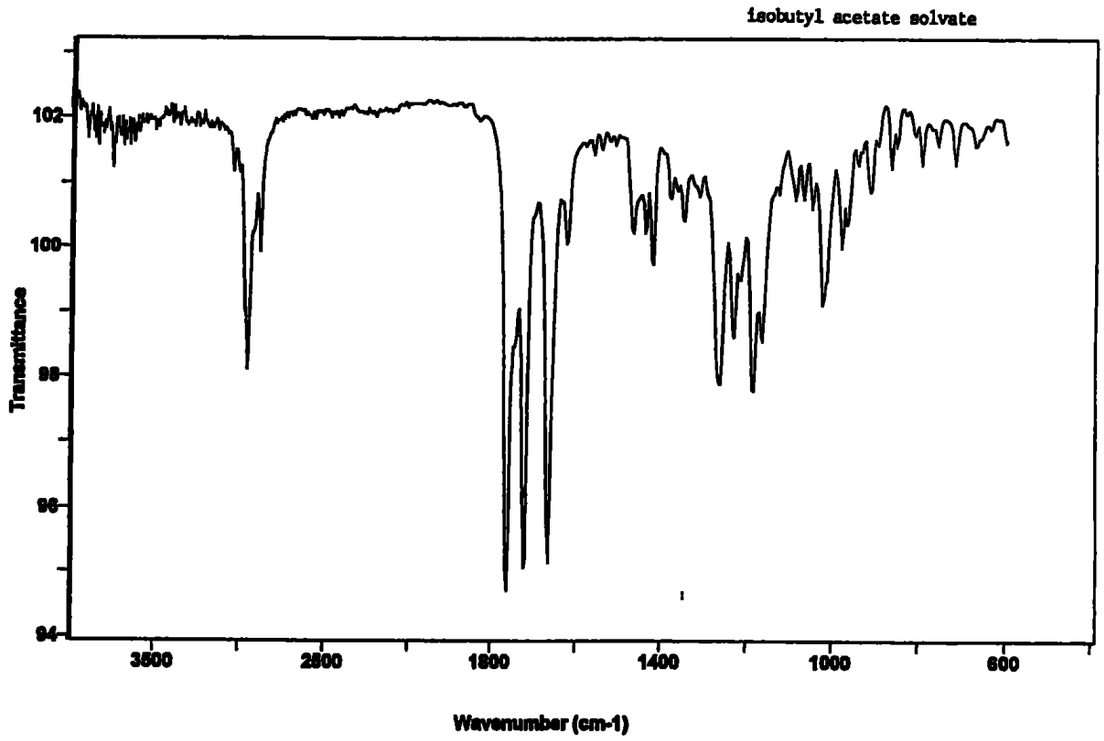

isobutyl acetate solvate
Transmittance
102
100
98
96
94
3600
2800
1800
1400
1000
600
Wavenumber (cm-1)

Figure 5N

butyl acetate solvate

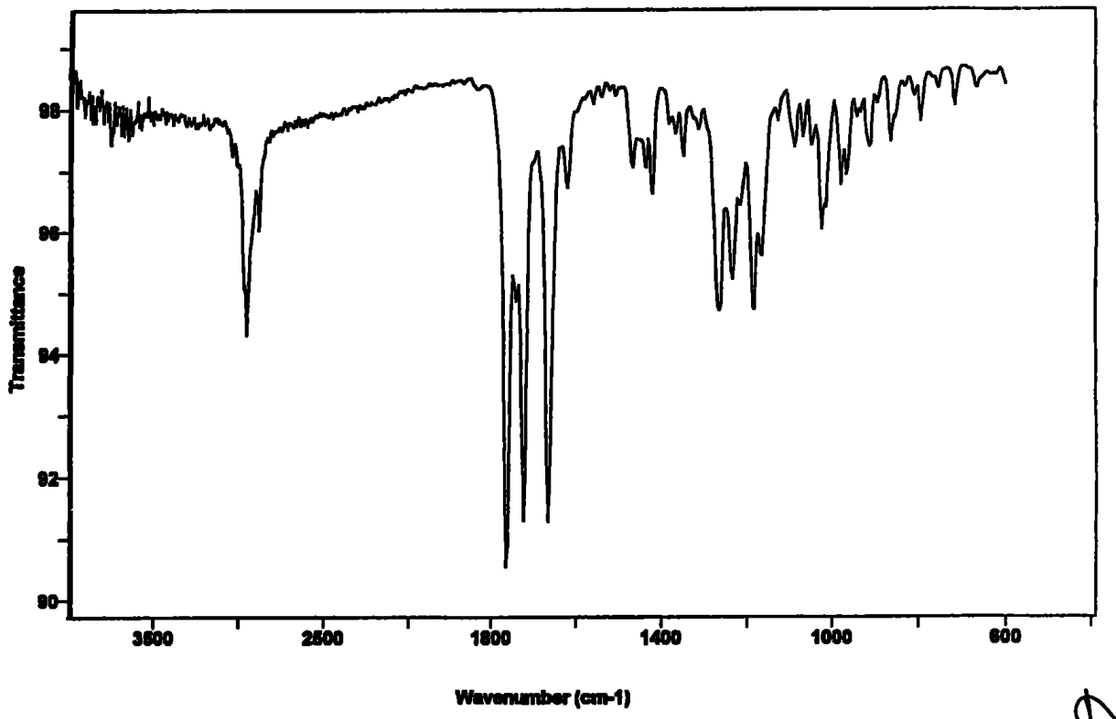


Figure 10

propyl acetate solvate

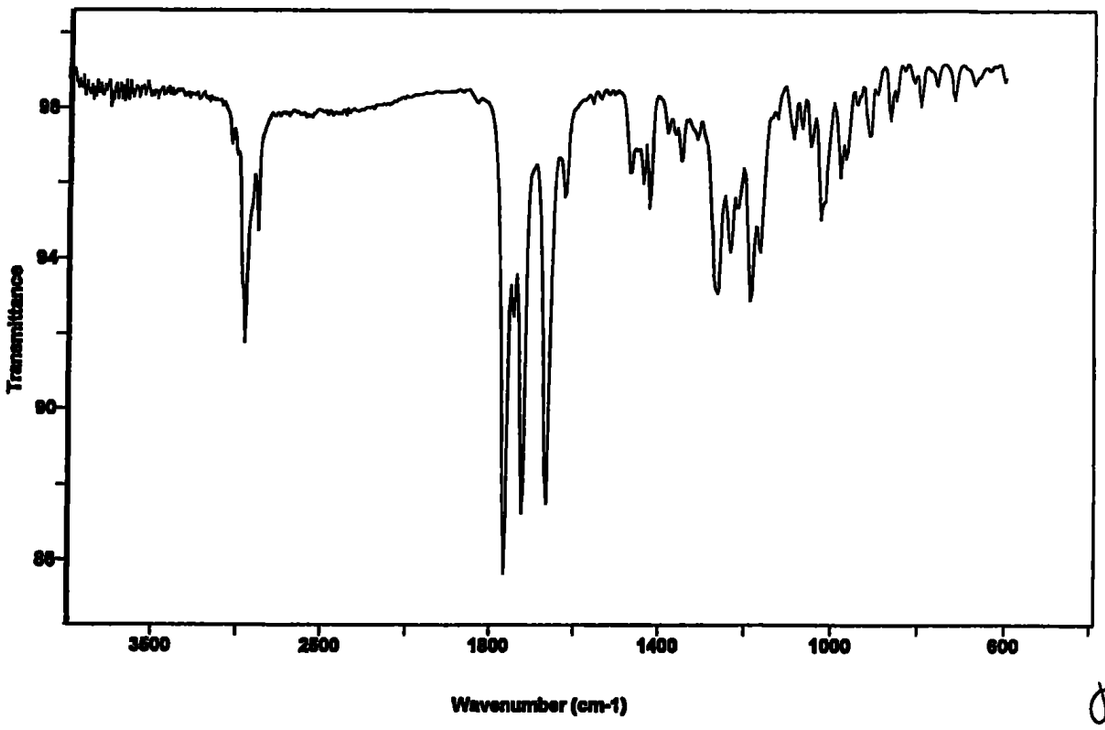


Figure 1-P

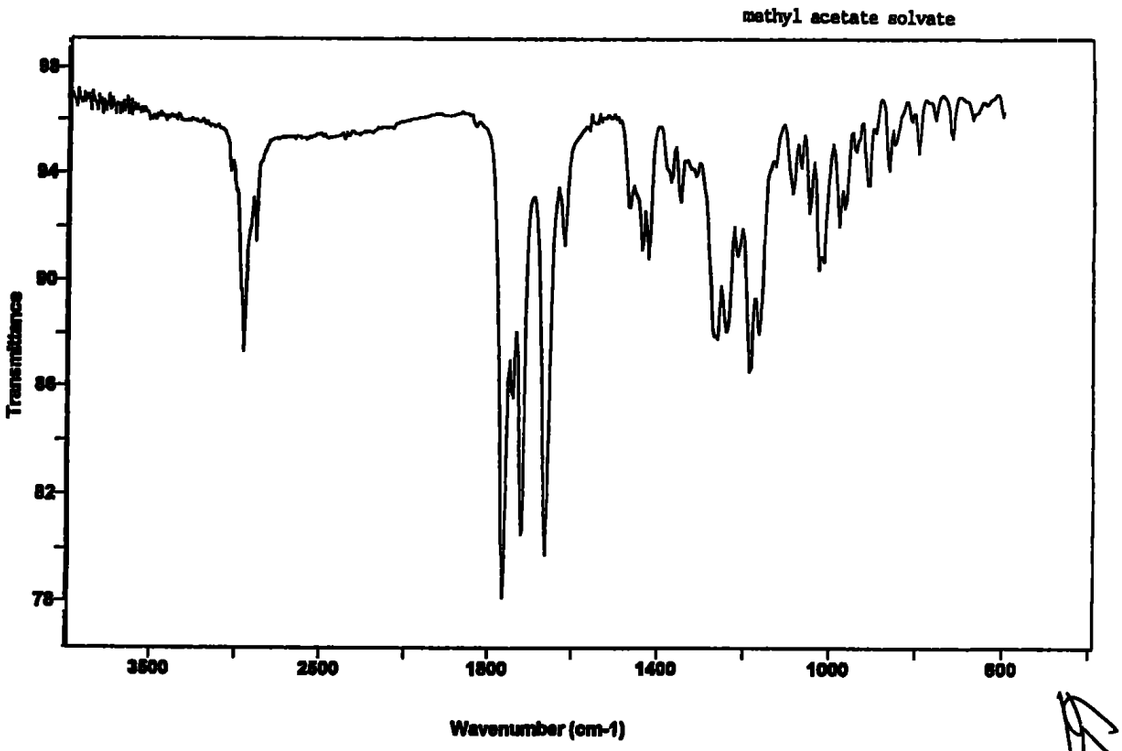

methyl acetate solvate
98
94
90
86
82
78
Transmittance
3500
2500
1800
1400
1000
800
Wavenumber (cm-1)

Figure 3-Q

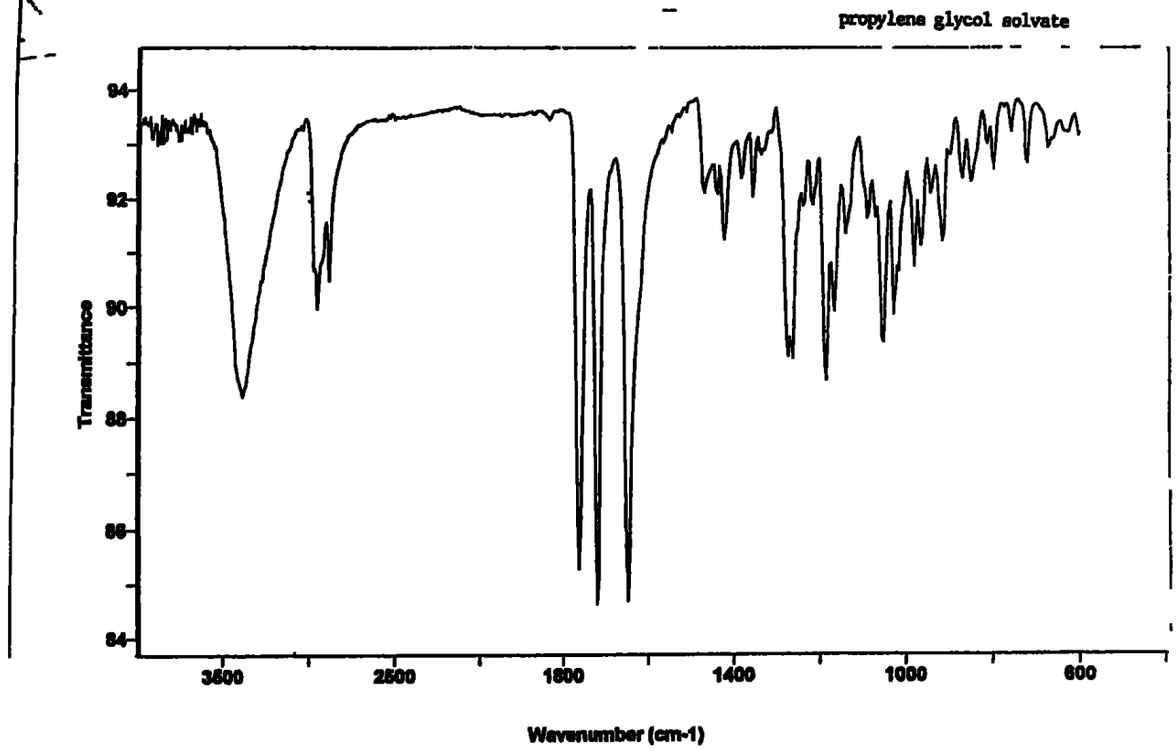

propylene glycol solvate
Transmittance
94
92
90
88
86
84
3500
2500
1800
1400
1000
600
Wavenumber (cm-1)

Figure 3-R

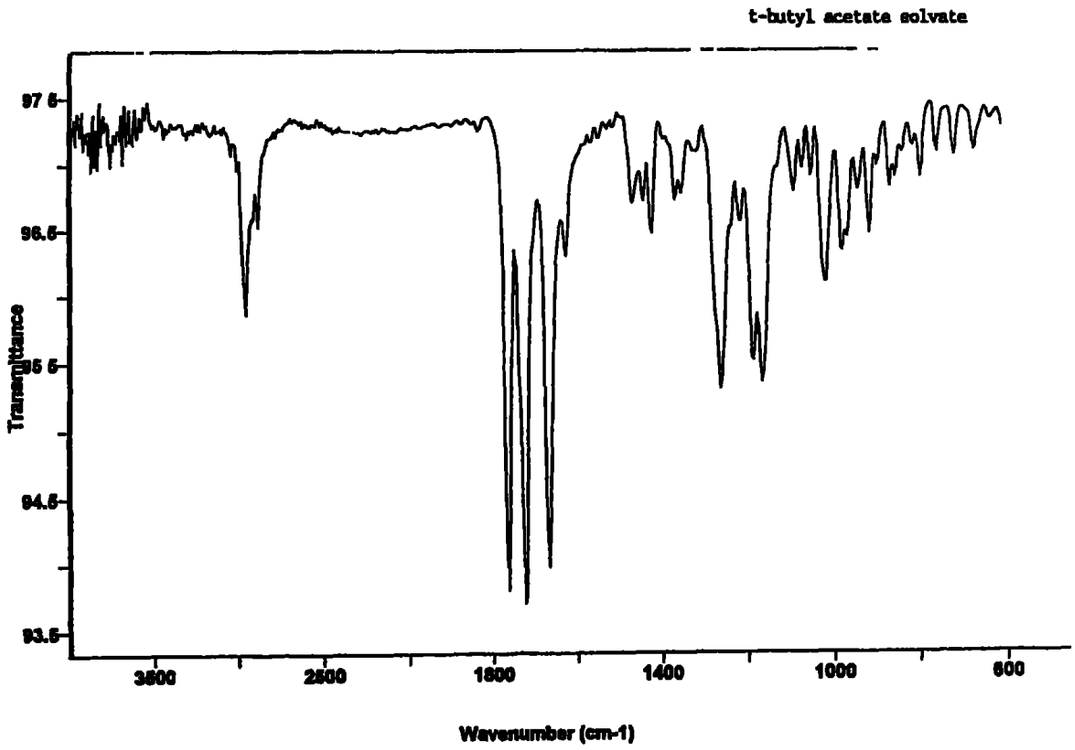

t-butyl acetate solvate
97.5
96.5
95.5
94.5
93.5
Transmittance
3500
2500
1800
1400
1000
600
Wavenumber (cm-1)

Figure 4

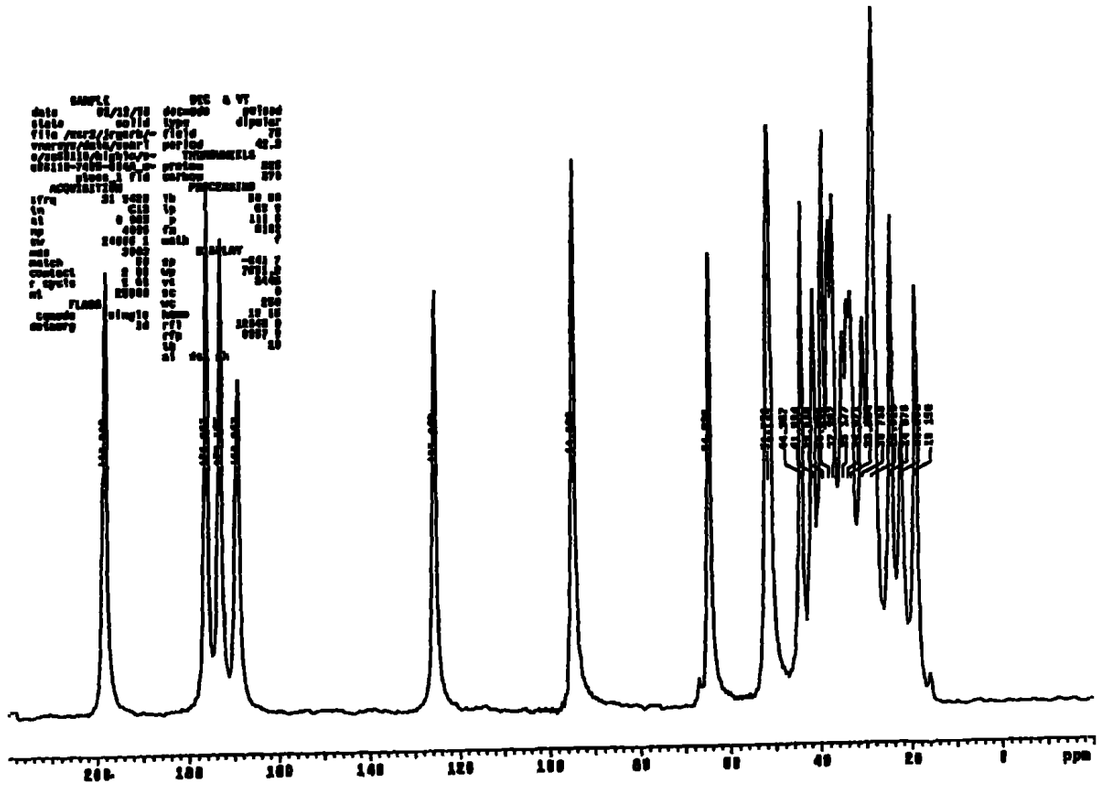


Figure 5

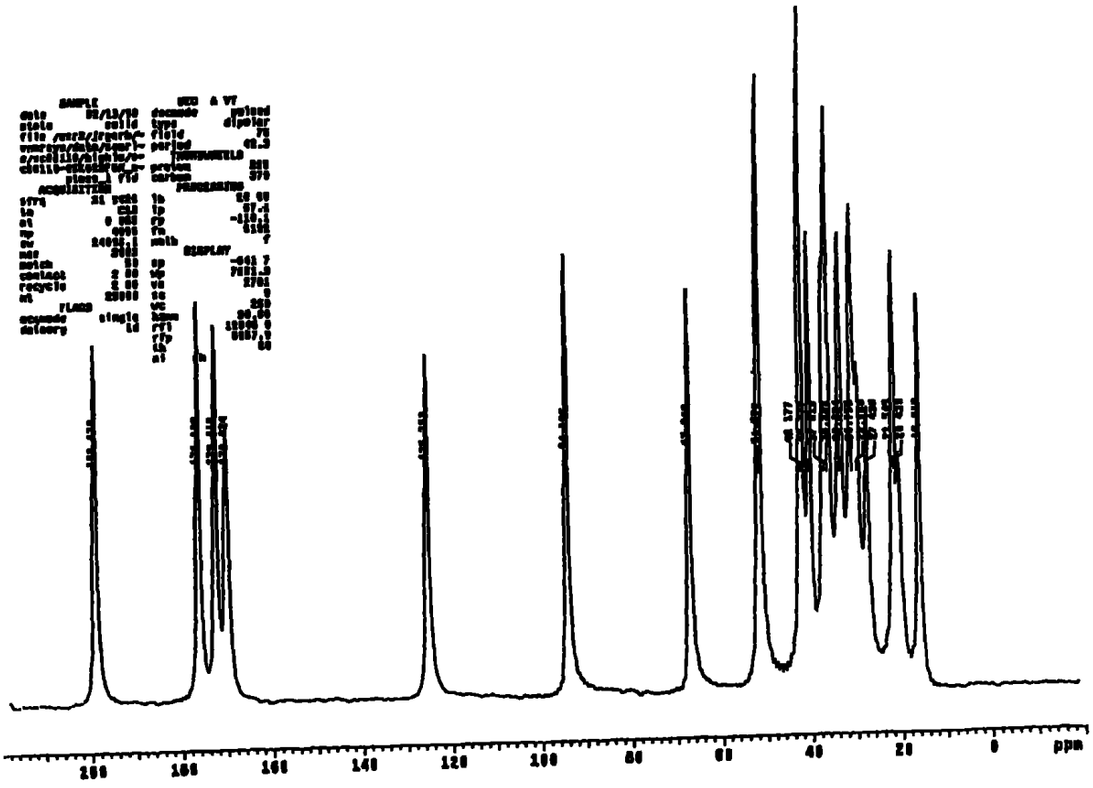


Figure 6-A

Size 3.3010 mg
Method 10 deg ramp to 300 deg

methyl ethyl ketone solvate

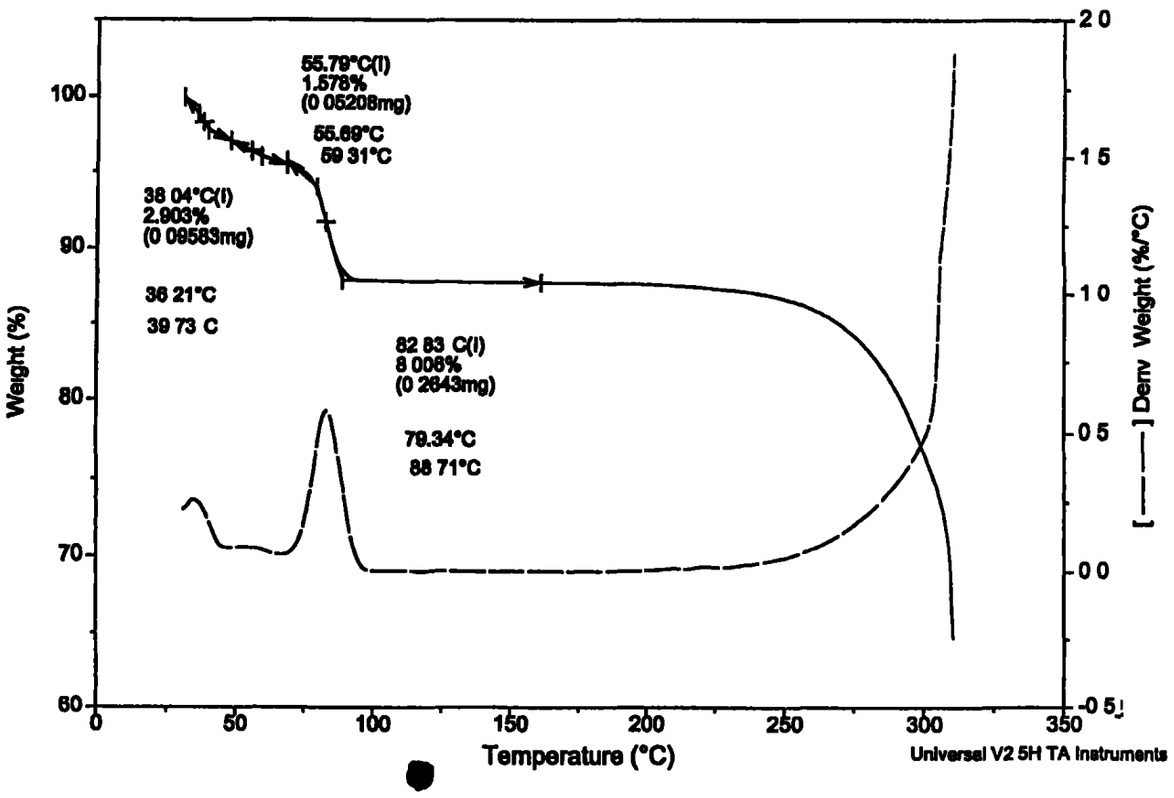
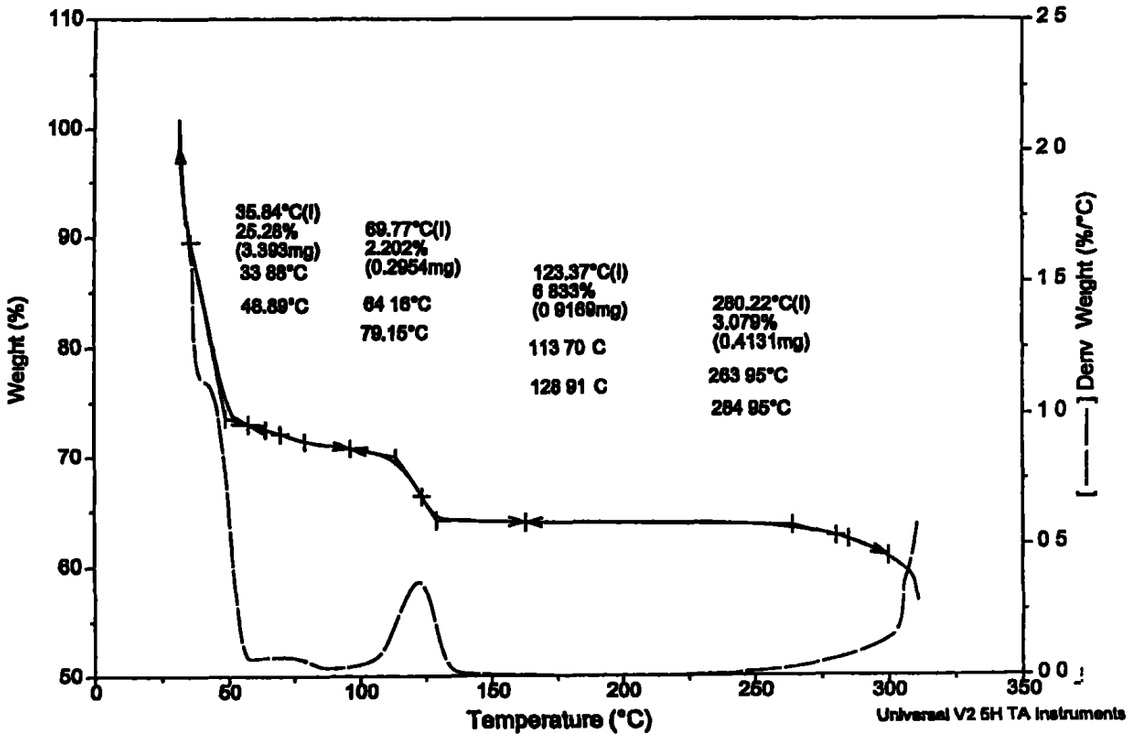


Figure 6-B

GG8021 8096S1D3

Size 13.4190 mg
Method 10 deg ramp to 300 deg

n-propyl alcohol solvate



649

Figure 6-C

[illegible]

Size 14 9110 mg
Method 10 deg ramp to 300 deg

tetrahydrofuran solvate

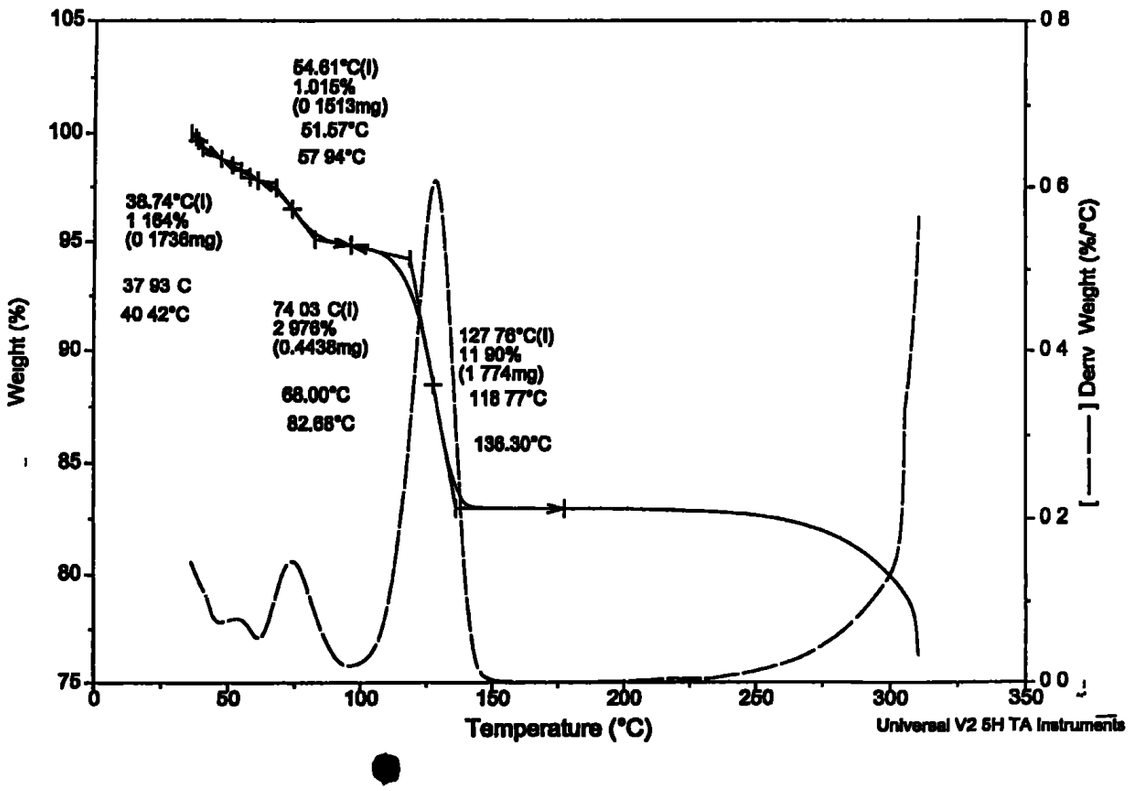


Figure 6-D

GG8021 8096010S

Size 9 5030 mg
Method 10 deg ramp to 300 deg

ethyl propionate solvate

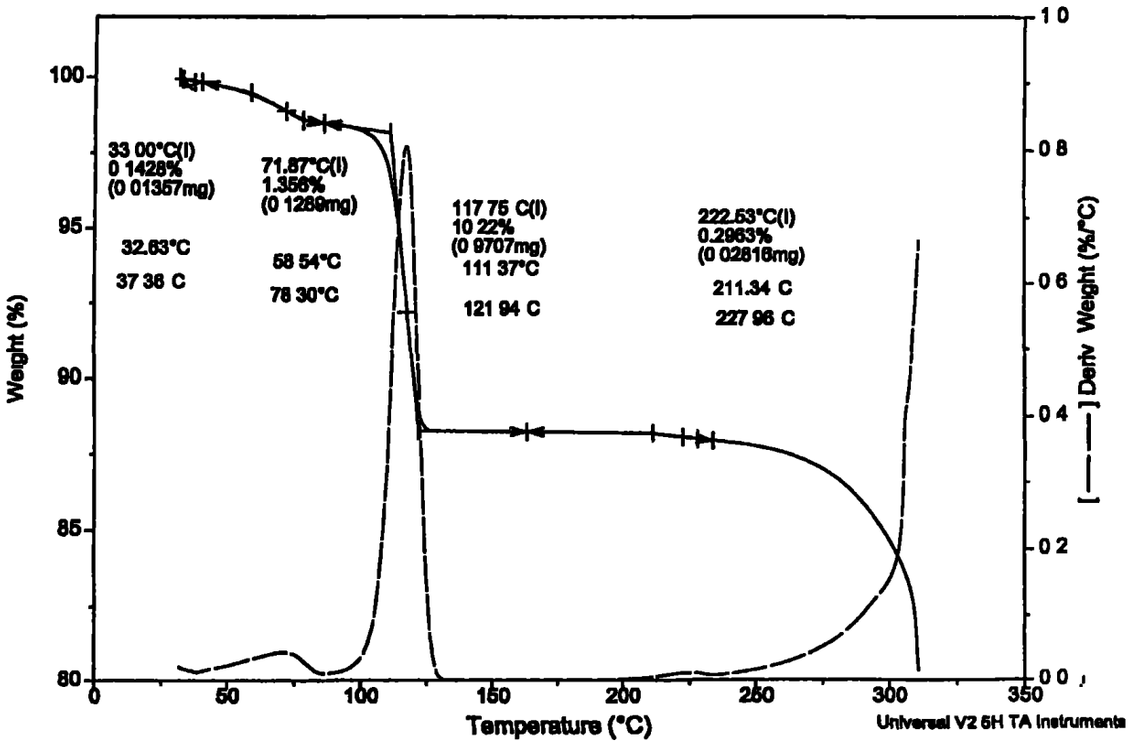


Figure 6-E

GG8027' 8096978 09

Size 8 4270 mg
Method 10 deg ramp to 300 deg

acetic acid solvate

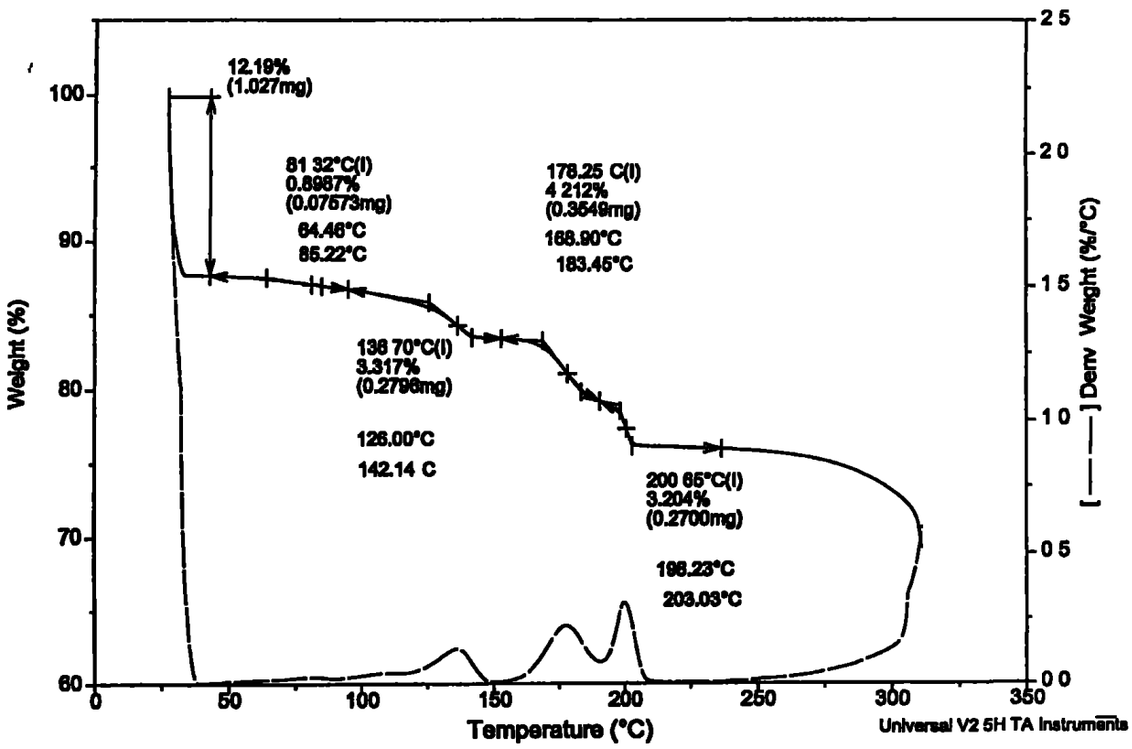


Figure 6-F

GG8021 8036S109

Size 3 9730 mg
Method 10 deg ramp to 300 deg

chloroform solvate

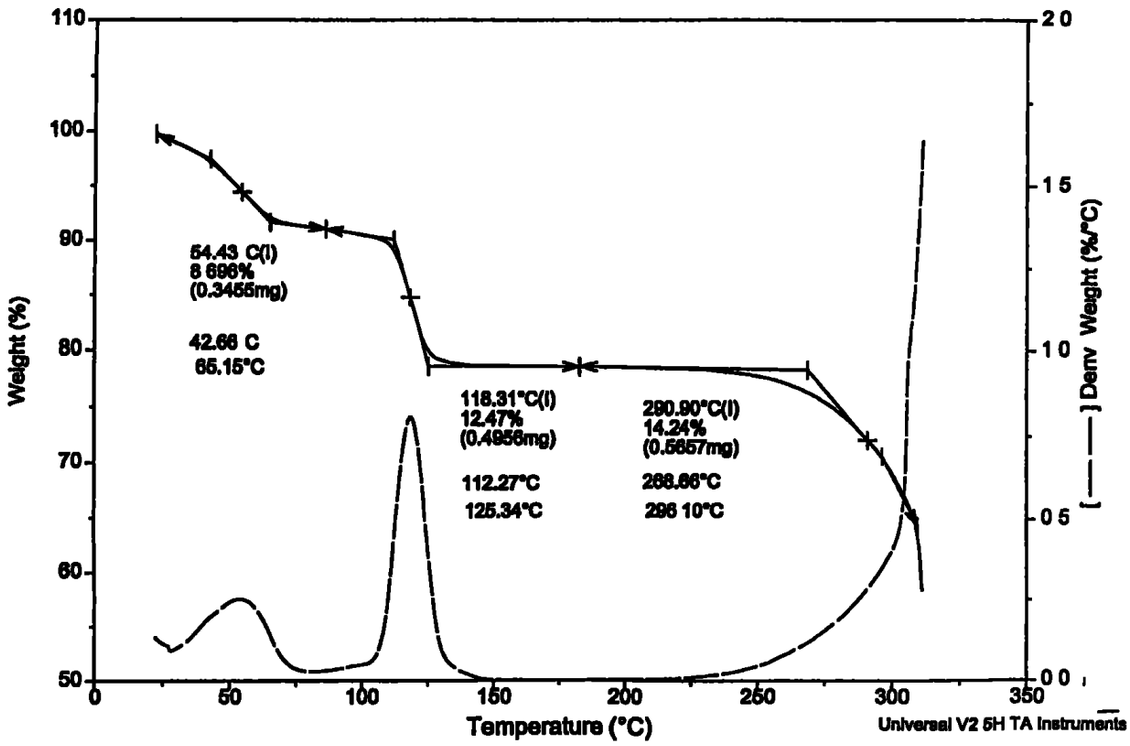


Figure 6-C

GG802T 8096910S

Size 2.2040 mg
Method 10 deg ramp to 300 deg

acetone solvate

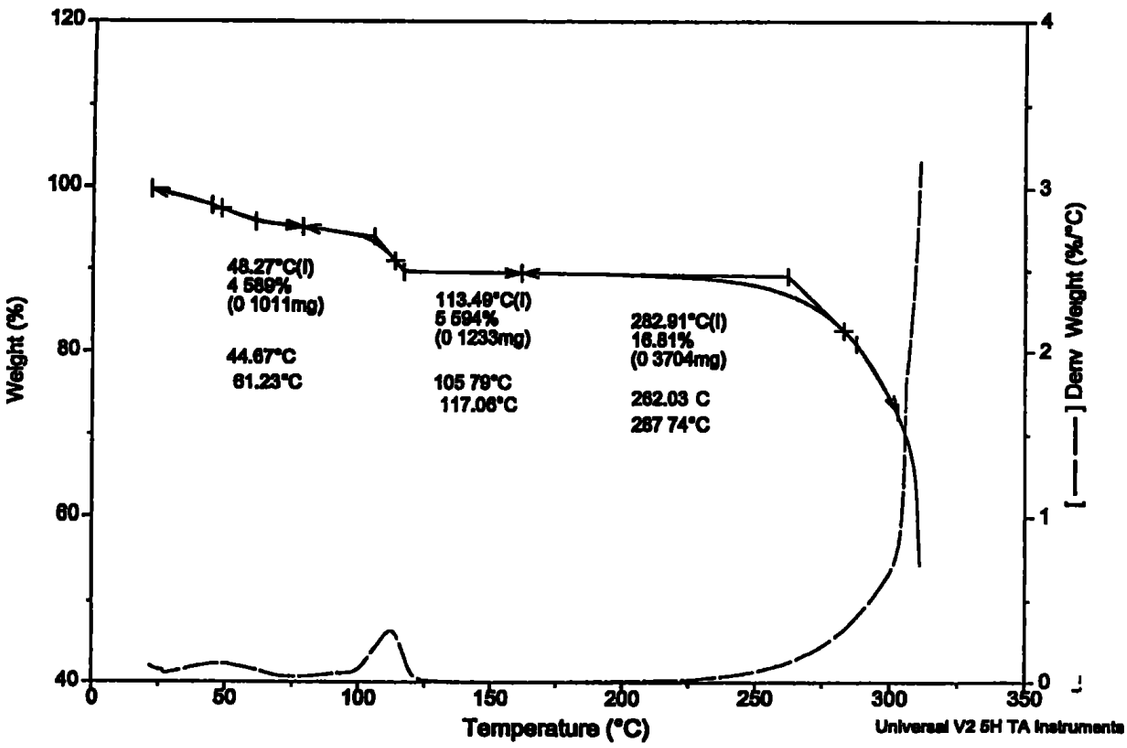


Figure 6-H

GG8021 80S6S103

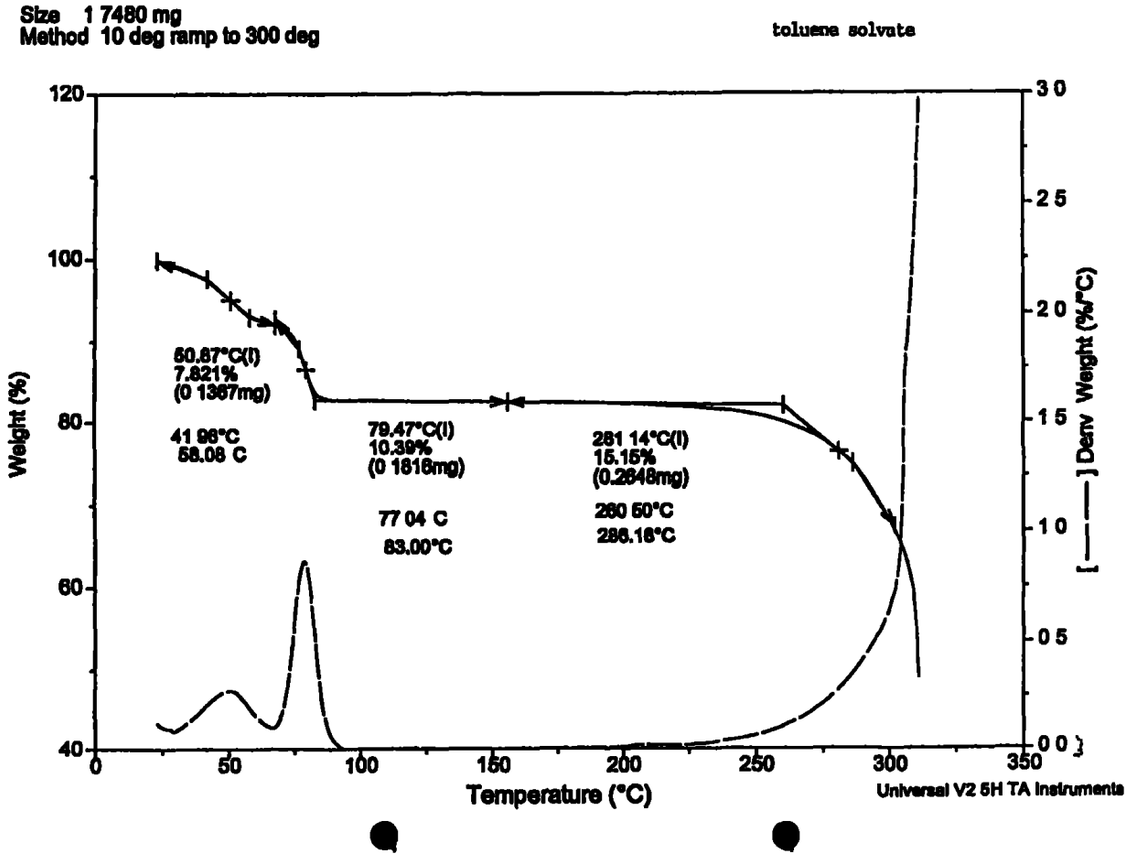


Figure 6-I

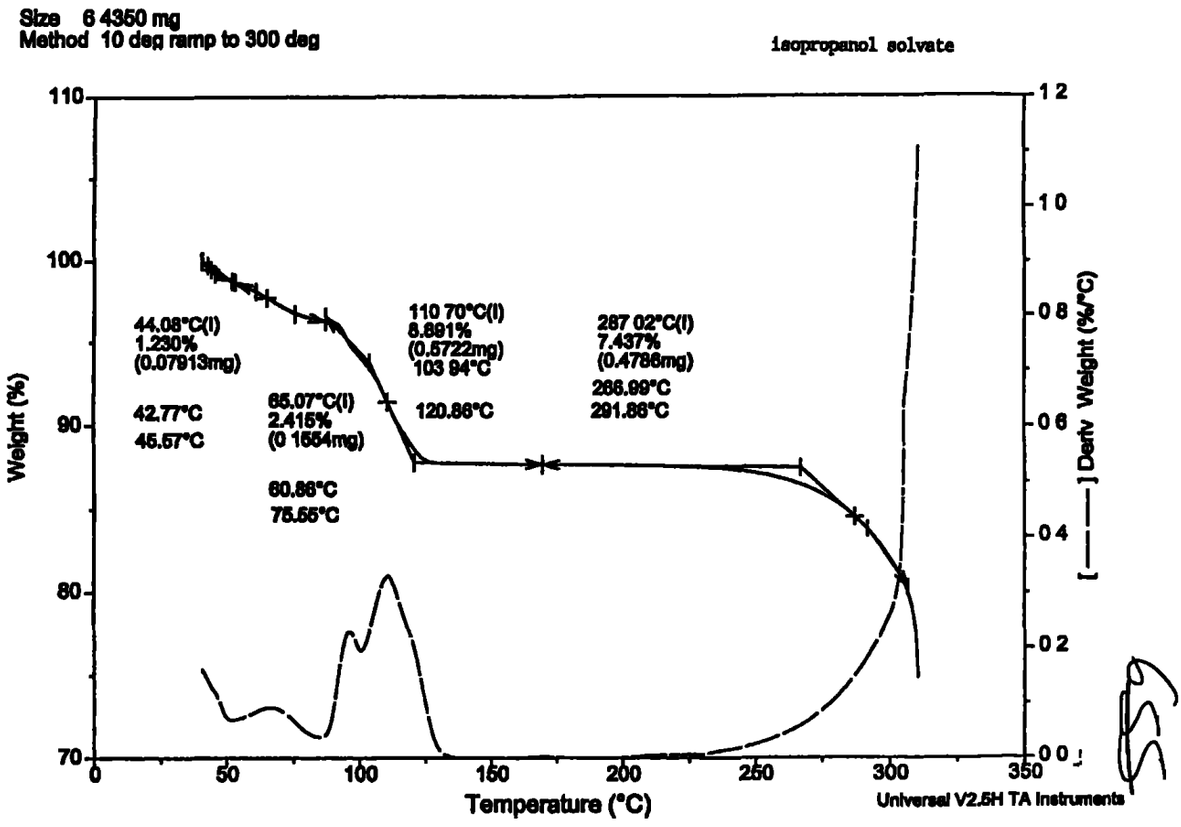

Size 6 4350 mg
Method 10 deg ramp to 300 deg
isopropanol solvate
44.08°C(I)
1.230%
(0.07913mg)
42.77°C
45.57°C
65.07°C(I)
2.415%
(0 1554mg)
60.86°C
75.55°C
110 70°C(I)
8.891%
(0.5722mg)
103 94°C
120.86°C
287 02°C(I)
7.437%
(0.4786mg)
286.99°C
291.86°C
Weight (%)
[——] Deriv Weight (%/°C)
Temperature (°C)
Universal V2.5H TA Instruments

Figure 6-J

Size. 6 1160 mg
Method 10 deg ramp to 300 deg

ethanol solvate

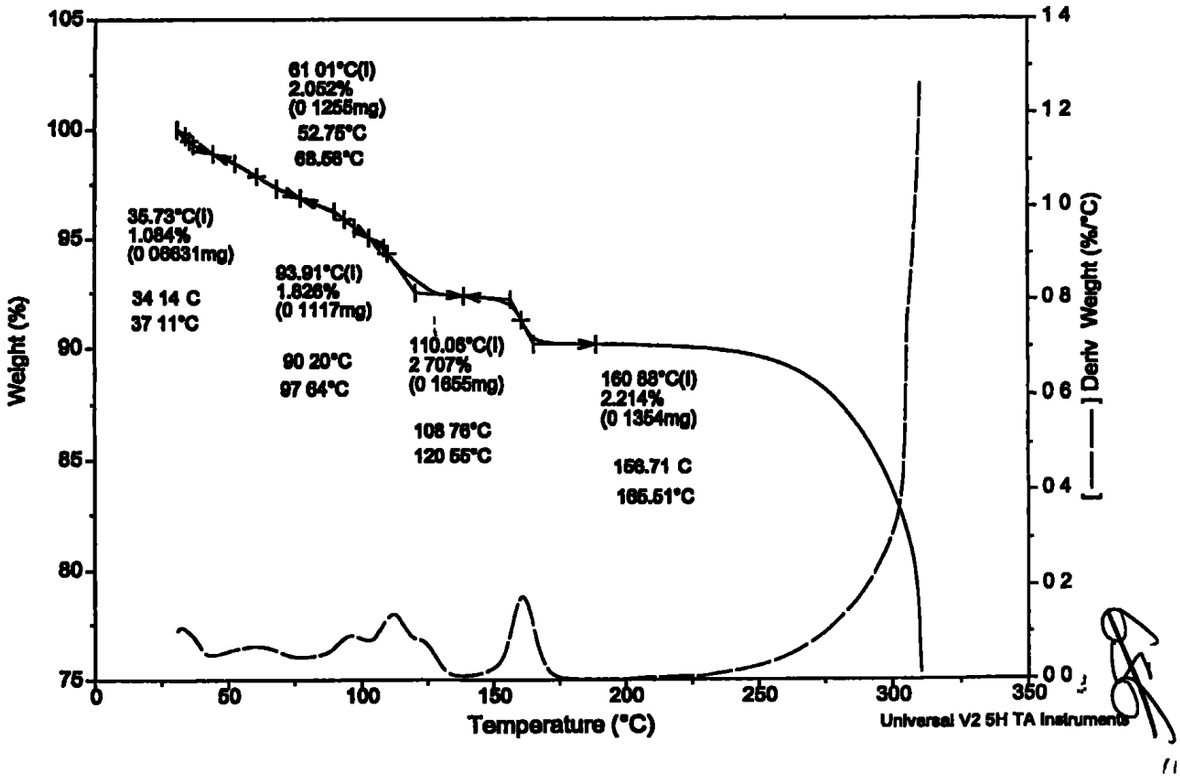


Figure 6-K

Size 4.9710 mg
Method 10 deg ramp to 300 deg

isobutyl acetate solvate

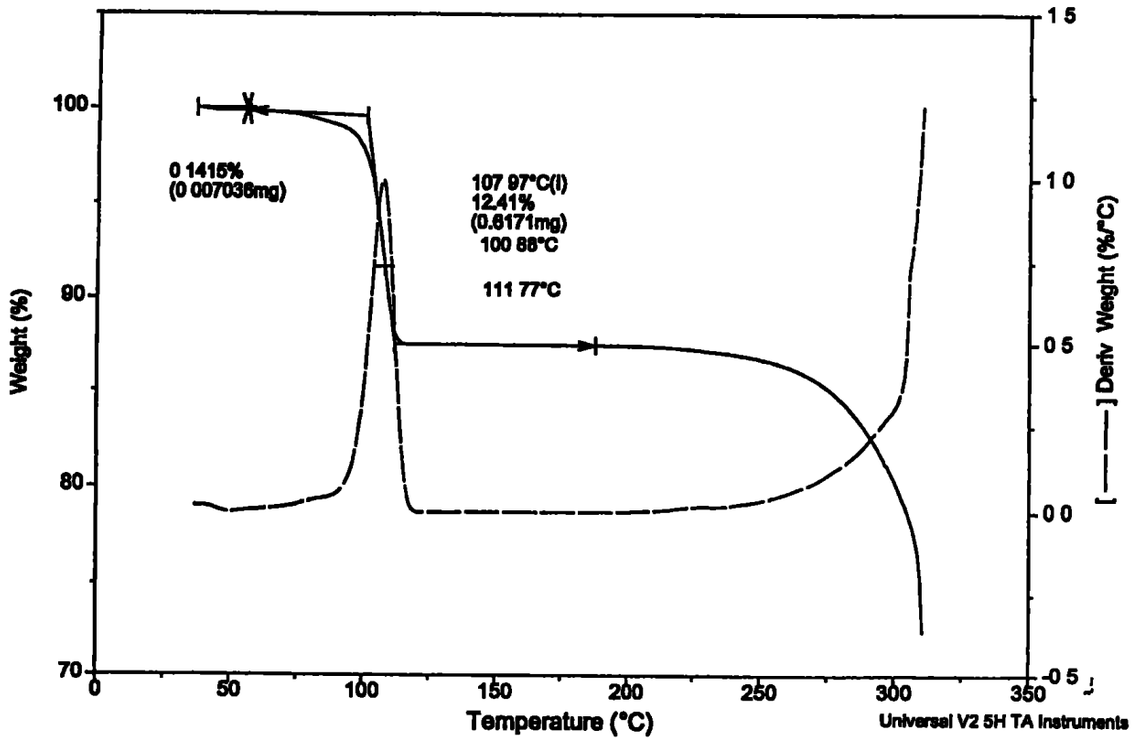


Figure [redacted]

GG8027'8096970S

Size 2 3240 mg
Method 10 deg ramp to 300 deg

butyl acetate solvate

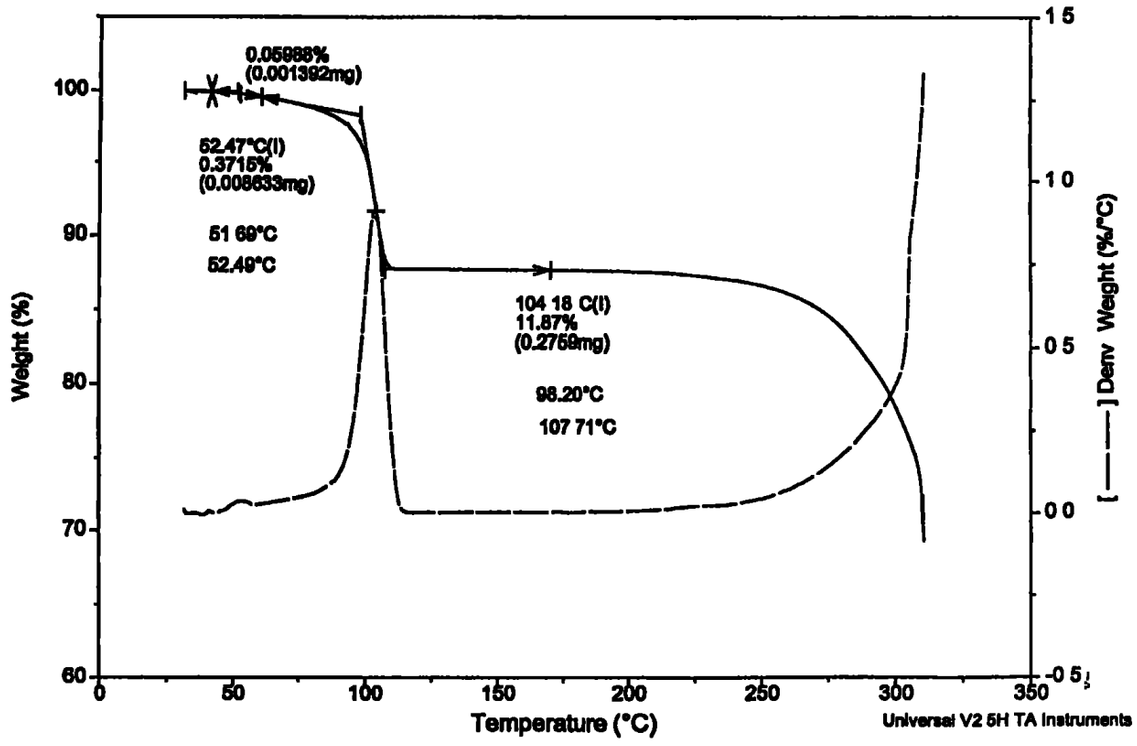


Figure [redacted]

GG8027 8096978

Size 2.7750 mg
Method 10 deg ramp to 300 deg

methyl acetate solvate

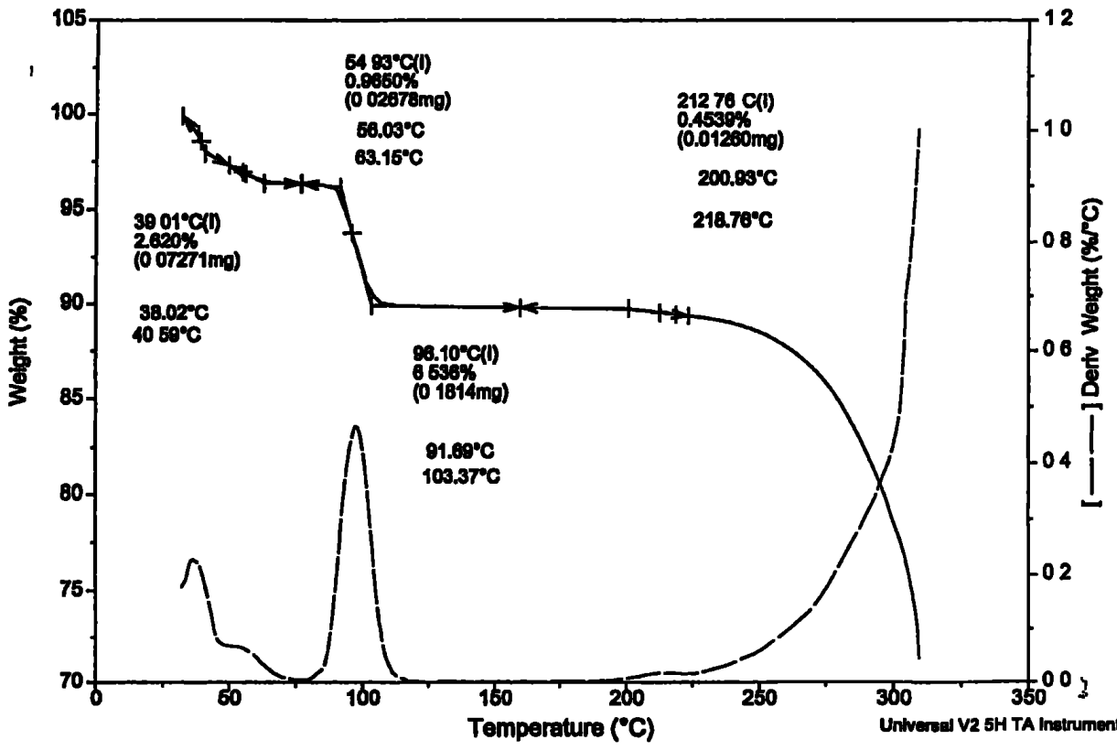


Figure 6-N

GG802T 8096T03

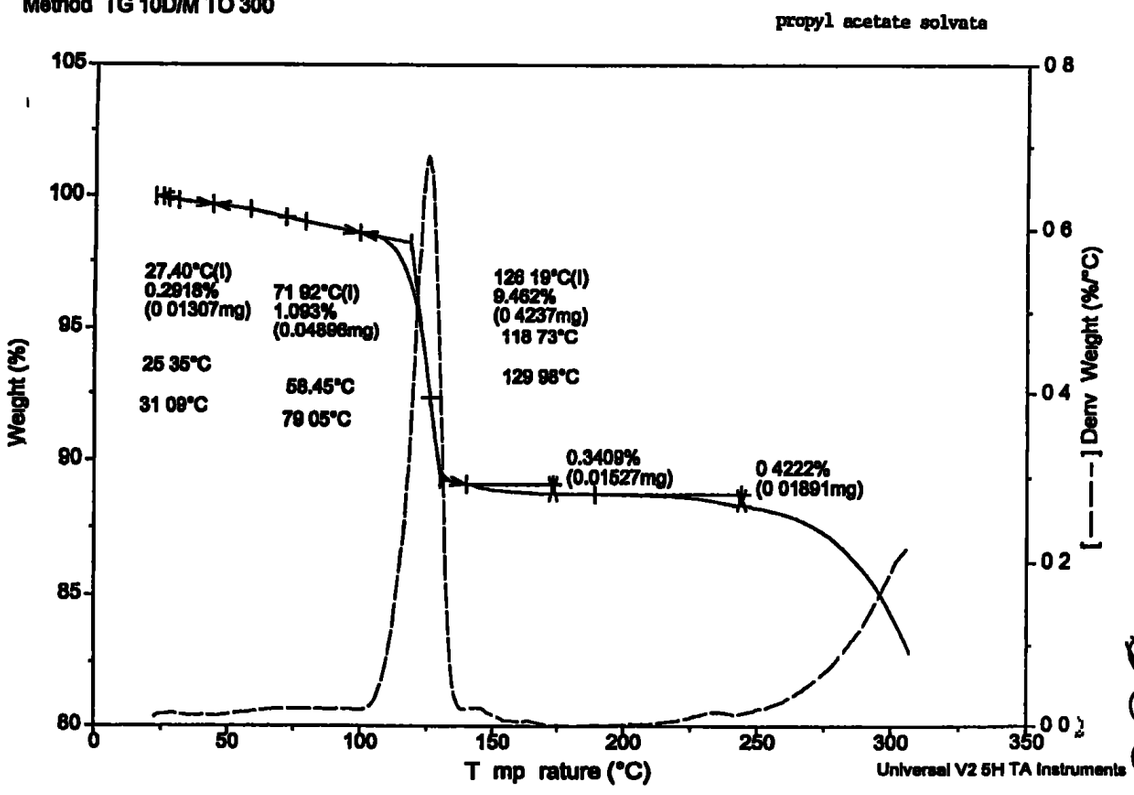


Figure 6-0

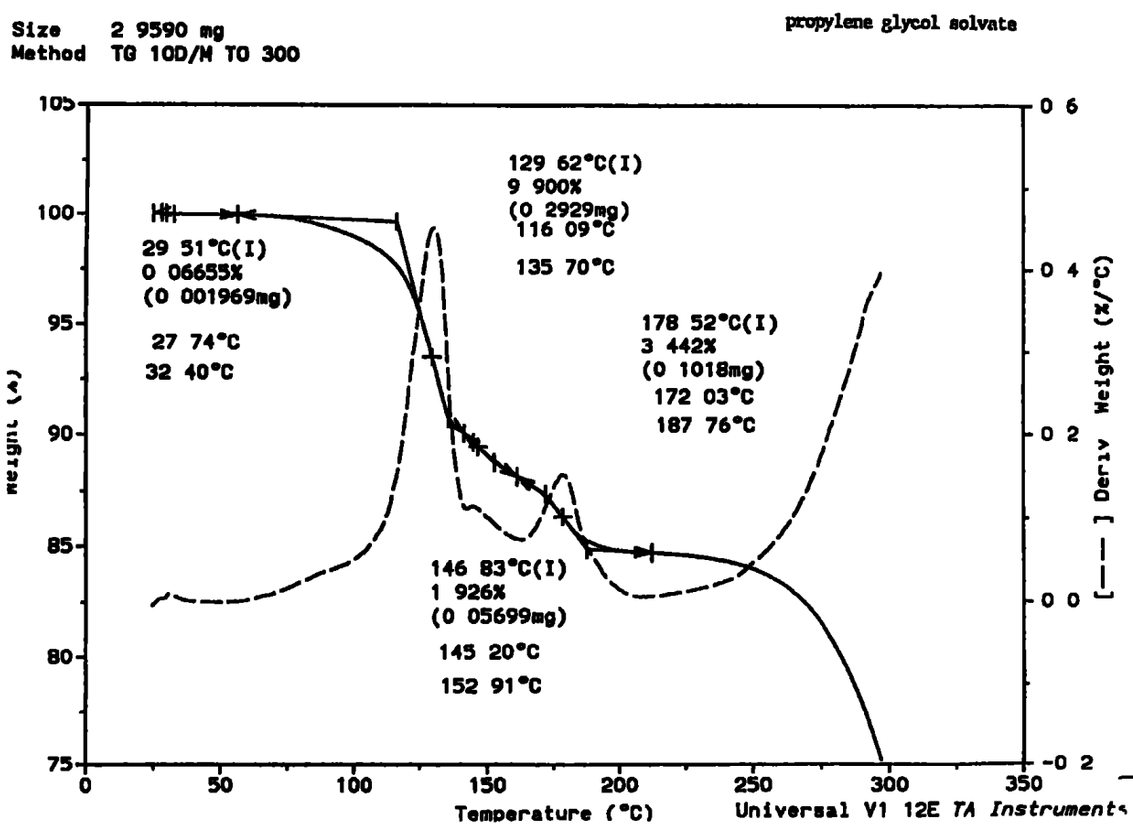

Size 2 9590 mg
Method TG 10D/M TO 300
propylene glycol solvate
129 62°C(I)
9 900%
(0 2929mg)
116 09°C
135 70°C
29 51°C(I)
0 06655%
(0 001969mg)
27 74°C
32 40°C
178 52°C(I)
3 442%
(0 1018mg)
172 03°C
187 76°C
146 83°C(I)
1 926%
(0 05699mg)
145 20°C
152 91°C
Weight (%)
[——] Deriv Weight (%/°C)
Temperature (°C)
Universal V1 12E TA Instruments

Figure 6-P

GGS02T 8D9G9TDS

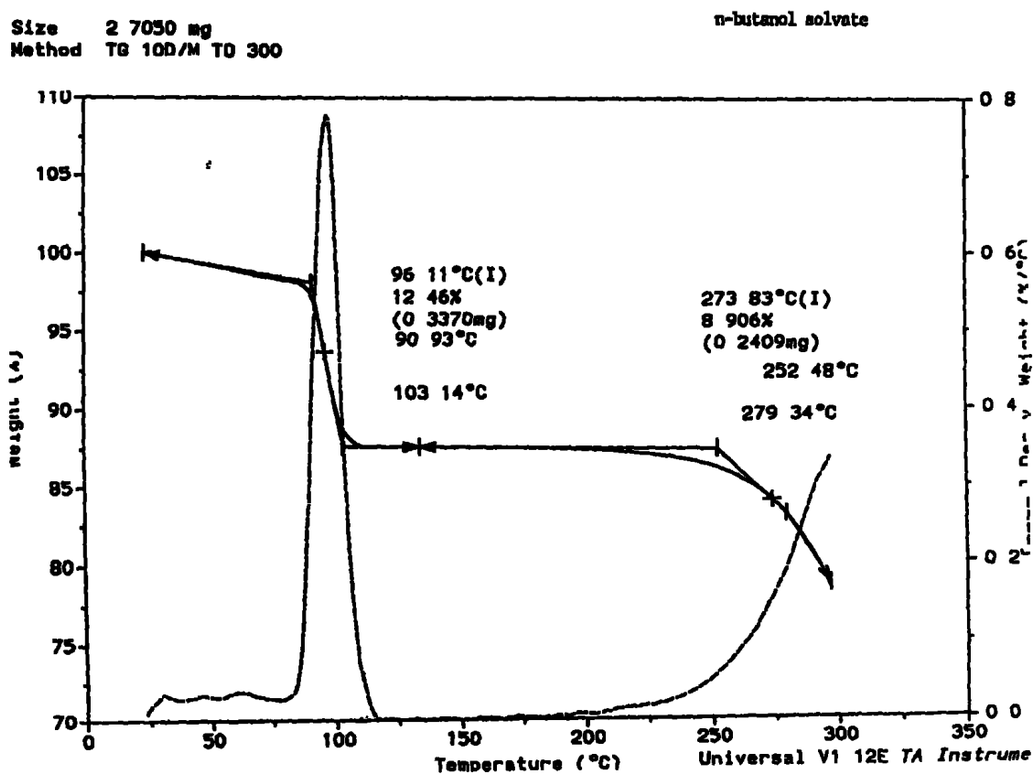


Figure 6-Q

GG8027 8059709

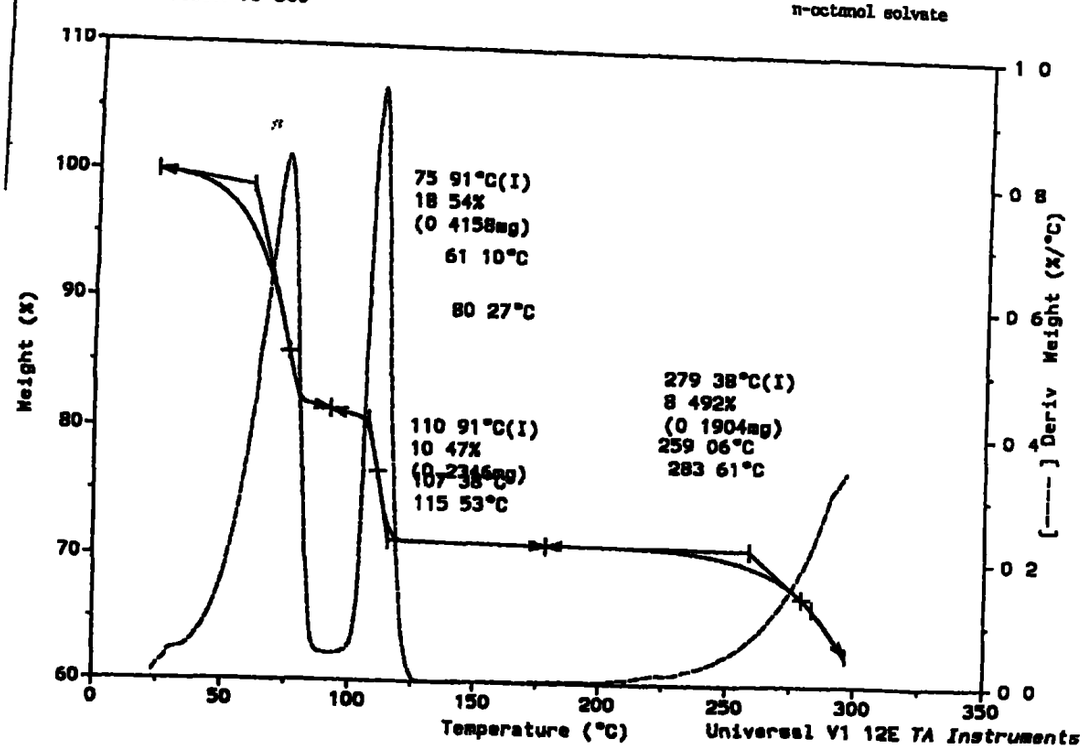


Figure 6-R

668027 8096908

Size 2 8630 mg
Method TG 10D/M TO 300

t-butyl acetate solvate

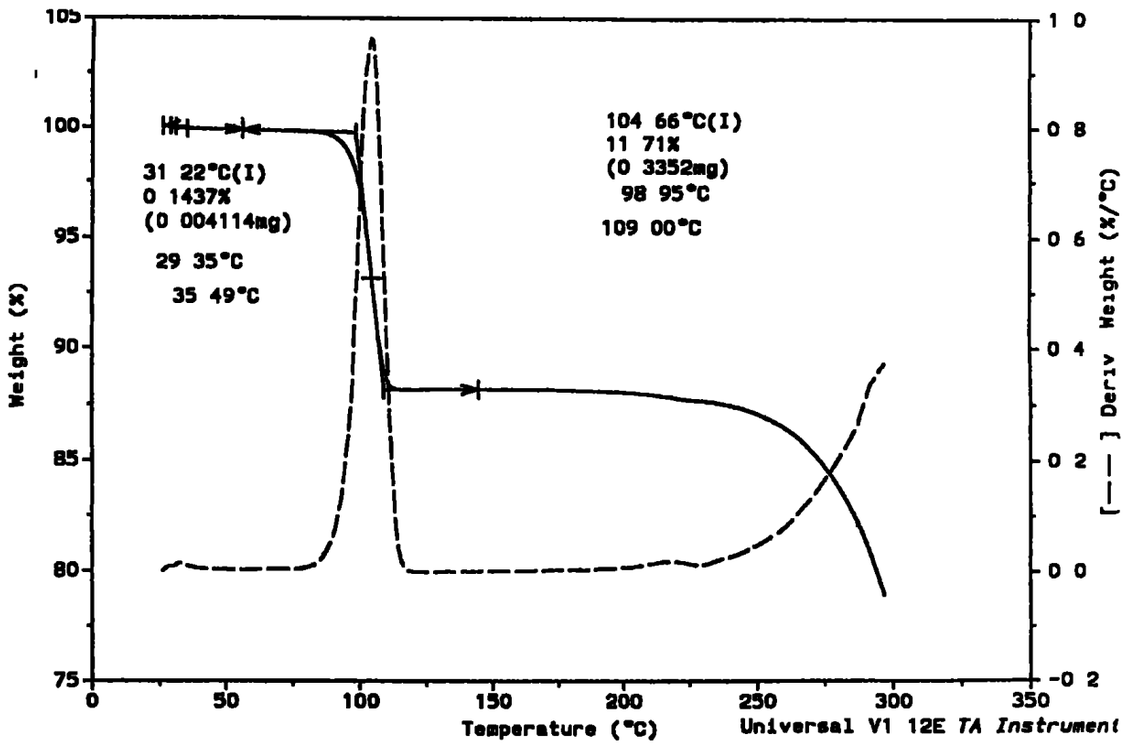


Figure 7

Figure 8

08/18/2008 12:08:27

6015956 2008 22

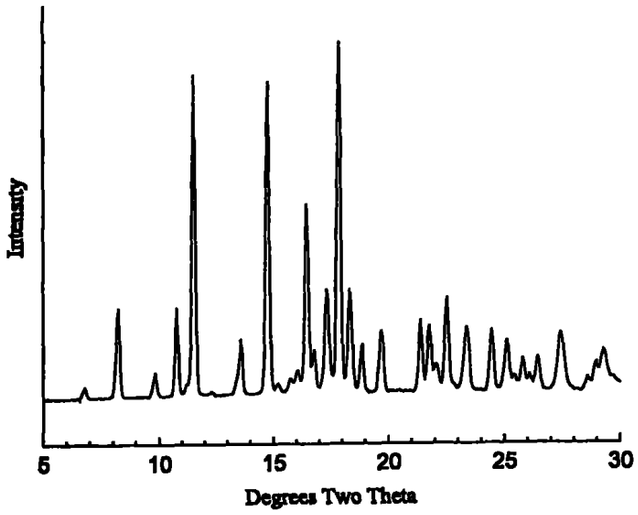


Figure 9

6681218 8036910S

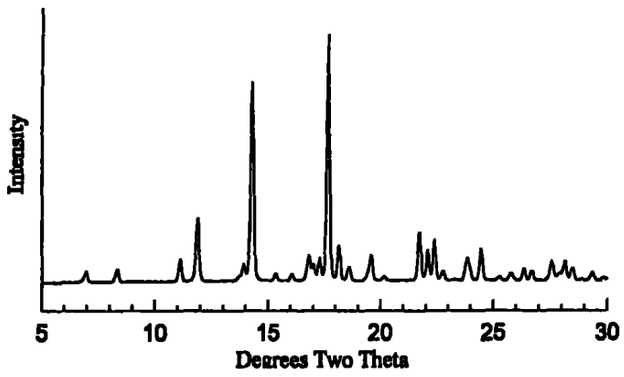


Figure 10

6618021 80926113

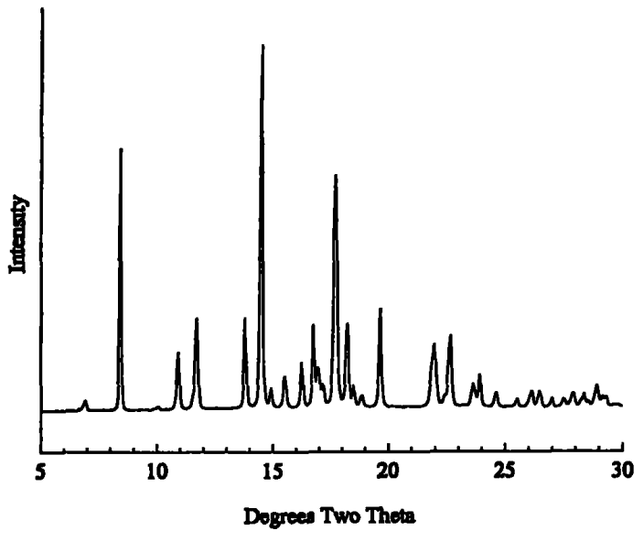


Figure 11

669
663

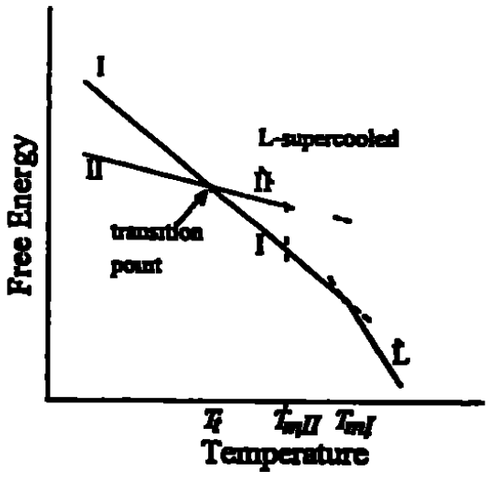


Figure 12

6015,508 12C8877

GG8021 8096910

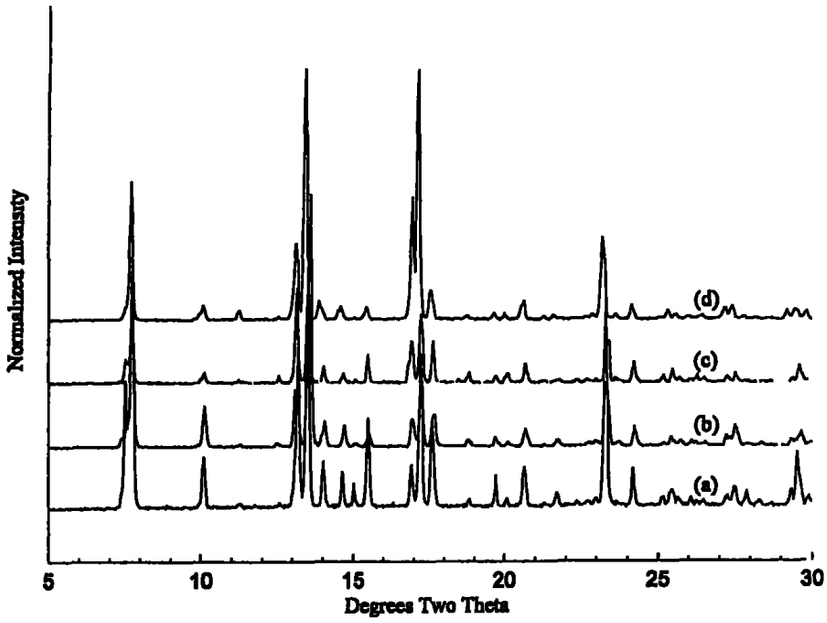


Figure 13

668021 8096910

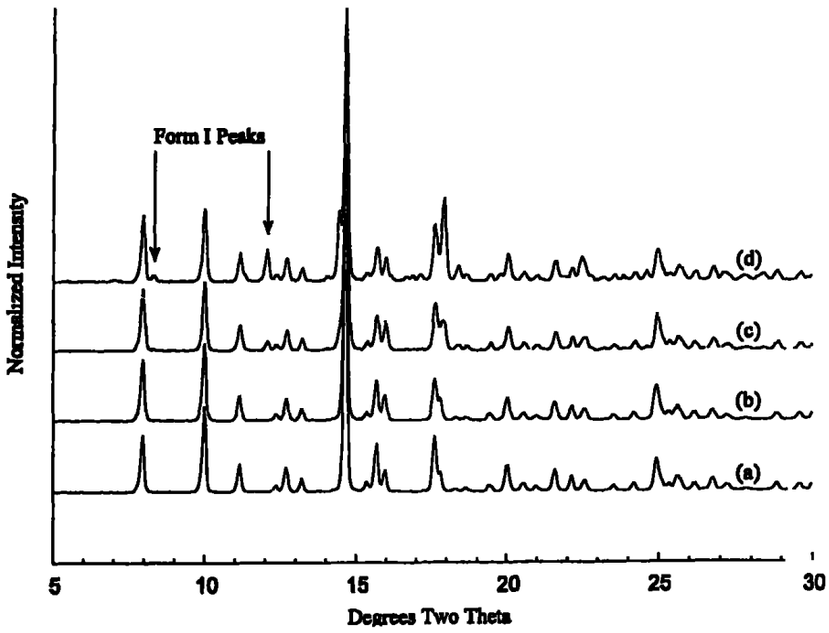


Figure 14

6668021 8096918

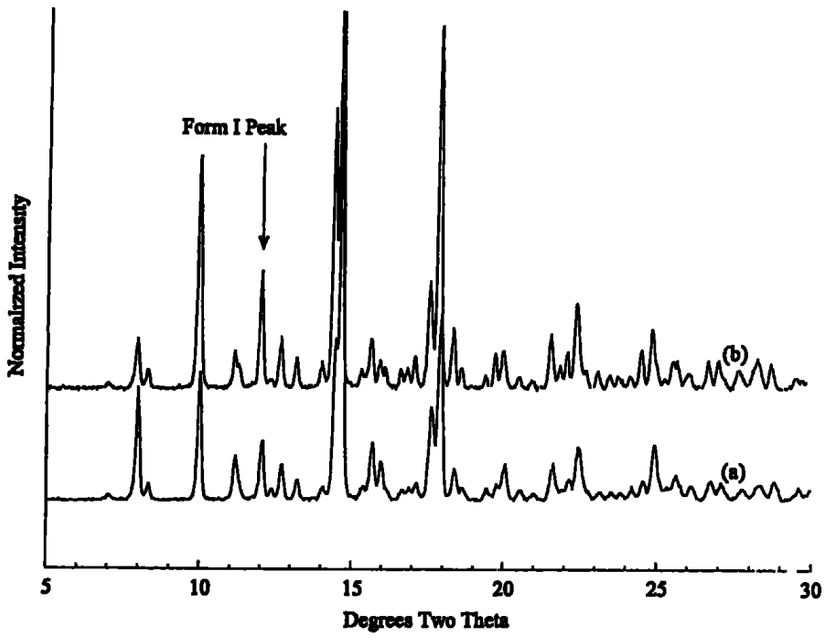


Figure 15

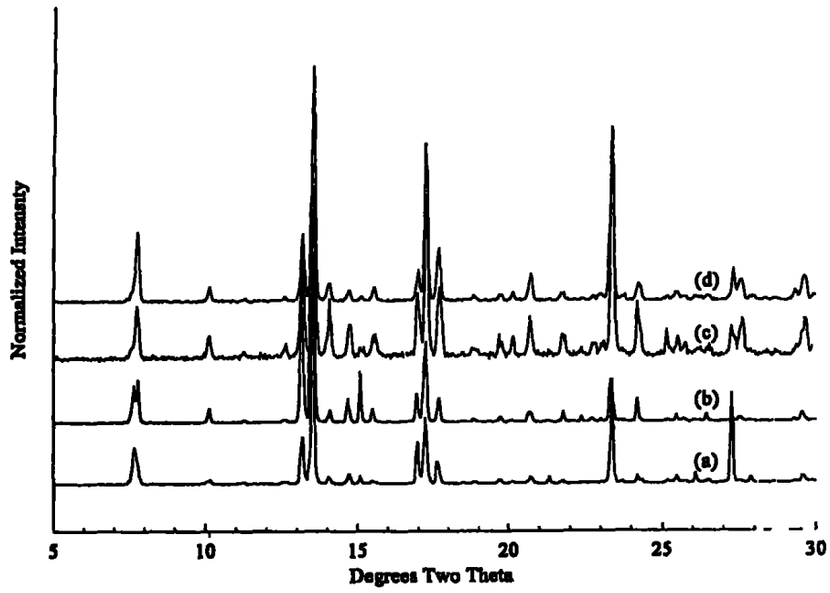


Figure 16

668027 8096910

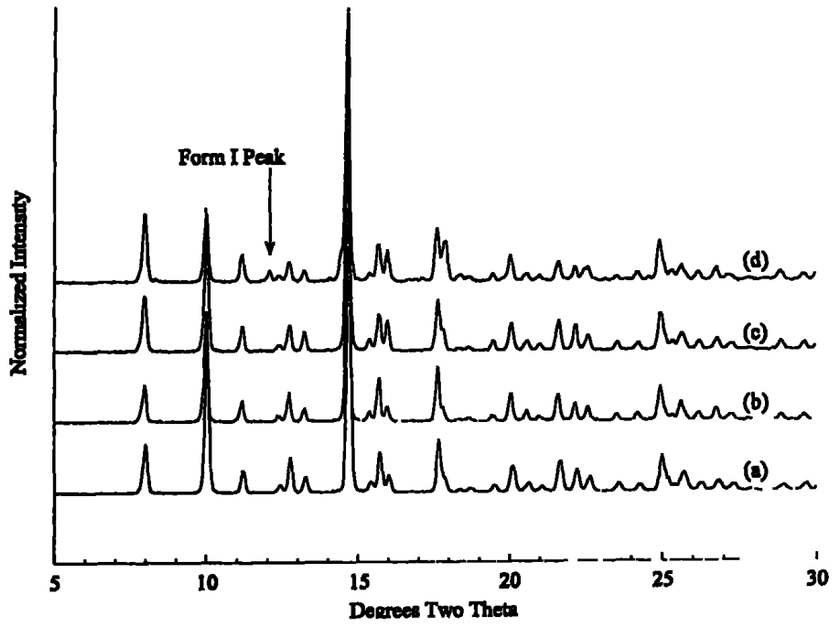


Figure 17

Figure 18

5015960 · 1208717

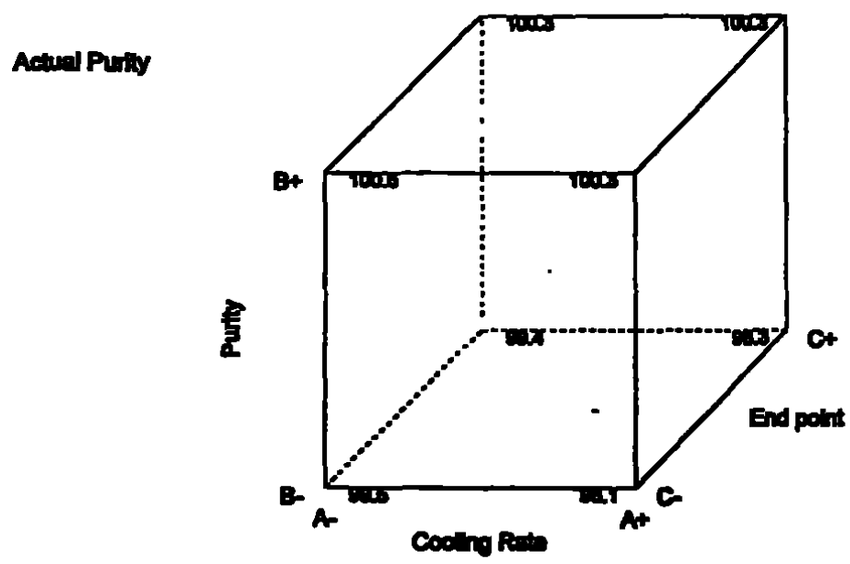
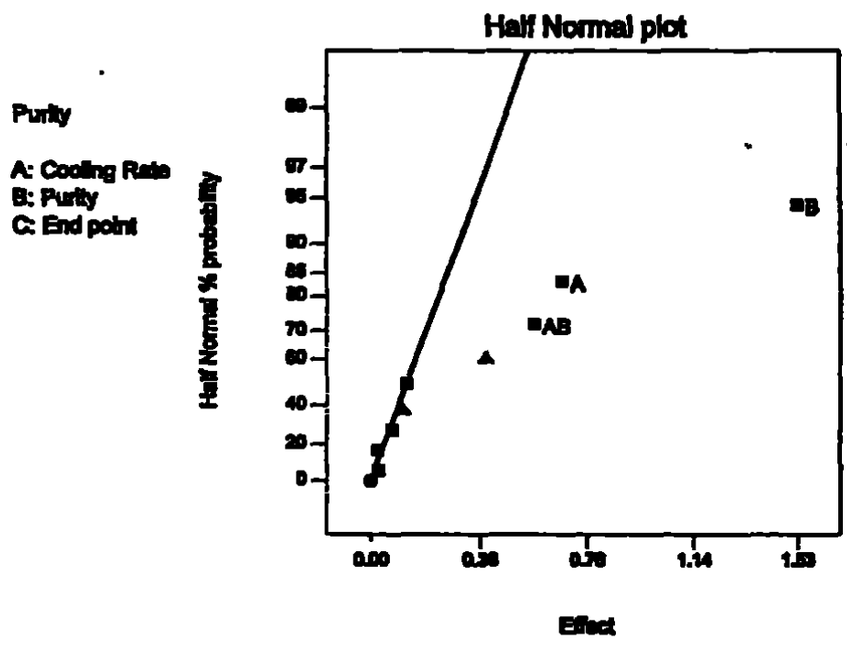
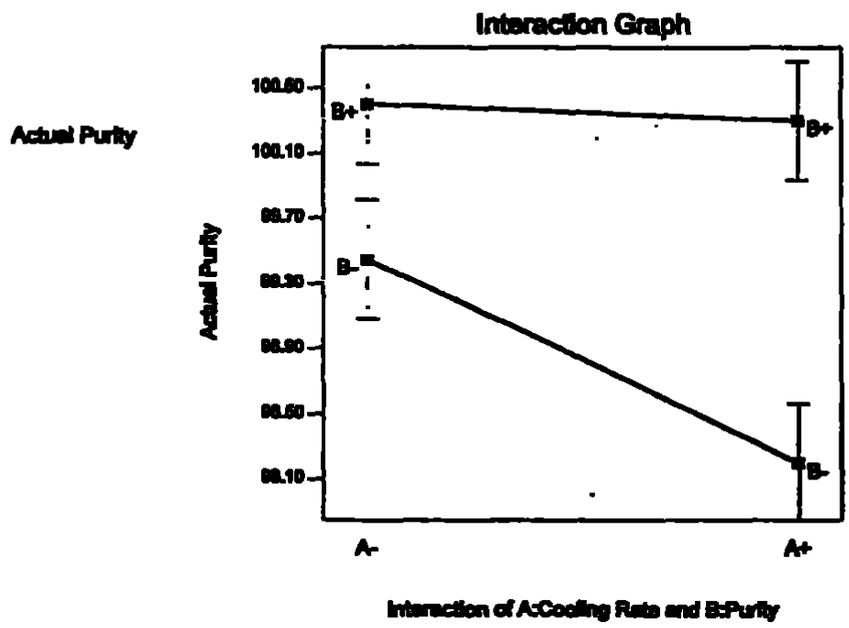


Figure 19



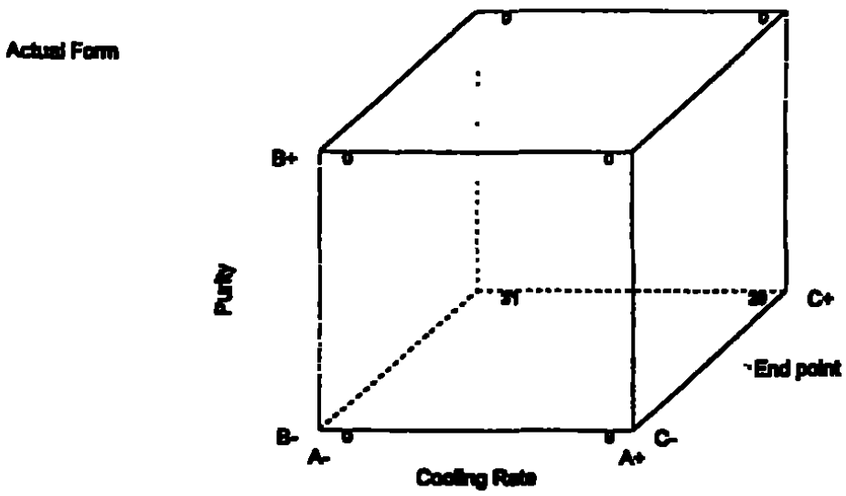
6,682,121 · 8956105

Figure 20



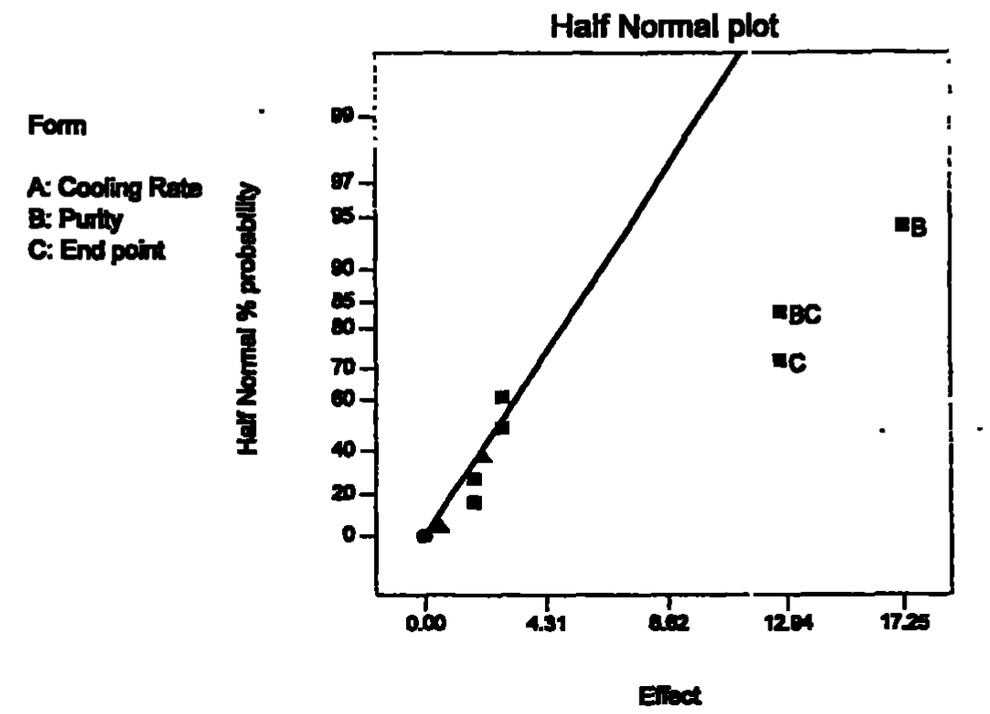
5015768 .12088/2

Figure 21



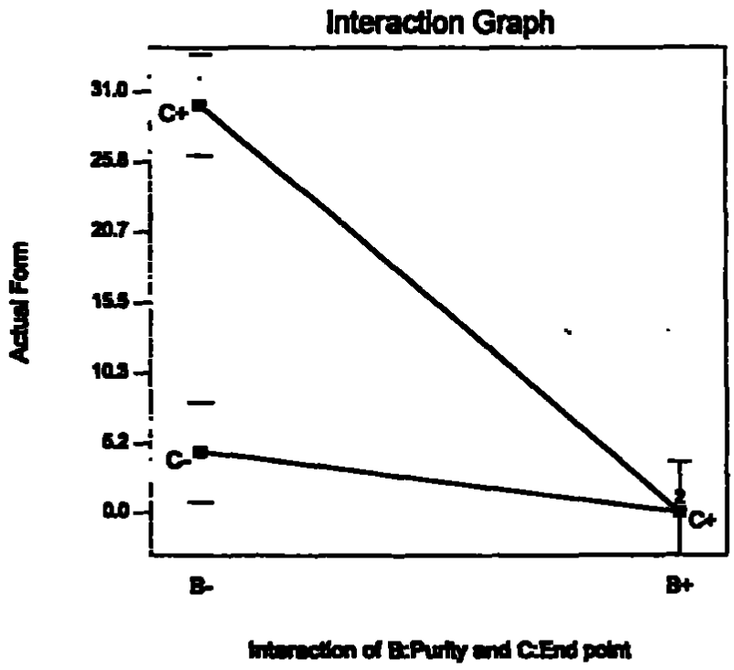
US005968379A

Figure 22



EOI6795US 12087977

Figure 23



02085308.1208277

6081960 .12/08/99

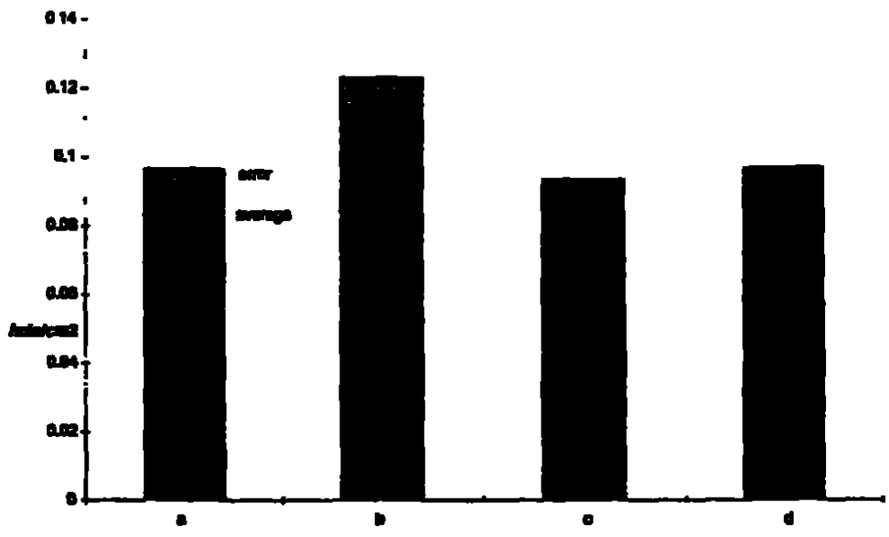


Figure 26:

Figure 2

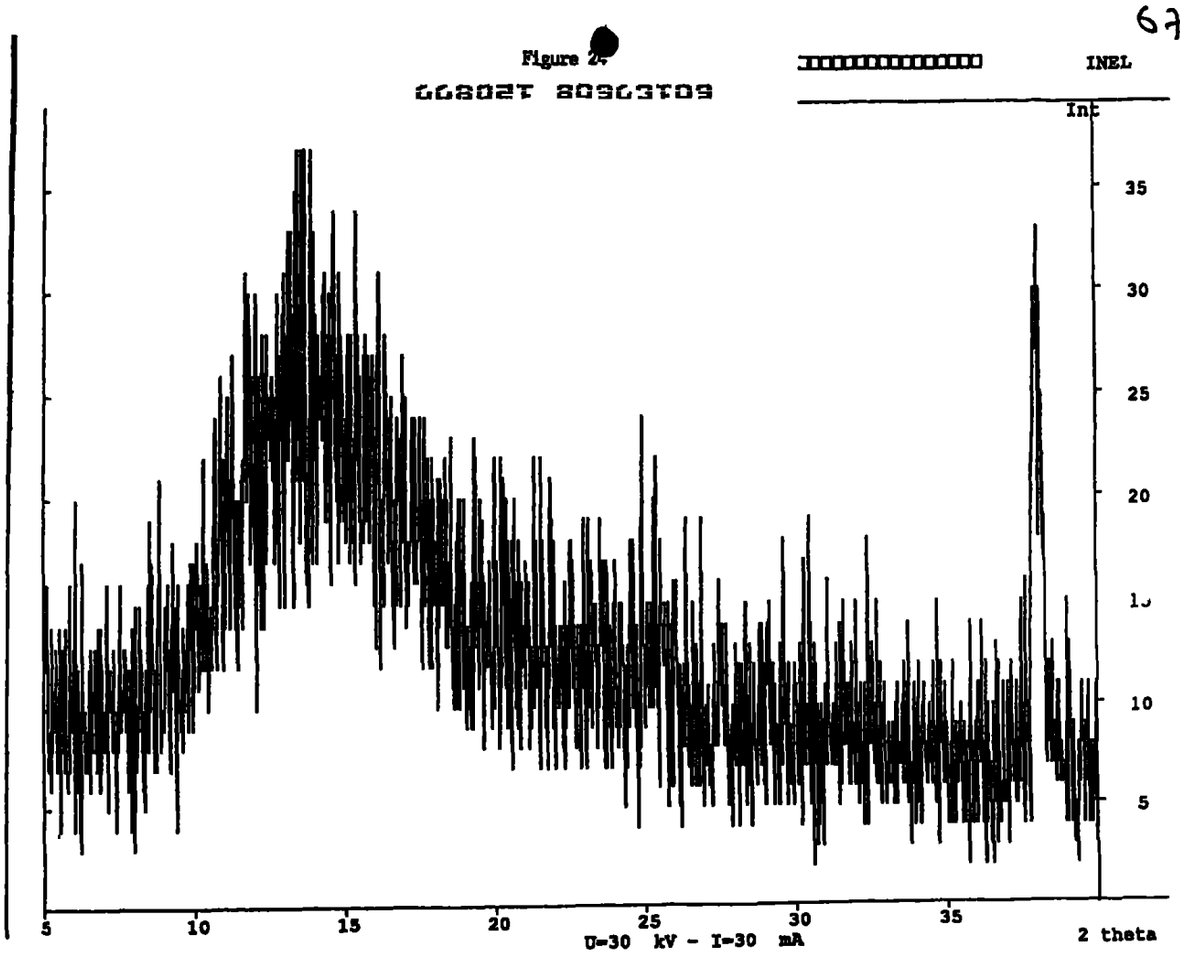
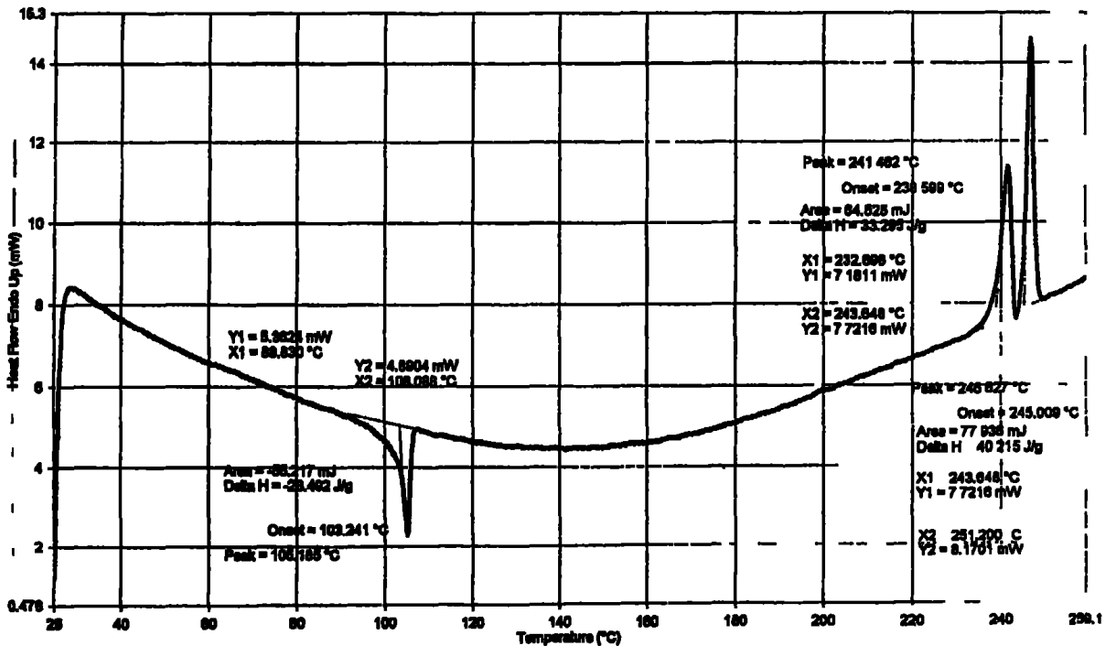


Figure 2[illegible]

[illegible]

Sample Weight: 1.838 mg
Comment: Eplerenone
Amorphous by freeze dry in water



PR 122641

LOS ESTADOS UNIDOS DE AMERICA

A TODOS AQUELLOS A QUIENES LA PRESENTE CONCIERNE:
DEPARTAMENTO DE COMERCIO DE LOS ESTADOS UNIDOS

Oficina de Patentes y Marcas de los Estados Unidos

5 de enero de 2001

LA PRESENTE ES PARA CERTIFICAR QUE LA ANEXA A ESTA ES UNA COPIA FIEL DE LOS REGISTROS DE LA OFICINA DE PATENTES Y MARCAS DE LOS ESTADOS UNIDOS DE LOS DOCUMENTOS DE LA SOLICITUD DE PATENTE IDENTIFICADA MAS ADELANTE QUE SATISFACEN LOS REQUISITOS PARA QUE SE OTORGUE UNA FECHA DE PRIORIDAD BAJO 35 USC 111..

SOLICITUD NUMERO: 60/169,608

FECHA DE PRESENTACION: *8 de diciembre de 1999*

Por la Autoridad del
COMISIONADO DE PATENTES Y MARCAS

Firmado por

W. MONTGOMERY

Funcionario Certificador

6/86/29

REIVINDICACIONES .

1. Partículas que comprenden una forma cristalina de eplerenona, donde dicha forma cristalina comprende un solvente, dicha forma cristalina no comprende metilen cloruro, etil éter o isopropanol, y al menos 90% de dichas partículas tienen un tamaño de partícula menor a 15 micras.

2. Las partículas de la reivindicación 1 donde dicha forma cristalina no comprende metilen cloruro, etil éter, isopropanol, metil etil cetona o etanol.

3. Las partículas de la reivindicación 1 donde dicha forma cristalina comprende un solvente seleccionado de un grupo consistente de metiletil cetona , 2-pentanona, ácido acético, acetona, butil acetato, cloroformo, etanol, isobutanol, isobutil acetato, metil acetato, etil propionato, n.butanol, n-octanol, n-propanol, propil acetato, propilen glicol, t-butanol, tetrahidrofurano, tolueno, y t-butil acetato.

4. Las partículas de la reivindicación 1 donde dicha forma cristalina comprende un solvente seleccionado del grupo consistente de 2-pentanona, ácido acético, acetona, butil acetato, cloroformo, etanol, isobutanol, isobutil acetato, metil

acetato, etil propionato, n-butanol, n-octanol, n-propanol, propil acetato, propilen glicol, t-butanol, tetrahidrofurano, tolueno y t-butil acetato.

5. Las partículas de la reivindicación 1 donde dicha forma cristalina comprende etanol.

6. Las partículas de la reivindicación 1 donde dicha forma cristalina comprende metiletil cetona.

7. Las partículas de la reivindicación 6 donde dicha forma cristalina tiene un patrón de difracción en polvo en rayos X con un pico de 13.6 ± 0.2 grados dos theta.

8. Las partículas de la reivindicación 6 donde dicha forma cristalina tiene un patrón de difracción en polvo en rayos X con picos de 7.6 ±0.2 y 13.6 ± 0.2 grados dos theta.

9. Las partículas de la reivindicación 6 donde dicha forma cristalina tiene un patrón de difracción en polvo en rayos X sustancialmente al ilustrado en la figura 1.C.

10. Las partículas de la reivindicación 6 donde dicha forma cristalina tiene un sistema cristalino ortorómbico.

11. Las Partículas de la reivindicación 6 donde dicha forma cristalina tiene un grupo espacial $P2_12_12_1$.

12. Las partículas de la reivindicación 6 donde dicha forma cristalina tiene valores de unidad de celda a, b, y c de 23.53 Å, de 8.16 Å y de 13.08 Å, respectivamente.

13. Las partículas de la reivindicación 6 donde dicha forma cristalina tiene valores de unidad de celda α, β, y γ cada uno de 90 .

14. Las partículas de la reivindicación 1 donde al menos 90 por ciento de dichas partículas tienen un tamaño de partícula inferior a 1 micra.

15. Las partículas de la reivindicaciones 14 donde al menos 90 por ciento de dichas partículas tienen un tamaño de partícula inferior a 800 nm.

16. Las partículas de la reivindicación 14 donde al menos 90 por ciento de dichas partículas tienen un tamaño de partícula de 10 nm hasta 1 micra.

17. Las partículas de la reivindicación 14 donde al menos 90% de dichas partículas tienen un tamaño de partícula de 100nm hasta 800 nm.

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18. Las partículas de la reivindicación 14 donde al menos 90% de dichas partículas tienen un tamaño de partícula de 200 nm hasta 800 nm.

19. Las partículas de la reivindicación 14 donde al menos 90% de dichas partículas tiene un tamaño de partícula entre 400nm hasta 800 nm.

20. Eplerenona que comprende las partículas de la reivindicación 1, donde dicha eplerenona tiene una fase de pureza de al menos 50 por ciento de eplerenona cristalina solvatada.

21. La eplerenona de la reivindicación 20 donde dicha eplerenona tiene una fase de pureza de al menos 90 por ciento de eplerenona cristalina solvatada.

22. La eplerenona de la reivindicación 20 donde dicha eplerenona es sustancialmente una fase pura de eplerenona cristalina solvatada.

23. La composición farmacéutica que comprende una cantidad terapéuticamente efectiva de las partículas de cualquiera de las reivindicaciones 1 a 22 en asociación con al menos un portador, adyuvante o diluyente farmacéuticamente aceptable.

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24. La composición de la reivindicación 23 donde la única forma cristalina de eplerenona presente en la composición es una forma de cualquiera de las reivindicaciones 1 a 6.

25. El método de tratar o prevenir una condición o desorden mediada de aldosterona en un sujeto, dicho método comprende la administración a un sujeto que tiene o es susceptible a dicha condición o desorden una cantidad terapéuticamente efectiva de las partículas de acuerdo con cualquiera de las reivindicaciones 1 a 22.

26. El método de la reivindicación 25 para ser usado en el tratamiento de hiperaldosteronismo.

27. El método de la reivindicación 25 para ser usado en el tratamiento de hipertensión.

28. El método de la reivindicación 25 para ser usado en el tratamiento de falla del corazón.

29. El método de la reivindicación 25 para ser usado en el tratamiento de cirrosis del hígado.

30. El método de la reivindicación 25 para ser usado en el tratamiento de una condición seleccionada de un grupo consistente de exceso de colágeno / fibrosis, hipertrofia benigna prostática y depresión.

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31. Las partículas de la reivindicación 1 donde dichas partículas además comprenden un compuesto seleccionado del grupo consistente de 7-metil hidrógeno 4α, 5α:9α, 11α-diepoxi-17-hidroxi-3-oxo-17α-pregnano-7α,21-dicarboxilato, γ-lactona; 7-metil hidrógeno 11α, 12α-epoxi-17-hidroxi-3-oxo-17α-pregn-4-ene-7α, 21-dicarboxilato, γ-lactona; y 7-metil hidrógeno 17-hidroxi-3-oxo-17α-pregna-4,9(11)-dien-7α, 21-dicarboxilato, γ- lactona.

32. Las partículas de la reivindicación 1 donde dichas partículas además comprenden 7-metil hidrógeno 4α,5α:9α, 11α-diepoxi-17-hidroxi-3-oxo-17α-pregnano-7α, 21-dicarboxilato, γ lactona.

COLO 12482-801-001-5

JJC-ID-10

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THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

January 17, 2001

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE UNDER 35 USC 111.

APPLICATION NUMBER: ***60/169,639***
FILING DATE: ***December 08, 1999***

By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

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Certifying Officer

12-09-99

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APPROV

12/08/99 JC590 U.S. PTO

Express Mail Label Number EL523767117US
Attorney Docket Number SRL 6176 (C-3256)

JC583 U.S. PTO 60/169639 12/08/99

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

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[x] Additional inventors are being named on the 3rd separately numbered sheet(s) attached hereto.

Title of the Invention: COMBINATIONS OF SOLID STATE FORMS OF EPLERENONE

Correspondence Address: Customer Number 000321

The application includes:

[x] Specification - Number of Pages 102

[x] Drawings - Number of Sheets 92

[ ] Small Entity Statement

[x] Return Receipt Postcard

[ ] Other _____

Method of Payment of Filing Fee:

[X] A check in the amount of $ 150.00 is enclosed to cover the Provisional Filing Fee.

[ ] The Commissioner is hereby authorized to charge filing fees and credit Deposit Account No. 19-1345.

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

[X] No
[ ] Yes, the name of the U.S. Government agency and the Government contract number are: _____

Respectfully submitted,

Scott A. Williams, Reg. No. 39,876

Date: 12/8/99

SAWskg

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12-09-99

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APPROV
6/96

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12/08/99 PTO
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Application Number TBA
Filing Date December 8, 1999
First Named Inventor Kathleen P. Barton
Group Art Unit TBA
Examiner Name TBA
Attorney Docket Number SRL 6176 (C-3256)

METHOD OF PAYMENT

1. [ ] The Commissioner is hereby authorized to charge the indicated fees to Deposit Account No. 19-1345, in the name of Senniger, Powers, Leavitt & Roedel.

[ ] The Commissioner is hereby authorized to charge any additional filing and claim fees under 37 CFR 1.16 and application processing fees under 37 CFR 1.17 to Deposit Account No. 19-1345, in the name of Senniger, Powers, Leavitt & Roedel.

2. [x] Check Enclosed. The Commissioner is hereby authorized to charge any under payment or credit any over payment to Deposit Account No. 19-1345, in the name of Senniger, Powers, Leavitt & Roedel.

FEE CALCULATION

1. [x] BASIC FILING FEE $150.00 (Type: Provisional)
Entity Status: large

2. [ ] CLAIM FEE $_____

Total Claims _____
Independent Claims _____
Multiple Dependent Claims _____

3. [ ] ADDITIONAL FEES $_____

[ ] Surcharge - late filing fee or oath
[ ] Surcharge - late provisional filing fee or cover sheet
[ ] Extension for reply within _____ month
[ ] Notice of Appeal
[ ] Filing a Brief in Support of an appeal
[ ] Request for Reexamination
[ ] Petitions to the Commissioner
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[ ] Recording each patent assignment per property
[ ] Other: _____

TOTAL AMOUNT OF PAYMENT $ 150.00

Scott A. Williams, Reg. No. 39,876 — Date 12/8/99

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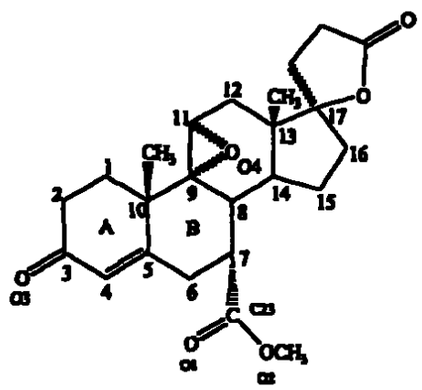
COMBINATIONS OF SOLID STATE FORMS OF EPLERENONE

Field of the Invention

This invention is in the field of aldosterone receptor antagonist pharmaceutical agents and specifically relates to combinations of crystalline and amorphous solid state forms of eplerenone, methods of preparing such combinations of eplerenone, pharmaceutical compositions comprising such combinations of eplerenone, and methods for the treatment and/or prophylaxis of aldosterone-mediated conditions and/or disorders, including conditions and disorders associated with hyperaldosteronism such as hypertension.

Background of the Invention

The compound methyl hydrogen 9,11-epoxy-17-hydroxy-3-oxopregn-4-ene-7,21-dicarboxylate, γ-lactone 1 (also referred to herein as "eplerenone") was first reported in Grob et al., U.S. Patent 4,559,332 which discloses a class of 9,11-epoxy steroid compounds and their salts. Eplerenone is an aldosterone receptor antagonist that can be administered in a therapeutically effective amount where use of an aldosterone receptor antagonist is indicated, such as in the treatment of pathological conditions associated with hyperaldosteronism including hypertension, heart failure including cardiac insufficiency, and cirrhosis of the liver.



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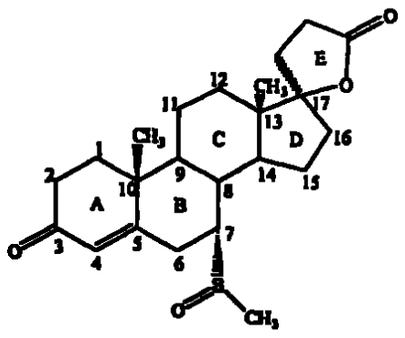
U.S. Patent 4,559,332 generally discloses the preparation of eplerenone and the preparation of pharmaceutical formulations comprising eplerenone.

Additional processes for the preparation of 9,11-epoxy steroid compounds and their salts, including eplerenone, are disclosed in Ng et al., WO97/21720 and Ng et al., WO98/25948.

Grob et al., "Steroidal Aldosterone Antagonists: Increased Selectivity of 9α,11-Epoxy Derivatives", Helvetica Chimica Acta, Vol. 80, pp. 566-585 (1997), discloses an X-ray crystal structure analysis of a solvate of eplerenone prepared by crystallizing eplerenone from a methylene chloride/diethyl ether solvent system.

De Gasparo et al., "Antialdosterones: Incidence and Prevention of Sexual Side Effects", J. Steroid Biochem., Vol. 32, No. 13, pp. 223-227 (1989), discloses the use of non-formulated eplerenone having a 20 micron particle size in a single dose study of eplerenone.

Spironolactone 2, a 20-spiroxane-steroid having activity as an aldosterone receptor antagonist, is commercially available for the treatment of hypertension. Spironolactone, however, has antiandrogenic activity that can result in gynecomastia and impotence in men. It also has weak progestational activity that can produce menstrual irregularities in women. Accordingly, there is interest in the development of additional active aldosterone receptor antagonists such as eplerenone that do not interact with other steroid receptor systems such as glucocorticoid, progestin and androgen steroid receptor systems and/or that provide for a broader range of treatment.



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Agafonov et al., "Polymorphism of Spironolactone", J. Pharm. Sciences, Vol. 80, No. 2, pp 181-185 (1991), discloses an acetonitrile solvate, an ethanol solvate, an ethyl acetate solvate, a methanol solvate and two non-solvated polymorphic crystalline forms of spironolactone. Brittan, Polymorphism in Pharmaceutical Solids, pp. 114-116, 207, 235, and 261 (Marcel Dekker, Inc. 1999), likewise discloses these solid state forms of spironolactone.

Summary of the Invention

A first aspect of the invention comprises novel combinations of a first solid state form of eplerenone and a second solid state form of eplerenone wherein said solid state forms of eplerenone are selected from the Form I crystalline form of eplerenone; the Form II crystalline form of eplerenone; a crystalline form of eplerenone comprising a solvent; and amorphous eplerenone. These solid state forms of eplerenone possess activity as aldosterone receptor antagonists and have unique properties relative to each other. Selection of appropriate combinations of these solid state forms of eplerenone can help to reduce processing,

storage and formulation problems encountered with respect to eplerenone.

A second aspect of the invention comprises combinations comprising particles of a first solid state form of eplerenone and a second solid state form of eplerenone wherein at least 90% of the particles have a particle size less than about 400 microns.

A third aspect of the invention comprises pharmaceutical compositions comprising a first solid state form of eplerenone and a second solid state form of eplerenone.

A fourth aspect of the invention comprises a method for the prophylaxis and/or treatment of an aldosterone-mediated condition and/or disorder comprising administering to a subject a therapeutically-effective amount of a first solid state form of eplerenone and a second solid state form of eplerenone, either alone or in combination with other active ingredients.

A fifth aspect of the invention comprises a novel amorphous form of eplerenone.

A sixth aspect of the invention comprises particles of the amorphous eplerenone wherein at least 90% of the particles have a particle size less than about 400 microns.

A seventh aspect of the invention comprises methods for the preparation of the novel amorphous form of eplerenone.

Additional aspects of the invention are discussed throughout the specification of this application.

Brief Description of the Drawings

Fig. 1-A shows X-ray powder diffraction patterns of Form I eplerenone.

Fig. 1-B shows X-ray powder diffraction patterns of Form II eplerenone.

Fig. 1-C shows X-ray powder diffraction patterns of the methyl ethyl ketone solvate of eplerenone.

Figs. 1-D through 1-O show X-ray powder diffraction patterns for the following eplerenone solvates: n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, acetone solvate, toluene solvate, isopropanol solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, methyl acetate solvate, and propyl acetate solvate, respectively.

Fig. 2-A shows a differential scanning calorimetry (DSC) thermogram of non-milled Form II directly crystallized from methyl ethyl ketone.

Fig. 2-B shows a differential scanning calorimetry (DSC) thermogram of non-milled Form II prepared by desolvation of a solvate obtained by crystallization of a high purity eplerenone from methyl ethyl ketone.

Fig. 2-C shows a differential scanning calorimetry (DSC) thermogram of Form II prepared by crystallizing a solvate from a solution of high purity eplerenone in methyl ethyl ketone, desolvating the solvate to yield Form II, and milling the resulting Form II.

Fig. 2-D shows a differential scanning calorimetry (DSC) thermogram of non-milled Form I prepared by desolvation of a solvate obtained by digestion of low purity eplerenone from appropriate solvents.

Figs. 2-E through 2-T show DSC thermograms for the following eplerenone solvates: n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, chloroform solvate, acetone solvate, toluene

solvate, isopropanol solvate, t-butyl acetate solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, methyl acetate solvate, propyl acetate solvate, n-butanol solvate, and n-octanol solvate, respectively.

Fig. 3-A shows the infrared spectra (diffuse reflectance, DRIFTS) of Form I eplerenone.

Fig. 3-B shows the infrared spectra (diffuse reflectance, DRIFTS) of Form II eplerenone.

Fig. 3-C shows the infrared spectra (diffuse reflectance, DRIFTS) of the methyl ethyl ketone solvate of eplerenone.

Fig. 3-D shows the infrared spectra (diffuse reflectance, DRIFTS) of eplerenone in chloroform solution.

Figs. 3-E through 3-Q show infrared absorption spectra for the following eplerenone solvates: n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, acetone solvate, toluene solvate, isopropanol solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, propyl acetate solvate, methyl acetate solvate, propylene glycol solvate, and t-butyl acetate solvate respectively.

Fig. 4 shows ^{13}C NMR spectra for Form I of eplerenone.

Fig. 5 shows ^{13}C NMR spectra for Form II of eplerenone.

Figs. 6-A through 6-R show thermogravimetry analysis profiles for the following eplerenone solvates: methyl ethyl ketone solvate, n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, chloroform solvate, acetone solvate, toluene solvate, isopropanol solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, methyl acetate solvate, propyl acetate solvate, propylene glycol solvate, n-butanol

solvate, n-octanol solvate, and t-butyl acetate solvate, respectively.

Fig. 7 is a scanning electron micrograph of Form II eplerenone prepared by desolvation of the methyl ethyl ketone solvate.

Fig. 8 is a scanning electron micrograph of Form II eplerenone prepared by direct crystallization from ethyl acetate.

Fig. 9 shows an X-ray powder diffraction pattern of a crystalline form of 7-methyl hydrogen 4α,5α:9α,11α-diepoxy-17-hydroxy-3-oxo-17α-pregnane-7α,21-dicarboxylate, γ-lactone isolated from methyl ethyl ketone.

Fig. 10 shows an X-ray powder diffraction pattern of the crystalline form of 7-methyl hydrogen 11α,12α-epoxy-17-hydroxy-3-oxo-17α-pregn-4-ene-7α,21-dicarboxylate, γ-lactone isolated from isopropanol.

Fig. 11 shows an X-ray powder diffraction pattern of the crystalline form of 7-methyl hydrogen 17-hydroxy-3-oxo-17α-pregna-4,9(11)-diene-7α,21-dicarboxylate, γ-lactone isolated from n-butanol.

Fig. 12 illustrates the Gibbs free energy-temperature relationship for enantiotropically related polymorphs.

Fig. 13 shows the X-ray powder diffraction patterns for the wet cake (methyl ethyl ketone solvate) obtained from (a) 0%, (b) 1%, (c) 3%, and (d) 5% diepoxide-doped methyl ethyl ketone crystallizations.

Fig. 14 shows the X-ray powder diffraction patterns for the dried solids obtained from (a) 0%, (b) 1%, (c) 3%, and (d) 5% diepoxide-doped methyl ethyl ketone crystallizations.

Fig. 15 shows the X-ray powder diffraction patterns for the dried solids from the methyl ethyl ketone crystallization

with 3% doping of diepoxide (a) without grinding of the solvate prior to drying, and (b) with grinding of the solvate prior to drying.

Fig. 16 shows the X-ray powder diffraction patterns for the wet cake (methyl ethyl ketone solvate) obtained from (a) 0%, (b) 1%, (c) 5%, and (d) 10% 11,12-epoxide-doped methyl ethyl ketone crystallizations.

Fig. 17 shows the X-ray powder diffraction patterns for the dried solids obtained from (a) 0%, (b) 1%, (c) 5%, and (d) 10% 11,12-epoxide-doped methyl ethyl ketone crystallizations.

Fig. 18 shows a cube plot of product purity, starting material purity, cooling rate and endpoint temperature based on the data reported in Table X-7A.

Fig. 19 shows a half normal plot prepared using the cube plot of Fig. 18 to determine those variables having a statistically significant effect on the purity of the final material.

Fig. 20 is an interaction graph based on the results reported in Table X-7A showing the interaction between starting material purity and cooling rate on final material purity.

Fig. 21 shows a cube plot of Form I weight fraction, starting material purity, cooling rate and endpoint temperature based on the data reported in Table X-7A.

Fig. 22 shows a half normal plot prepared using the cube plot of Fig. 21 to determine those variables having a statistically significant effect on the purity of the final material.

Fig. 23 is an interaction graph based on the results reported in Table X-7A showing the interaction between

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starting material purity and endpoint temperature on final material purity.

Fig. 24 shows an X-ray diffraction pattern of amorphous eplerenone.

Fig. 25 shows a DSC thermogram of amorphous eplerenone.

Fig. 26 shows the dissolution rates measured for four polymorph samples.

Detailed Description of the Preferred Embodiments

As with all pharmaceutical compounds and compositions, the chemical and physical properties of eplerenone are important in its commercial development. These properties include, but are not limited to: (1) packing properties such as molar volume, density and hygroscopicity, (2) thermodynamic properties such as melting temperature, vapor pressure and solubility, (3) kinetic properties such as dissolution rate and stability (including stability at ambient conditions, especially to moisture and under storage conditions), (4) surface properties such as surface area, wettability, interfacial tension and shape, (5) mechanical properties such as hardness, tensile strength, compactibility, handling, flow and blend; and (6) filtration properties. These properties can affect, for example, the processing and storage of pharmaceutical compositions comprising eplerenone. Solid state forms of eplerenone that provide an improvement in one or more of these properties relative to other solid state forms of eplerenone are desirable.

The present invention comprises such novel solid state forms of eplerenone. Specifically, these novel solid state forms include solvated crystalline forms, non-solvated and

non-hydrated crystalline forms (designated "Form I" and "Form II"), and an amorphous form of eplerenone. Each solid state form of eplerenone described in the present application possesses one or more of the above-described advantageous chemical and/or physical properties relative to the other solid state forms described herein or otherwise disclosed in the literature.

In one embodiment of the invention, the solid state form comprises Form I eplerenone. Form I exhibits a faster dissolution rate (approximately 30% higher) at lower temperatures (i.e., temperatures below the enantiotropic transition temperature as later discussed) than, for example, Form II. Where dissolution of eplerenone in the gastrointestinal tract is the rate-controlling step for delivery of the eplerenone to the target cells, faster dissolution generally results in improved bioavailability. Form I, therefore, can provide an improved bioavailability profile relative to Form II. In addition, selection of a solid state form of eplerenone having a faster dissolution rate likewise provides greater flexibility in the selection of excipients for, and in the formulation of, immediate release pharmaceutical compositions relative to other solid state forms having a slower dissolution rate.

In another embodiment of the invention, the solid state form comprises Form II eplerenone. Form II possesses greater physical stability at lower temperatures (i.e., at temperatures below the enantiotropic transition temperature as later discussed) than, for example, Form I. Solid state forms of eplerenone such as Form II that do not require the use of special processing or storage conditions, and that avoid the need for frequent inventory replacement, are

desirable. For example, selection of a solid state form of eplerenone that is physically stable during the manufacturing process (such as during milling of eplerenone to obtain a material with reduced particle size and increased surface area) can avoid the need for special processing conditions and the increased costs generally associated with such special processing conditions. Similarly, selection of a solid state form of eplerenone that is physically stable at different conditions of storage (especially considering the different possible storage conditions during the lifetime of the eplerenone product) can help avoid polymorphic or other degradative changes in the eplerenone that can lead to product loss or deterioration of product efficacy. Therefore, the selection of a solid state form of eplerenone such as Form II having greater physical stability provides a meaningful benefit over less stable eplerenone forms.

In another embodiment of the invention, the solid state form of eplerenone comprises a solvated crystalline form of eplerenone. Such solvated crystalline forms are useful as intermediates in the preparation of Form I and Form II eplerenone. An additional benefit from the use of a solvated crystalline form as an intermediate is the "intrinsic micronizing" of the crystal that results upon desolvation as discussed later in this application. Such "intrinsic micronizing" can reduce or eliminate milling requirements. Further, where additional milling is still required, it is easier to mill certain solvates before the desolvation step than to mill Form I or Form II after desolvation of the solvate.

Pharmaceutically acceptable solvated crystalline forms also can be used directly in pharmaceutical compositions.

These solvated crystalline forms preferably do not comprise methylene chloride, isopropanol or ethyl ether; and, in yet another embodiment, do not comprise methylene chloride, isopropanol, ethyl ether, methyl ethyl ketone or ethanol; and, in yet another embodiment, do not comprise methylene chloride, isopropanol, ethyl ether, methyl ethyl ketone, ethanol, ethyl acetate or acetone. More preferably, the solvated crystalline forms are substantially exclusive of solvents that are not pharmaceutically-acceptable solvents. Because Form I and Form II typically are more physically stable than the crystalline solvates at room temperature and under atmospheric pressure, the solvated crystalline forms used in such compositions generally comprise a pharmaceutically acceptable higher boiling and/or hydrogen bonding solvent such as, but not limited to, butanol. It is believed that the solvated crystalline forms collectively can offer a range of different dissolution rates and, where dissolution of eplerenone in the gastrointestinal tract is the rate-controlling step for delivery of the eplerenone to the target cells, bioavailabilities relative to Form I and Form II.

In another embodiment of the invention, the solid state form of eplerenone comprises an amorphous form of eplerenone. Amorphous eplerenone is useful as an intermediate in the preparation of Form I and Form II eplerenone. In addition, it is believed that amorphous eplerenone possesses a different dissolution rate and, where dissolution of eplerenone in the gastrointestinal tract is the rate-controlling step for delivery of the eplerenone to the target cells, bioavailability relative to Form I and Form II.

In another embodiment, the invention comprises combinations of solid state eplerenone forms selected from

the group consisting of Form I eplerenone, Form II eplerenone, solvated crystalline eplerenone and amorphous eplerenone. Such combinations are useful, for example, in the preparation of pharmaceutical compositions having differing dissolution profiles, including controlled release compositions.

In addition, depending upon the intended use of the solid state form of eplerenone, processing considerations may favor the selection of a specific solid state form of eplerenone or a specific combination of such solid state forms. Pure phase Form II, for example, generally is more easily prepared than pure phase Form I. A mixture of Form I and Form II, however, generally is more easily prepared than phase pure Form II and allows for the use of a low purity eplerenone starting material. Use of a solvated crystalline form instead of Form I or Form II in a composition eliminates a processing step, desolvation, for those processes that otherwise would proceed by desolvation of the solvated crystalline form. Alternatively, the desolvation step can be eliminated, for example, if Form II is directly crystallized from an appropriate solvent without the intervening preparation and desolvation of the intermediate solvated crystalline form. These processes are described in greater detail later in the application.

Although each of the above solid state eplerenone embodiments can embrace a broad range of eplerenone particle sizes, it has been discovered that reduction of the particle size of the solid state form of eplerenone to a D_{90} particle size (later defined herein) between about 25 microns to about 400 microns can improve the bioavailability of unformulated eplerenone and pharmaceutical compositions comprising that

solid state form of eplerenone. Accordingly, the D_{90} particle size of the unformulated eplerenone or the eplerenone used as a starting material in the pharmaceutical composition generally is less than about 400 microns, preferably less than about 200 microns, more preferably less than about 150 microns, still more preferably less than about 100 microns, and still more preferably less than about 90 microns. In one embodiment, the D_{90} particle size is between about 40 microns to about 100 microns. In another embodiment, the D_{90} particle size is between about 30 microns to about 50 microns. In another embodiment, the D_{90} particle size is between about 50 microns to about 150 microns. In another embodiment, the D_{90} particle size is between about 75 microns to about 125 microns.

Definitions

The term "amorphous" as applied to eplerenone refers to a solid state wherein the eplerenone molecules are present in a disordered arrangement and do not form a distinguishable crystal lattice or unit cell. When subjected to X-ray powder diffraction, amorphous eplerenone does not produce any characteristic crystalline peaks.

Where reference is made in this application to the "boiling point" of a substance or solution, the term "boiling point" means the boiling point of the substance or solution under the applicable process conditions.

The term "crystalline form" as applied to eplerenone refers to a solid state form wherein the eplerenone molecules are arranged to form a distinguishable crystal lattice (i) comprising distinguishable unit cells, and (ii) yielding diffraction peaks when subjected to X-ray radiation.

The term "crystallization" as used throughout this application can refer to crystallization and/or recrystallization depending upon the applicable circumstances relating to the preparation of the eplerenone starting material.

The term "digestion" means a process in which a slurry of solid eplerenone in a solvent or mixture of solvents is heated at the boiling point of the solvent or mixture of solvents under the applicable process conditions.

The term "direct crystallization" as used herein refers to the crystallization of eplerenone directly from a suitable solvent without the formation and desolvation of an intermediate solvated crystalline solid state form of eplerenone.

The term "particle size" as used herein refers to particle size as measured by conventional particle size measuring techniques well known in the art, such as laser light scattering, sedimentation field flow fractionation, photon correlation spectroscopy, or disk centrifugation. The term "D_{90} particle size" means the particle size of at least 90% of the particles as measured by such conventional particle size measuring techniques.

The term "DSC" means differential scanning calorimetry.

The term "HPLC" means high pressure liquid chromatography.

The term "IR" means infra red.

The term "min" means minute.

The term "psi" means pounds per square inch absolute.

The term "purity" means the chemical purity of eplerenone according to conventional HPLC assay. As used herein, "low purity eplerenone" generally means eplerenone that contains an

effective amount of a Form I growth promoter and/or a Form II growth inhibitor. As used herein, "high purity eplerenone" generally means eplerenone that does not contain, or contains less than an effective amount of, a Form I growth promoter and/or a Form II growth inhibitor.

The term "phase purity" means the solid state purity of eplerenone with regard to a particular crystalline or amorphous form of the eplerenone as determined by the infrared spectroscopy analytical methods described herein.

The term "XPRD" means X-ray powder diffraction.

The term "rpm" means revolutions per minute.

The term "TGA" means thermogravimetric analysis.

The term "T_m" means melting temperature.

Characterization of Crystalline Form

1. Molecular Conformation

Single crystal X-ray analysis indicates that the eplerenone molecular conformation differs between Form I and Form II, particularly with respect to the orientation of the ester group at the 7-position of the steroid ring. The orientation of the ester group can be defined by the C8-C7-C23-02 torsion angle.

In the Form I crystal lattice, the eplerenone molecule adopts a conformation in which the methoxy group of the ester is approximately aligned with the C-H bond at the 7-position and the carbonyl group is approximately positioned over the center of the B-steroid ring. The C8-C7-C23-02 torsion angle is approximately -73.0° in this conformation. In this orientation, the carbonyl oxygen atom of the ester group (01) is in close contact with the oxygen atom of the 9,11-epoxide

ring (04). The 01-04 distance is about 2.97 Å, which is just below the van der Waal's contact distance of 3.0 Å (assuming van der Waal's radii of 1.5Å for the oxygen).

In the Form II crystal lattice, the eplerenone molecule adopts a conformation in which the ester group is rotated approximately 150° relative to that of Form I and has a C8-C7-C23-02 torsion angle of approximately +76.9°. In this orientation, the methoxy group of the ester is directed toward the 4,5-alkene segment of the A-steroid ring. In this orientation, the distance between either oxygen atom of the ester group (01,02) and the oxygen atom of the 9,11-epoxide ring is increased relative to the distance determined for Form I. The 02-04 distance is approximately 3.04Å, falling just above the van der Waal's contact distance. The 01-04 distance is about 3.45Å.

The eplerenone molecule appears to adopt a conformation characteristic of Form II in the solvated crystalline forms analyzed by single crystal X-ray diffraction to date.

2. X-Ray Powder Diffraction

The various crystalline forms of eplerenone were analyzed with either a Siemens D5000 powder diffractometer or an Inel Multipurpose Diffractometer. For the Siemens D500 powder diffractometer, the raw data was measured for 2q values from 2 to 50, with steps of 0.020 and step periods of two seconds. For the Inel Multipurpose Diffractometer, samples were placed in an aluminum sample holder and raw data was collected for 30 minutes at all two theta values simultaneously.

Tables 1A, 1B and 1C set out the significant parameters of the main peaks in terms of 2q values and intensities for

the Form I (prepared by desolvation of the ethanol solvate obtained by digestion of low purity eplerenone), Form II (prepared by desolvation of the methyl ethyl ketone solvate obtained by recrystallization of high purity eplerenone), and methyl ethyl ketone solvate (prepared by room temperature slurry conversion of high purity eplerenone in methyl ethyl ketone) crystalline forms of eplerenone, respectively (X-ray radiation at a wavelength of 1.54056 Angstroms).

Minor shifts in peak positioning may be present in the diffraction patterns of Form I and Form II as a result of imperfections in the spacing of the crystal diffraction planes due to the route of manufacture of Form I and Form II (i.e. desolvation of a solvate). In addition, Form I is isolated from a solvate prepared by digestion of crude eplerenone. This method results in a lower overall chemical purity (approximately 90%) of the Form I. Finally, the solvated forms of eplerenone are expected to show some shifting in the positioning of the diffraction peaks due to the increased mobility of the solvent molecules within the solvent channels in the crystal lattice.

TABLE 1A: FORM I DATA

Angle 2-theta	d-spacing Angstrom	Intensity	Intensity %
6.994	12.628	1188	7.2
8.291	10.655	2137	13
10.012	8.827	577	3.5
11.264	7.849	1854	11.3
12.04	7.344	7707	46.8
14.115	6.269	3121	19
14.438	6.13	15935	96.8
15.624	5.703	637	3.9
16.169	5.477	1349	8.2
16.699	5.305	1663	10.1
16.94	5.23	1692	10.3
17.147	5.167	2139	13
17.66	5.018	6863	41.8
17.91	4.949	16455	100
18.379	4.823	3108	18.9
18.658	4.752	1216	7.4
19.799	4.48	1499	9.1
20.235	4.385	383	2.3
21.707	4.091	1267	7.7
21.8	4.073	1260	7.7
21.959	4.044	1279	7.8
22.461	3.955	4264	25.9
23.191	3.832	1026	6.2
23.879	3.723	1000	6.1
24.599	3.616	1688	10.3
25.837	3.445	931	5.7
26.034	3.42	686	4.2
26.868	3.316	912	5.5
27.093	3.288	1322	8
27.782	3.209	1236	7.5
28.34	3.147	1845	11.2
28.861	3.091	957	5.8
29.866	2.9892	745	4.5
30.627	2.9166	992	6
31.108	2.8726	1205	7.3
33.215	2.6951	1287	7.8
33.718	2.656	802	4.9
34.434	2.6024	914	5.6

TABLE 1B: FORM II DATA

Angle 2-Theta	d-spacing Angstrom	Intensity Cps	Intensity %
7.992	11.054	11598	26.6
10.044	8.799	12048	27.6
11.206	7.889	4929	11.3
12.441	7.109	1747	4
12.752	6.936	4340	9.9
13.257	6.673	2444	5.6
14.705	6.019	43646	100
15.48	5.727	2670	6.1
15.727	5.63	7982	18.3
16.016	5.529	3519	8.1
17.671	5.015	8897	20.4
17.9	4.951	2873	6.6
18.352	4.83	612	1.4
18.703	4.74	669	1.8
19.524	4.543	1126	2.6
20.103	4.413	3753	8.6
20.63	4.302	1451	3.3
21.067	4.214	876	2
21.675	4.097	2760	6.3
22.232	3.995	1951	4.5
22.652	3.922	1657	3.8
23.624	3.763	827	1.9
24.279	3.663	1242	2.8
25.021	3.556	5144	11.8
25.485	3.492	1702	3.9
25.707	3.463	2493	5.7
26.251	3.392	1371	3.1
26.85	3.318	1970	4.5
27.319	3.262	1029	2.4
27.931	3.192	440	1
27.989	3.187	440	1
28.937	3.083	1128	2.6
29.703	3.005	1211	2.8
30.173	2.9594	1506	3.5
30.584	2.9208	1602	3.7
30.885	2.8928	1550	3.6
31.217	2.8628	1068	2.4
31.805	2.8285	1038	2.4
32.059	2.7895	1211	2.8
32.64	2.7412	684	1.6
32.747	2.7324	758	1.7
33.46	2.6758	506	1.2
34.194	2.6201	1085	2.5
34.545	2.5943	915	2.1

TABLE 1C: METHYL ETHYL KETONE DATA

Angle 2-Theta	d-spacing Angstrom	Intensity Cps	Intensity %
7.584	11.648	5629	32.6
7.753	11.393	15929	92.3
10.161	8.707	2877	16.7
11.31	7.817	701	4.1
12.648	6.994	1027	5.9
13.193	6.705	15188	88
13.558	6.526	14225	82.4
14.074	6.287	1966	11.4
14.746	6.002	2759	16
15.185	5.837	801	4.6
15.548	5.694	1896	11
17.031	5.202	7980	46.2
17.28	5.127	17267	100
17.708	5.005	6873	39.8
18.556	4.778	545	3.2
18.871	4.699	1112	6.4
19.766	4.488	1704	9.9
20.158	4.401	1396	8.1
20.725	4.282	2644	15.3
21.767	4.076	1127	6.5
22.06	4.026	451	2.6
22.864	3.886	1542	8.9
23.412	3.796	14185	82.2
23.75	3.743	1154	6.7
24.288	3.662	3063	17.7
25.253	3.524	1318	7.6
25.603	3.49	1736	10.1
26.761	3.455	1225	7.1
26.176	3.402	1346	7.8
26.548	3.355	1098	6.4
27.357	3.257	1944	11.3
27.805	3.229	2118	12.3
27.9	3.195	858	5
28.376	3.142	583	3.4
28.749	3.103	763	4.4
29.3	3.046	1162	6.8
29.679	3.006	2606	15.1
30.402	2.9377	2184	12.8
30.739	2.9063	648	3.8

Graphical examples of the x-ray diffraction patterns for Form I, Form II, and the methyl ethyl ketone solvate

crystalline forms of eplerenone are shown in Figs. 1-A, 1-B, and 1-C, respectively. Form I shows distinguishing peaks at 7.0 ± 0.2, 8.3 ± 0.2, and 12.0 ± 0.2 degrees two theta. Form II shows distinguishing peaks at 8.0 ± 0.2, 12.4 ± 0.2, 12.8 ± 0.2, and 13.3 ± 0.2 degrees two theta. The methyl ethyl ketone solvated crystalline form shows distinguishing peaks at 7.6 ± 0.2, 7.8 ± 0.2, and 13.6 ± 0.2 degrees two theta.

Examples of the x-ray diffraction patterns are shown in Figs. 1-D through 1-O for the following solvate crystalline forms of eplerenone: n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, acetone solvate, toluene solvate, isopropanol solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, methyl acetate solvate, and propyl acetate solvate, respectively.

3. Melting/Decomposition Temperature

The temperatures of melting and/or decomposition of non-solvated eplerenone crystalline forms were determined using a TA Instruments 2920 differential scanning calorimeter. Each sample (1-2 mg) was placed in either a sealed or unsealed aluminum pan and heated at 10°C/minute. Melting/decomposition ranges were defined from the extrapolated onset to the maximum of the melting/decomposition endotherm.

The melting of the non-solvated eplerenone crystals forms (Form I and Form II) was associated with chemical decomposition and loss of trapped solvent from the crystal lattice. The melting/decomposition temperature also was affected by the manipulation of the solid prior to analysis. For example, non-milled Form II (approximate D_{90} particle size of about 180-450 microns) prepared by direct crystallization

from an appropriate solvent or from desolvation of a solvate obtained from crystallization of high purity eplerenone in an appropriate solvent or mixture of solvents generally had a melting range of about 237-242°C. Milled Form II (approximate D_{90} particle size of about 80-100 microns) (Form II prepared by crystallizing a solvate from a solution of high purity eplerenone in an appropriate solvent or mixture of solvents, desolvating the solvate to yield Form II, and milling the resulting Form II) generally had a lower and broader melting/decomposition range of about 223-234°C. Non-milled Form I (approximate D_{90} particle size of about 180-450 microns) prepared by desolvation of a solvate obtained by digestion of low purity eplerenone generally had a higher melting/decomposition range of about 247-251°C. Examples of the DSC thermograms of (a) non-milled Form II directly crystallized from methyl ethyl ketone, (b) non-milled Form II prepared by desolvation of a solvate obtained by crystallization of a high purity eplerenone from methyl ethyl ketone, (c) Form II prepared by milling a desolvated solvate obtained by crystallization of high purity eplerenone from methyl ethyl ketone, and (d) non-milled Form I prepared by desolvation of a solvate obtained by digestion of low purity eplerenone from methyl ethyl ketone are given in Figures 2-A, 2-B, 2-C and 2-D, respectively.

DSC thermograms of solvated forms of eplerenone were determined using a Perkin Elmer Pyris 1 differential scanning calorimeter. Each sample (1-10 mg) was placed in an unsealed aluminum pan and heated at 10°C/minute. One or more endothermal events at lower temperatures were associated with enthalpy changes that occurred as solvent was lost from the solvate crystal lattice. The highest temperature endotherm

or endotherms were associated with the melting/decomposition of Form II or Form I eplerenone. Examples of the DSC thermograms are shown in Figs. 2-E through 2-T for the following solvated crystalline forms of eplerenone: n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, chloroform solvate, acetone solvate, toluene solvate, isopropanol solvate, t-butyl acetate solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, methyl acetate solvate, propyl acetate solvate, n-butanol solvate, and n-octanol solvate, respectively.

4. Infrared Absorption Spectroscopy

Infrared absorption spectra of the non-solvated forms of eplerenone (Form I and Form II) were obtained with a Nicolet DRIFT (diffuse reflectance infrared fourier transform) Magna System 550 spectrophotometer. A Spectra-Tech Collector system and a microsample cup were used. Samples (5%) were analyzed in potassium bromide and scanned from 400-4000 cm^{-1}. Infrared absorption spectra of eplerenone in dilute chloroform solution (3%) or in the solvated crystal forms were obtained with a Bio-rad FTS-45 spectrophotometer. Chloroform solution samples were analyzed using a solution cell of 0.2 mm path length with sodium chloride salt plates. Solvate FTIR spectra were collected using an IBM micro-MIR (multiple internal reflectance) accessory. Samples were scanned from 400-4000 cm^{-1}. Examples of the infrared absorption spectra of (a) Form I, (b) Form II, (c) the methyl ethyl ketone solvate, and (d) eplerenone in chloroform solution are shown in Figures 3-A, 3-B, 3-C and 3-D, respectively.

Table 2 discloses illustrative absorption bands for

eplerenone in the Form I, Form II, and methyl ethyl ketone solvate crystal forms. Illustrative absorption bands for eplerenone in chloroform solution are also disclosed for comparison. Differences between Form I and either Form II or the methyl ethyl ketone solvate were observed, for example, in the carbonyl region of the spectrum. Form I has an ester carbonyl stretch of approximately 1739 cm^{-1} while both Form II and the methyl ethyl ketone solvate have the corresponding stretch at approximately 1724 and 1722 cm^{-1}, respectively. The ester carbonyl stretch occurs at approximately 1727 cm^{-1} in the eplerenone in chloroform solution. The change in stretching frequency of the ester carbonyl between Form I and Form II reflects the change in orientation of the ester group between the two crystal forms. In addition, the stretch of the ester of the conjugated ketone in the A-steroid ring shifts from approximately 1664-1667 cm^{-1} in either Form I or the methyl ethyl ketone solvate to approximately 1655 cm^{-1} in Form II. The corresponding carbonyl stretch occurs at approximately 1665 cm^{-1} in dilute solution.

Another difference between Form I and Form II was seen in the C-H bending region. Form I has an absorption at approximately 1399 cm^{-1} which is not observed in Form II, the methyl ethyl ketone solvate, or the eplerenone in chloroform solution. The 1399 cm^{-1} stretch occurs in the region of CH_2 scissoring for the C2 and C21 methylene groups adjacent to carbonyl groups.

Table 2

Absorption Region	Form I (cm^{-1})	Form II (cm^{-1})	Methyl Ethyl Ketone Solvate (cm^{-1})	Eplerenone in Chloroform (cm^{-1})
ν C=O(lactone)	1773	1775	1767	1768
ν C=O(ester)	1739	1724	1722	1727
ν C=O(3keto)	1664	1655	1667	1665
ν C=C (3,4-olefin)	1619	1619	1622	1623
δ_{as}CH3, δCH2, δCH2(α to carbonyl)	1460, 1444, 1426	1467, 1438, 1422, 1399	1467, 1438, 1422	1464, 1438, 1422
δ_{s}CH3	1380	1381	~1380	1378

Examples of infrared absorption spectra are shown in Figs. 3-E through 3-S for the following solvated crystalline forms of eplerenone: n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, acetone solvate, toluene solvate, isopropanol solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, propyl acetate solvate, methyl acetate solvate, propylene glycol solvate, and t-butyl acetate solvate respectively.

5. Nuclear Magnetic Resonance

^{13}C NMR spectra were obtained at a field of 31.94 MHz. Examples of the ^{13}C NMR spectra of Form I and Form II eplerenone are shown in Figs. 4 and 5, respectively. The Form I eplerenone analyzed to obtain the data reflected in Fig. 4 was not phase pure and included a small amount of Form II eplerenone. Form I is most clearly distinguished by the carbon resonances at around 64.8 ppm, 24.7 ppm and 19.2 ppm.

Form II is most clearly distinguished by the carbon resonances at around 67.1 ppm and 16.0 ppm.

6. Thermogravimetry

Thermogravimetric analysis was performed using a TA Instruments TGA 2950 thermogravimetric analyzer. Samples were placed in an unsealed aluminum pan under nitrogen purge. Starting temperature was 25°C with the temperature increased at a rate of about 10°C/minute.

Examples of thermogravimetry analysis profiles are shown in Figs. 6-A through 6-R for the following solvated crystalline forms of eplerenone: methyl ethyl ketone solvate, n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, chloroform solvate, acetone solvate, toluene solvate, isopropanol solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, methyl acetate solvate, propyl acetate solvate, propylene glycol solvate, n-butanol solvate, n-octanol solvate, and t-butyl acetate solvate, respectively.

7. Microscopy

Hot-stage microscopy was performed on single crystals of the methyl ethyl ketone solvate using a Linkam THMS 600 Hot Stage with Zeiss Universal Polarized Light Microscope. Under polarized light at room temperature the solvate crystal was birefringent and translucent indicating that the crystal lattice was highly ordered. As the temperature increased to about 60°C, noticeable defects began to emerge along the long crystal dimension. A scanning electron micrograph of Form II eplerenone obtained by desolvation of the methyl ethyl ketone solvate is shown in Fig. 7 and reveals surface defects,

pores, cracks and fractures within the crystal lattice. A scanning electron micrograph of Form II eplerenone obtained by direct crystallization from ethyl acetate is shown in Fig. 8 and does not exhibit similar surface defects, pores, cracks and fractures within the crystal lattice.

8. Unit Cell Parameters

Tables 3A, 3B and 3C below summarize the unit cell parameters determined for Form I, Form II, and several solvated crystalline forms.

725 x10

TABLE 3A

Parameter	Form I	Form II	Methyl ethyl ketone Solvate
Crystal system	Ortho-rhombic	Monoclinic	Orthorhombic
Space group	$P2_12_12_1$	$P2_1$	$P2_12_12_1$
a	21.22 Å	8.78 Å	23.53 Å
b	15.40 Å	11.14 Å	8.16 Å
c	6.34 Å	11.06 Å	13.08 Å
α	90°	90°	90°
β	90°	93.52°	90°
γ	90°	90°	90°
Z	4	2	4
Volume (Å)	2071.3	1081.8	2511.4
ρ (calcu-lated)	1.329 g/cm³	1.275 g/cm³	1.287 g/cm³
R	0.0667	0.062	0.088

TABLE 3B

Parameter	Acetone Solvate	Toluene Solvate	Butyl Acetate Solvate[1]
Crystal system	Ortho-rhombic	Ortho-rhombic	Ortho-rhombic
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
a	23.31 Å	23.64 Å	23.07 Å
b	13.13 Å	13.46 Å	13.10 Å
c	8.28 Å	8.16 Å	8.24 Å
α	90°	90°	90°
β	90°	90°	90°
γ	90°	90°	90°
Z	4	4	4
Volume (Å)	2533.7	2596.6	2490.0
ρ (calcu-lated)	1.239 g/cm³	1.296 g/cm³	1.334 g/cm³
R	0.058	0.089	0.093

[1]The solvate molecules were not completely refined due to disorder of the solvent molecules in the channels.

TABLE 3C

Parameter	Isobutyl Acetate Solvate[1]	Isopropanol Solvate[1]	Ethanol Solvate[1]
Crystal system	Ortho-rhombic	Ortho-rhombic	Ortho-rhombic
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
a	23.19 Å	23.15 Å	23.51 Å
b	12.95 Å	12.73 Å	13.11 Å
c	8.25 Å	8.25 Å	8.27 Å
α	90°	90°	90°
β	90°	90°	90°
γ	90°	90°	90°
z	4	4	4
Volume (Å)	2476.4	2433.2	2548.6
ρ (calculated)	1.337 g/cm³	1.296 g/cm³	1.234 g/cm³
R	0.098	0.152	0.067

[1]The solvate molecules were not refined completely due to disorder of the solvent molecules in the channels.

Additional information on selected solvated crystalline forms of eplerenone is reported in Table 4 below. The unit cell data reported in Table 3A above for the methyl ethyl ketone solvate also are representative of the unit cell parameters for many of these additional eplerenone crystalline solvates. Most of the eplerenone crystalline solvates tested are substantially isostructural to each other. While there may be some minor shifting in the X-ray powder diffraction peaks from one solvated crystalline form to the next due to the size of the incorporated solvent molecule, the overall diffraction patterns are substantially the same and the unit cell parameters and molecular positions are substantially identical for most of the solvates tested.

TABLE 4

Solvent	Stoichiometry (Solvent: Eplerenone)	Isostructural to Methyl Ethyl Ketone Solvate?	Desolvation Temperature[1] (°C)
Methyl Ethyl Ketone	1:1	N/A	89
2-Pentanone	---	---	---
Acetic Acid	1:2	Yes	203
Acetone	1:1	Yes	117
Butyl Acetate	1:2	Yes	108
Chloroform	---	Yes	125
Ethanol	1:1	Yes	166
Isobutanol	---	---	---
Isobutyl Acetate	1:2	Yes	112
Isopropanol	1:1	Yes	121
Methyl Acetate	1:1	Yes	103
Ethyl Propionate	1:1	Yes	122
n-Butanol	1:1	Yes	103
n-Octanol	---	Yes	116
n-Propanol	1:1	Yes	129
Propyl Acetate	1:1	Yes	130
Propylene Glycol	---	Yes	188
t-Butanol	---	---	---
Tetrahydrofuran	1:1	Yes	136
Toluene	1:1	Yes	83
t-Butyl Acetate	---	Yes	109

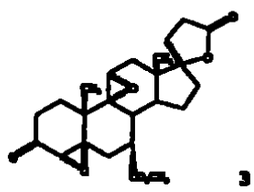
[1]Defined as the extrapolated desolvation temperature from the final solvent weight loss step as determined by thermogravimetric analysis at a heating rate of 10°C/minute under nitrogen purge. Desolvation temperatures, however, can be affected by the method of manufacture of the solvate. Different methods can produce different numbers of nucleation sites capable of initiating desolvation in the solvate at lower temperatures.

The unit cell of the solvate is composed of four eplerenone molecules. The stoichiometry of the eplerenone molecules and solvent molecules in the unit cell is also reported in Table 4 above for a number of solvates. The unit cell of Form I is composed of four eplerenone molecules. The unit cell of Form II is composed of two eplerenone molecules. The solvate unit cells are converted during desolvation into

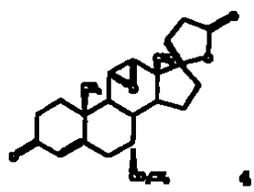
Form I and/or Form II unit cells when the eplerenone molecules undergo translation and rotation to fill the spaces left by the solvent molecules. Table 4 also reports the desolvation temperatures for a number of different solvates.

9. Crystal Properties of Impurity Molecules

Selected impurities in eplerenone can induce the formation of Form I during the desolvation of the solvate. In particular, the effect of the following two impurity molecules was evaluated: 7-methyl hydrogen 4α,5α:9α,11α-diepoxy-17-hydroxy-3-oxo-17α-pregnane-7α,21-dicarboxylate, γ-lactone 3 (the "diepoxide"); and 7-methyl hydrogen 11α,12α-epoxy-17-hydroxy-3-oxo-17α-pregn-4-ene-7α,21-dicarboxylate, γ-lactone 4 (the "11,12-epoxide").



3



4

The effect of these impurity molecules on the eplerenone crystalline form resulting from desolvation is described in greater detail in the examples of this application.

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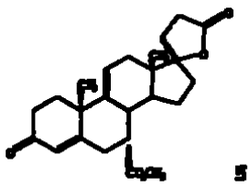
Given the similarity in single crystal structure of 7-methyl hydrogen 17-hydroxy-3-oxo-17α-pregna-4,9(11)-diene-7α,21-dicarboxylate, γ-lactone 5 (the "9,11-olefin") and Form I, it is hypothesized that the 9,11-olefin also can induce the formation of Form I during the desolvation of the solvate.



5

A single crystal form was isolated for each impurity compound. Representative X-ray powder diffraction patterns for the crystal forms isolated for the diepoxide, 11,12-epoxide and 9,11-olefin are given in Figs. 9, 10 and 11, respectively. The X-ray powder diffraction pattern of each impurity molecule is similar to the X-ray powder diffraction pattern of Form I, suggesting that Form I and the three impurity compounds have similar single crystal structures.

Single crystals of each impurity compound also were isolated and subjected to X-ray structure determination to verify that these three compounds adopt single crystal structures similar to that of Form I. Single crystals of the diepoxide were isolated from methyl ethyl ketone. Single crystals of the 11,12-epoxide were isolated from isopropanol. Single crystals of the 9,11-olefin were isolated from n-butanol. Crystal structure data determined for the crystalline form of each impurity compound are given in Table 5. The resulting crystal system and cell parameters were substantially the same for the Form I, diepoxide, 11,12-epoxide, and 9,11-olefin crystalline forms.

TABLE 5

Parameter	Form I	Diepoxide	11,12 Epoxide	9,11 olefin
Crystal system	Ortho-rhombic	Ortho-rhombic	Ortho-rhombic	Ortho-rhombic
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
a	21.22 Å	21.328 Å	20.90 Å	20.90 Å
b	15.40 Å	16.16 Å	15.55 Å	15.74 Å
c	6.34 Å	6.15 Å	6.38 Å	6.29 Å
α	90°	90°	90°	90°
β	90°	90°	90°	90°
γ	90°	90°	90°	90°
z	4	4	4	4
Volume (Å)	2071.3	2119.0	2073.2	2069.3
ρ (calculated)	1.329 g/cm³	1.349 g/cm³	1.328 g/cm³	1.279 g/cm³
R	0.0667	0.0762	0.0865	0.0764

The four compounds reported in Table 5 crystallize into the same space group and have similar cell parameters (i.e., they are isostructural). It is hypothesized that the diepoxide, 11,12-epoxide and 9,11-olefin adopt a Form I conformation. The relative ease of isolation of a Form I packing (directly from solution) for each impurity compound, indicates that the Form I lattice is a stable packing mode for this series of structurally similar compounds.

Preparation of Eplerenone

The eplerenone starting material used to prepare the novel crystalline forms of the present invention can be prepared using the methods set forth in Ng et al., WO97/21720; and Ng et al., WO98/25948, particularly scheme 1 set forth in WO97/21720 and WO98/25948.

Preparation of Crystalline Forms

1. Preparation of Solvated Crystalline Form

The solvated crystalline forms of eplerenone can be prepared by crystallization of eplerenone from a suitable solvent or a mixture of suitable solvents. A suitable solvent or mixture of suitable solvents generally comprises an organic solvent or a mixture of organic solvents that solubilizes the eplerenone together with any impurities at an elevated temperature, but upon cooling, preferentially crystallizes the solvate. The solubility of eplerenone in such solvents or mixtures of solvents generally is about 5 to about 200 mg/mL at room temperature. The solvent or mixtures of solvents preferably are selected from those solvents previously used in the process to prepare the eplerenone starting material, particularly those solvents that would be pharmaceutically acceptable if contained in the final pharmaceutical composition comprising the eplerenone crystalline form. For example, a solvent system comprising methylene chloride that yields a solvate comprising methylene chloride generally is not desirable.

Each solvent used preferably is a pharmaceutically acceptable solvent, particularly a Class 2 or Class 3 solvent as defined in "Impurities: Guideline For Residual Solvents", International Conference On Harmonisation Of Technical Requirements For Registration Of Pharmaceuticals For Human Use (Recommended for Adoption at Step 4 of the ICH Process on July 17, 1997 by the ICH Steering Committee). Still more preferably, the solvent or mixture of solvents is selected from the group consisting of methyl ethyl ketone, 1-propanol,

2-pentanone, acetic acid, acetone, butyl acetate, chloroform, ethanol, isobutanol, isobutyl acetate, methyl acetate, ethyl propionate, n-butanol, n-octanol, isopropanol, propyl acetate, propylene glycol, t-butanol, tetrahydrofuran, toluene, methanol and t-butyl acetate. Still more preferably, the solvent is selected from the group consisting of methyl ethyl ketone and ethanol.

In another embodiment of this process, the solvent or mixture of solvents is selected from the group consisting of 1-propanol, 2-pentanone, acetic acid, acetone, butyl acetate, chloroform, isobutanol, isobutyl acetate, methyl acetate, ethyl propionate, n-butanol, n-octanol, propyl acetate, propylene glycol, t-butanol, tetrahydrofuran, toluene, methanol and t-butyl acetate.

In another embodiment of this process, the solvent or mixture of solvents is selected from the group consisting of 1-propanol, 2-pentanone, acetic acid, acetone, butyl acetate, chloroform, isobutanol, isobutyl acetate, methyl acetate, ethyl propionate, n-butanol, n-octanol, n-propanol, propyl acetate, propylene glycol, t-butanol, tetrahydrofuran, toluene, methanol and t-butyl acetate.

To prepare the solvated crystalline form of eplerenone, an amount of the eplerenone starting material is solubilized in a volume of the solvent and cooled until crystals form. The solvent temperature at which the eplerenone is added to the solvent generally will be selected based upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, this solvent temperature typically is at least about 25°C, preferably from about 30°C to the boiling point of the solvent, and more preferably from about 25°C below the

boiling point of the solvent to the boiling point of the solvent.

Alternatively, hot solvent may be added to the eplerenone and the mixture can be cooled until crystals form. The solvent temperature at the time it is added to the eplerenone generally will be selected based upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, the solvent temperature typically is at least 25°C, preferably from about 50°C to the boiling point of the solvent, and more preferably from about 15°C below the boiling point of the solvent to the boiling point of the solvent.

The amount of the eplerenone starting material mixed with a given volume of solvent likewise will depend upon the solubility curve of the solvent or mixture of solvents. Typically, the amount of eplerenone added to the solvent will not completely solubilize in that volume of solvent at room temperature. For most of the solvents described herein, for example, the amount of eplerenone starting material mixed with a given volume of solvent usually is at least about 1.5 to about 4.0 times, preferably about 2.0 to about 3.5 times, and more preferably about 2.5 times, the amount of eplerenone that will solubilize in that volume of solvent at room temperature.

After the eplerenone starting material has completely solubilized in the solvent, the solution typically is cooled slowly to crystallize the solvated crystalline form of eplerenone. For most of the solvents described herein, for example, the solution is cooled at a rate slower than about 20°C/minute, preferably at a rate of about 10°C/minute or slower, more preferably at a rate of about 5°C/minute or

slower, and still more preferably at a rate of about 1°C/minute or slower.

The endpoint temperature at which the solvated crystalline form is harvested will depend upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, the endpoint temperature typically is less than about 25°C, preferably less than about 5°C, and more preferably less than about -5°C. Decreasing the endpoint temperature generally favors the formation of the solvated crystalline form.

Alternatively, other techniques may be used to prepare the solvate. Examples of such techniques include, but are not limited to, (i) dissolving the eplerenone starting material in one solvent and adding a co-solvent to aid in the crystallization of the solvate crystalline form, (ii) vapor diffusion growth of the solvate, (iii) isolation of the solvate by evaporation, such as rotary evaporation, and (iv) slurry conversion.

The crystals of the solvated crystalline form prepared as described above can be separated from the solvent by any suitable conventional means such as by filtration or centrifugation. Increased agitation of the solvent system during crystallization generally results in smaller crystal particle sizes.

2. Preparation of Form II From Solvate

Form II eplerenone can be prepared directly from the solvated crystalline form by desolvation. Desolvation can be accomplished by any suitable desolvation means such as, but not limited to, heating the solvate, reducing the ambient pressure surrounding the solvate, or combinations thereof.

If the solvate is heated to remove the solvent, such as in an oven, the temperature of the solvate during this process typically does not exceed the enantiotropic transition temperature for Form I and Form II. This temperature preferably does not exceed about 150°C.

The desolvation pressure and time of desolvation are not narrowly critical. The desolvation pressure preferably is about one atmosphere or less. As the desolvation pressure is reduced, however, the temperature at which the desolvation can be carried out and/or the time of desolvation likewise is reduced. Particularly for solvates having higher desolvation temperatures, drying under vacuum will permit the use of lower drying temperatures. The time of desolvation need only be sufficient to allow for the desolvation, and thus the formation of Form II, to reach completion.

To ensure the preparation of a product that comprises substantially all Form II, the eplerenone starting material typically is a high purity eplerenone, preferably substantially pure eplerenone. The eplerenone starting material used to prepare Form II eplerenone generally is at least 90% pure, preferably at least 95% pure, and more preferably at least 99% pure. As discussed in greater detail elsewhere in this application, certain impurities in the eplerenone starting material can adversely affect the yield and Form II content of the product obtained from the process.

The crystallized eplerenone product prepared in this manner from a high purity eplerenone starting material generally comprises at least 10% Form II, preferably at least 50% Form II, more preferably at least 75% Form II, still more preferably at least 90% Form II, still more preferably at

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least about 95% Form II, and still more preferably substantially phase pure Form II.

3. Preparation of Form I From Solvate

A product comprising Form I can be prepared in substantially the same manner as set forth above for the preparation of Form II by (i) using a low purity eplerenone starting material instead of a high purity eplerenone starting material, (ii) seeding the solvent system with phase pure Form I crystals, or (iii) a combination of (i) and (ii).

A. Use Of Impurities As Growth Promoters and Inhibitors

The presence and amount of selected impurities in the eplerenone starting material, rather than the total amount of all impurities in the eplerenone starting material, affect the potential for Form I crystal formation during the desolvation of the solvate. The selected impurity generally is a Form I growth promoter or Form II growth inhibitor. It may be contained in the eplerenone starting material, contained in the solvent or mixture of solvents before the eplerenone starting material is added, and/or added to the solvent or mixture of solvents after the eplerenone starting material is added. Bonafede et al., "Selective Nucleation and Growth of an Organic Polymorph by Ledge-Directed Epitaxy on a Molecular Crystal Substate", J. Amer. Chem. Soc., Vol. 117, No. 30 (August 2, 1995) discusses the use of growth promoters and growth inhibitors in polymorph systems and is incorporated by reference herein. For the present invention, the impurity generally comprises a compound having a single crystal structure substantially identical to the single crystal structure of Form I. The impurity preferably is a compound

having an X-ray powder diffraction pattern substantially identical to the X-ray powder diffraction pattern of Form I, and more preferably is selected from the group consisting of the diepoxide, the 11,12-epoxide, the 9,11-olefin and combinations thereof.

The amount of impurity needed to prepare Form I crystals typically can depend, in part, upon the solvent or mixture of solvents and the solubility of the impurity relative to eplerenone. In the crystallization of Form I from a methyl ethyl ketone solvent, for example, the weight ratio of diepoxide to low purity eplerenone starting material typically is at least about 1:100, preferably at least about 3:100, more preferably between about 3:100 and about 1:5, and still more preferably between about 3:100 and about 1:10. The 11,12-epoxide has a higher solubility in methyl ethyl ketone than the diepoxide and generally requires a larger amount of the 11,12-epoxide generally is necessary to prepare Form I crystals. Where the impurity comprises the 11,12-epoxide, the weight ratio of the diepoxide to the low purity eplerenone starting material typically is at least about 1:5, more preferably at least about 3:25, and still more preferably between about 3:25 and about 1:5. Where both the diexpoxide and the 11,12-epoxide impurities are used in the preparation of the Form I crystals, the weight ratio of each impurity to the eplerenone starting material may be lower than the corresponding ratio when only that impurity is used in the preparation of the Form I crystals.

A mixture of Form I and Form II is generally obtained when a solvate comprising the selected impurity is desolvated. The weight fraction of Form I in the product resulting from the initial desolvation of the solvate

typically is less than about 50%. Further treatment of this product by crystallization or digestion, as discussed below, generally will increase the weight fraction of Form II in the product.

B. Seeding

Form I crystals also can be prepared by seeding the solvent system with phase pure Form I crystals (or a Form I growth promoter and/or Form II growth inhibitor as previously discussed above) prior to crystallization of the eplerenone. The eplerenone starting material can be either a low purity eplerenone or a high purity eplerenone. When the resulting solvate prepared from either starting material is desolvated, the weight fraction of Form I in the product typically is at least about 70% and may be as great as about 100%.

The weight ratio of Form I seed crystals added to the solvent system to the eplerenone starting material added to the solvent system generally is at least about 0.75:100, preferably between about 0.75:100 to about 1:20, and more preferably between about 1:100 to about 1:50. The Form I seed crystals can be prepared by any of the methods discussed in this application for the preparation of Form I crystals, particularly the preparation of Form I crystals by digestion as discussed below.

The Form I seed crystals may be added at one time, in multiple additions or substantially continually over a period of time. The addition of the Form I seed crystals, however, generally is completed before the eplerenone begins to crystallize from solution, i.e., the seeding is completed before the cloud point (the lower end of the metastable zone) is reached. Seeding typically is performed when the solution

temperature ranges from about 0.5°C above the cloud point to about 10°C above the cloud point, preferably within about 2°C to about 3°C above the cloud point. As the temperature above the cloud point at which the seeds are added increases, the amount of seeding needed for crystallization of Form I crystals generally increases.

The seeding preferably occurs not only above the cloud point, but within the metastable zone. Both the cloud point and the metastable zone are dependent on the eplerenone solubility and concentration in the solvent or mixture of solvents. For a 12 volume dilution of methyl ethyl ketone, for example, the high end of the metastable zone generally is between about 70°C to about 73°C and the lower end of the metastable zone (i.e., the cloud point) is between about 57°C and 63°C. For a concentration of 8 volumes of methyl ethyl ketone, the metastable zone is even narrower because the solution is supersaturated. At this concentration, the cloud point of the solution occurs at about 75°C to about 76°C. Because the boiling point of methyl ethyl ketone is about 80° C under ambient conditions, seeding for this solution typically occurs between about 76.5°C and the boiling point.

An illustrative non-limiting example of seeding with Form I is set forth below in Example 7.

The crystallized eplerenone product obtained using a Form I growth promoter or Form II growth inhibitor, and/or Form I seeding generally comprises at least 2% Form I, preferably at least 5% Form I, more preferably at least 7% Form I, and still more preferably at least about 10% Form I.

The remaining crystallized eplerenone product generally is Form II.

C. Form I Prepared By Grinding Eplerenone

In yet another alternative, it has been discovered that a small amount of Form I can be prepared by suitable grinding eplerenone. Concentrations of Form I in ground eplerenone as high as about 3% have been observed.

4. Preparation of Form II From Solvate Prepared From Low Purity Eplerenone

As discussed above, crystallization of low purity eplerenone to form a solvate followed by desolvation of the solvate generally yields a product comprising both Form I and Form II. A product having a greater Form II content can be prepared from low purity eplerenone in substantially the same manner as set forth above for the preparation of Form I by seeding the solvent system with phase pure Form II crystals, or by using a Form II growth promoter and/or Form I growth inhibitor. The seeding protocol and the weight ratio of the amount of Form II seed crystals added to the solvent system to the amount of the eplerenone starting material added to the solvent system generally are similar to those ratios previously discussed above for the preparation of Form I eplerenone by seeding with phase pure Form I crystals.

The crystallized eplerenone product prepared in this manner generally comprises at least 10% Form II, preferably at least 50% Form II, more preferably at least 75% Form II, more preferably at least 90% Form II, still more preferably at least about 95% Form II, and still more preferably substantially phase pure Form II.

The seeding protocols described in this section and in the prior section relating to the preparation of Form I eplerenone also may allow for improved control of the particle size of the crystallized eplerenone.

5. Crystallization of Form II Directly From Solution

Form II eplerenone also can be prepared by the direct crystallization of eplerenone from a suitable solvent or mixture of solvents without the formation of an intermediate solvate and the accompanying need for desolvation. Typically, (i) the solvent has a molecular size that is incompatible with the available channel space in the solvate crystal lattice, (ii) the eplerenone and any impurities are soluble in the solvent at elevated temperatures, and (iii) upon cooling, results in the crystallization of the non-solvated Form II eplerenone. The solubility of eplerenone in the solvent or mixture of solvents generally is about 5 to about 200 mg/mL at room temperature. The solvent or mixture of solvents preferably comprises one or more solvents selected from the group consisting of methanol, ethyl acetate, isopropyl acetate, acetonitrile, nitrobenzene, water and ethyl benzene.

To crystallize Form II eplerenone directly from solution, an amount of the eplerenone starting material is solubilized in a volume of the solvent and cooled until crystals form. The solvent temperature at which the eplerenone is added to the solvent generally will be selected based upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, this solvent temperature typically is at least about 25°C, preferably from about 30°C to the boiling point of the

solvent, and more preferably from about 25°C below the boiling point of the solvent to the boiling point of the solvent.

Alternatively, hot solvent may be added to the eplerenone and the mixture can be cooled until crystals form. The solvent temperature at the time it is added to the eplerenone generally will be selected based upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, the solvent temperature typically is at least 25°C, preferably from about 50°C to the boiling point of the solvent, and more preferably from about 15°C below the boiling point of the solvent to the boiling point of the solvent.

The amount of the eplerenone starting material mixed with a given volume of solvent likewise will depend upon the solubility curve of the solvent or mixture of solvents. Typically, the amount of eplerenone added to the solvent will not completely solubilize in that volume of solvent at room temperature. For most of the solvents described herein, for example, the amount of eplerenone starting material mixed with a given volume of solvent usually is at least about 1.5 to about 4.0 times, preferably about 2.0 to about 3.5 times, and more preferably about 2.5 times, the amount of eplerenone that will solubilize in that volume of solvent at room temperature.

To ensure the preparation of a product that comprises substantially phase pure Form II, the eplerenone starting material generally is a high purity eplerenone. The eplerenone starting material preferably is at least 65% pure, more preferably at least 90% pure, still more preferably at least 98% pure, and still more preferably at least 99% pure.

After the eplerenone starting material has completely solubilized in the solvent, the solution typically is cooled slowly to crystallize the solvated crystalline form of eplerenone. For most of the solvents described herein, for example, the solution is cooled at a rate slower than about 1.0°C/minute, preferably at a rate of about 0.2°C/minute or slower, and more preferably at a rate between about 5°C/minute and about 0.1°C/minute.

The endpoint temperature at which the Form II crystals are harvested will depend upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, the endpoint temperature typically is less than about 25°C, preferably less than about 5°C, and more preferably less than about -5°C.

Alternatively, other techniques may be used to prepare the Form II crystals. Examples of such techniques include, but are not limited to, (i) dissolving the eplerenone starting material in one solvent and adding a co-solvent to aid in the crystallization of Form II eplerenone, (ii) vapor diffusion growth of Form II eplerenone, (iii) isolation of Form II eplerenone by evaporation, such as rotary evaporation, and (iv) slurry conversion.

The crystals of the solvated crystalline form prepared as described above can be separated from the solvent by any suitable conventional means such as by filtration or centrifugation.

In addition, Form II eplerenone also can be prepared by digesting (as described below) a slurry of high purity eplerenone in methyl ethyl ketone and filtering the digested eplerenone at the boiling point of the slurry.

6. Preparation of Form I Directly From Solution

It is hypothesized that if the crystallization is performed above the enantiotropic transition temperature (T_t) for Form I and Form II, particularly if Form I growth promoters or Form II growth inhibitors are present or the solvent is seeded with phase pure Form I crystals, Form I should crystallize directly from solution since Form I is more stable at these higher temperatures. The solvent system used preferably comprises a high boiling solvent such as nitrobenzene. Suitable Form I growth promoters would include, but would not be limited to, the diepoxide and the 11,12-olefin.

7. Digestion of Eplerenone With A Solvent

The solvated crystalline forms, Form I and Form II of eplerenone also can be prepared by digestion of an eplerenone starting material in a suitable solvent or mixture of solvents. In the digestion process, a slurry of eplerenone is heated at the boiling point of the solvent or mixture of solvents. For example, an amount of eplerenone starting material is combined with a volume of solvent or mixture of solvents, heated to reflux, and the distillate is removed while an additional amount of the solvent is added simultaneously with the removal of the distillate. Alternatively, the distillate can be condensed and recycled without the addition of more solvent during the digestion process. Typically, once the original volume of solvent has been removed or condensed and recycled, the slurry is cooled and solvated crystals form. The solvated crystals can be separated from the solvent by any suitable conventional means such as by filtration or centrifugation. Desolvation of the

solvate as previously described yields either Form I or Form II eplerenone depending upon the presence or absence of the selected impurities in the solvated crystals.

A suitable solvent or mixture of solvents generally comprises one or more of the solvents previously disclosed herein. The solvent may be selected, for example, from the group consisting of methyl ethyl ketone and ethanol.

The amount of eplerenone starting material added to the solvent used in the digestion process generally is sufficient to maintain a slurry (i.e., the eplerenone in the solvent or mixture of solvents is not completely solubilized) at the boiling point of the solvent or mixture of solvents. Illustrative values include, but are not limited to, about one gram of eplerenone per four mL methyl ethyl ketone and about one gram of eplerenone per eight mL ethanol.

The solution generally is cooled slowly once solvent turnover is complete to crystallize the solvated crystalline form of eplerenone. For the solvents tested, for example, the solution is cooled at a rate slower than about 20°C/minute, preferably about 10°C/minute or slower, more preferably about 5°C/minute or slower, and still more preferably about 1°C/minute or slower.

The endpoint temperature at which the solvated crystalline form is harvested will depend upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, the endpoint temperature typically is less than about 25°C, preferably less than about 5°C, and more preferably less than about -5°C.

If a product comprising primarily or exclusively Form II is desired, a high purity eplerenone starting material

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typically is digested. The high purity eplerenone starting material preferably is at least 98% pure, more preferably at least 99% pure, and still more preferably at least 99.5% pure. The digested eplerenone product prepared in this manner generally comprises at least 10% Form II, preferably at least 50% Form II, more preferably at least 75% Form II, more preferably at least 90% Form II, still more preferably at least about 95% Form II, and still more preferably substantially phase pure Form II.

If a product comprising primarily or exclusively Form I is desired, a low purity eplerenone starting material typically is digested. The low purity eplerenone starting material generally contains only as much Form I growth promoter and/or Form II growth inhibitor as is needed to yield Form I. Preferably, the low purity eplerenone starting material is at least 65% pure, more preferably at least 75% pure, and still more preferably at least 80% pure. The digested eplerenone product prepared in this manner generally comprises at least 10% Form I, preferably at least 50% Form I, more preferably at least 75% Form I, more preferably at least 90% Form I, still more preferably at least about 95% Form I, and still more preferably substantially phase pure Form I.

8. Preparation of Amorphous Eplerenone

Amorphous eplerenone can be prepared in small quantities by suitable comminution of solid eplerenone, such as by crushing, grinding and/or micronizing. Phase pure amorphous eplerenone can be prepared, for example, by lyophilizing a solution of eplerenone, particularly an aqueous solution of

eplerenone. These processes are illustrated in Examples 13 and 14 below.

Additional Processing Considerations

1. Thermodynamic Stability Considerations

Form II is more thermodynamically stable than Form I at ambient temperatures. As described in Example 5 below, when an organic slurry containing equivalent amounts of Form I and Form II was allowed to stand overnight at room temperature and the residual solids were then collected and analyzed by X-ray powder diffraction, the analytical results indicated that the eplerenone had completely converted to Form II. The differential scanning calorimetry ("DSC") data (discussed previously) indicate that Form I is more thermodynamically stable than Form II at higher temperatures since it has the higher melting/decomposition temperature. Taken together, the slurry conversion and DSC data indicate that Form I and Form II are related enantiotropically, i.e., a change in the stability relationship between the two polymorphs occurs around a transition temperature (T_t), with Form II being the more stable at lower temperatures. Fig. 12 shows the Gibbs free energy-temperature relationship generally observed for enantiotropically related polymorphs such as Form I and Form II, wherein I and II refer to the crystal forms, T_t refers to the transition temperature, T_m refers to the melting points of Form I and Form II, and L represents the liquid or melt state.

Accordingly, processing temperatures preferably are maintained below the transition temperature during the preparation of compositions comprising Form II. For example, drying temperatures employed for desolvation typically are

less than about 150°C, preferably less than about 125°C, more preferably less than about 115°C, more preferably less than about 110°C, and still more preferably between about 80°C to about 110°C. In addition, cooling (such as by use of liquid nitrogen) may be necessary during particle size reduction process steps to maintain the temperature of the Form II crystals below the transition temperature.

2. Intrinsic Micronizing Considerations

The method used to prepare crystalline eplerenone may affect the properties of the resulting crystal. For example, Form II prepared by desolvation of a solvate exhibits a higher incidence of surface defects, pores, cracks and fractures within the crystal lattice than Form II prepared by direct crystallization from solution. This "intrinsic micronizing" of the desolvated crystal results in an increase in both the available surface area of the crystal and the dissolution rate of the crystal. Dissolution times, therefore, can be shortened by selection of Form II crystals prepared by desolvation, lengthened by selection of Form II crystals prepared by direct crystallization, or otherwise adjusted by selection of an appropriate combination of Form II crystals prepared by desolvation and Form II crystals prepared by direct crystallization.

This "intrinsic micronizing" also may effectively reduce or eliminate the need for crystal particle size reduction during processing steps where Form II crystals prepared by desolvation are used in the preparation of the pharmaceutical composition. One disadvantage to using such Form II crystals, however, is the need for a desolvation step that is

not required for Form II crystals prepared by direct crystallization.

Product-By-Process Solid State Forms

The present invention also comprises each of the solid state eplerenone forms prepared in accordance with the processes disclosed in this application. In particular, the invention comprises solvated eplerenone, Form I eplerenone, Form II eplerenone, and combinations thereof, prepared as set forth in this application.

Combinations of Solid State Forms

Also embraced within this invention are combinations comprising a first solid state form of eplerenone and a second solid state form of eplerenone, wherein the first and second solid state forms of eplerenone are selected from Form I, Form II, solvated eplerenone and amorphous eplerenone. In general, the weight ratio of said first solid state form to said second solid state form preferably is at least about 1:9, more preferably is at least about 1:1, more preferably at least about 2:1, more preferably at least about 5:1, and still more preferably at least about 9:1.

In one embodiment, for example, the first solid state form is Form I and the second solid state form is Form II.

In another embodiment, for example, the first solid state form is Form II and the second solid state form is Form I.

Pharmaceutical Compositions

Also embraced within this invention is a class of pharmaceutical compositions comprising a first solid state

form of eplerenone and a second solid state form of eplerenone in association with one or more pharmaceutically-acceptable carriers and/or diluents and/or adjuvants (collectively referred to herein as "carrier" materials) and, if desired, other active ingredients. The first solid state form of eplerenone and the second solid state form of eplerenone are selected from Form I, Form II, solvated eplerenone and amorphous eplerenone. In general, the weight ratio of said first solid state form to said second solid state form preferably is at least about 1:9, more preferably is at least about 1:1, more preferably at least about 2:1, more preferably at least about 5:1, and still more preferably at least about 9:1.

In one embodiment of the invention, the entire amount of eplerenone contained in the composition is present as phase pure Form II.

In another embodiment of the invention, the entire amount of eplerenone contained in the composition is present as phase pure Form I.

In still another embodiment of the invention, the entire amount of eplerenone contained in the composition is present as phase pure solvated crystalline eplerenone.

In still another embodiment of the invention, the entire amount of eplerenone contained in the composition is present as amorphous eplerenone.

In still another embodiment of the invention, the composition comprises both Form I and Form II. The ratio of the amount of Form II to Form I in the composition generally is between about 1:20 to about 20:1. In other embodiments, for example, this ratio is between about 10:1 to about 1:10;

about 5:1 to about 1:5; about 2:1 to about 1:2; or is about 1:1.

In still another embodiment of the invention, the composition comprises a first solid state form of eplerenone and a second solid state form of eplerenone, wherein the first and second solid state forms of eplerenone are selected from Form I, Form II, solvated eplerenone and amorphous eplerenone. In general, the weight ratio of said first solid state form to said second solid state form preferably is at least about 1:9, preferably about 1:1, more preferably at least about 2:1, more preferably at least about 5:1, and still more preferably at least about 9:1.

In each of the above combination and pharmaceutical composition embodiments, the D_{90} particle size of the eplerenone used as a starting material in the pharmaceutical composition generally is between about 25 microns to about 400 microns, preferably less than about 200 microns, more preferably less than about 150 microns, still more preferably less than about 100 microns, and still more preferably less than about 90 microns. In one embodiment, the D_{90} particle size is between about 40 microns to about 100 microns. In another embodiment, the D_{90} particle size is between about 30 microns to about 50 microns. In another embodiment, the D_{90} particle size is between about 50 microns to about 150 microns. In another embodiment, the D_{90} particle size is between about 75 microns to about 125 microns.

These solid state forms of eplerenone of the present invention may be administered by any suitable route, preferably in the form of a pharmaceutical composition adapted to such a route, and in a dose effective for the treatment intended. The solid state forms of eplerenone and

compositions thereof may, for example, be administered orally, intravascularly, intraperitoneally, subcutaneously, intramuscularly or topically.

The amount of eplerenone that is administered and the dosage regimen for treating a condition or disorder with the solid state forms of eplerenone and/or compositions of this invention depends on a variety of factors, including the age, weight, sex and medical condition of the subject, the severity of the disease, the route and frequency of administration, and the particular compound employed, and thus may vary widely. The pharmaceutical compositions may contain eplerenone in the range of about 0.1 to 2000 mg, preferably in the range of about 0.5 to 500 mg and most preferably between about 1 and 200 mg. A daily dose of about 0.01 to 100 mg/kg body weight, preferably between about 0.5 and about 20 mg/kg body weight and most preferably between about 0.1 to 10 mg/kg body weight, may be appropriate. The daily dose can be administered in one to four doses per day.

For oral administration, the pharmaceutical composition may be in the form of, for example, a tablet, capsule, or suspension. The pharmaceutical composition is preferably made in the form of a dosage unit containing a particular amount of eplerenone. Examples of such dosage units are tablets or capsules. The eplerenone may also be administered by injection of a suspension wherein, for example, saline, dextrose or water may be used as a suitable carrier.

Other formulations may be in the form of a topical ointment or cream, or a suppository, containing eplerenone in a total amount of, for example, 0.075 to 30% w/w, preferably 0.2 to 20% w/w and most preferably 0.4 to 15% w/w. When formulated in an ointment, the eplerenone may be employed

with either paraffinic or a water-miscible ointment base. Alternatively, the eplerenone may be formulated in a cream with an oil-in-water cream base. If desired, the aqueous phase of the cream base may include, for example at least 30% w/w of a polyhydric alcohol such as propylene glycol, butane-1,3-diol, mannitol, sorbitol, glycerol, polyethylene glycol and mixtures thereof. The topical formulation may desirably include a compound which enhances absorption or penetration of the eplerenone through the skin or other affected areas. Examples of such dermal penetration enhancers includs dimethylsulfoxide and related analogs.

The novel solid state forms of eplerenone also can be administered by a transdermal device. Preferably, topical administration will be accomplished using a patch either of the reservoir and porous membrane type or of a solid matrix variety. In either case, the eplerenone is delivered continuously from the reservoir or microcapsules through a membrane into the eplerenone permeable adhesive, which is in contact with the skin or mucosa of the recipient. If the eplerenone is absorbed through the skin, a controlled and predetermined flow of the eplerenone is administered to the recipient. In the case of microcapsules, the encapsulating agent may also function as the membrane.

The oily phase of the emulsions of this invention may be constituted from known ingredients in a known manner. While the phase may comprise merely an emulsifier, it may comprise a mixture of at least one emulsifier with a fat or an oil or with both a fat and an oil. Preferably, a hydrophilic emulsifier is included together with a lipophilic emulsifier which acts as a stabilizer. It is also preferred to include both an oil and a fat. Together, the emulsifier(s) with or

without stabilizer(s) make-up the so-called emulsifying wax, and the wax together with the oil and fat make up the so-called emulsifying ointment base which forms the oily dispersed phase of the cream formulations. Emulsifiers and emulsion stabilizers suitable for use in the formulation of the present invention include Tween 60, Span 80, cetostearyl alcohol, myristyl alcohol, glyceryl monostearate, and sodium lauryl sulfate, among others.

The choice of suitable oils or fats for the formulation is based on achieving the desired cosmetic properties, since the solubility of the eplerenone in most oils likely to be used in pharmaceutical emulsion formulations is very low. Thus, the cream should preferably be a non-greasy, non-staining and washable product with suitable consistency to avoid leakage from tubes or other containers.. Straight or branched chain, mono- or dibasic alkyl esters such as di-isoadipate, isocetyl stearate, propylene glycol diester of coconut fatty acids, isopropyl myristate, decyl oleate, isopropyl palmitate, butyl stearate, 2-ethylhexyl palmitate or a blend of branched chain esters may be used. These may be used alone or in combination depending on the properties required. Alternatively, high melting point lipids such as white soft paraffin and/or liquid paraffin or other mineral oils can be used.

Formulations suitable for topical administration to the eye also include eye drops wherein the eplerenone is suspended in suitable carrier, especially an aqueous carrier for the eplerenone. The eplerenone is preferably present in such formulations in a concentration of 0.5 to 20%, advantageously 0.5 to 10% and particularly about 1.5% w/w.

For therapeutic purposes, the novel solid state forms of eplerenone ordinarily are combined with one or more adjuvants appropriate to the indicated route of administration. If administered per os, the compound may be admixed with lactose, sucrose, starch powder, cellulose esters of alkanoic acids, cellulose alkyl esters, talc, stearic acid, magnesium stearate, magnesium oxide, sodium and calcium salts of phosphoric and sulfuric acids, gelatin, acacia gum, sodium alginate, polyvinylpyrrolidone, and/or polyvinyl alcohol, and then tableted or encapsulated for convenient administration. Such capsules or tablets may contain an immediate release formulation, or may contain a controlled-release formulation such as a dispersion of the eplerenone in hydroxypropylmethyl cellulose. Formulations for parenteral administration may be in the form of aqueous or non-aqueous isotonic sterile injection suspensions. These suspensions may be prepared from sterile powders or granules having one or more of the carriers or diluents mentioned for use in the formulations for oral administration. Other adjuvants and modes of administration are well and widely known in the pharmaceutical art.

Methods of Treatment or Prophylaxis

The present invention also comprises methods for the treatment and/or prophylaxis of an aldosterone-mediated condition and/or disorder such as hypertension in a subject, the methods comprising treating the subject having or susceptible to such condition or disorder with a therapeutically-effective amount of Form I, Form II, solvated crystalline form, or an amorphous form of eplerenone, or combinations thereof. These solid state forms of eplerenone

would be useful for the treatment and/or prophylaxis of a condition and/or disorder in a subject where administration of an aldosterone-antagonist is indicated including, but not limited to, the treatment of hyperaldosteronism such as hypertension, heart failure including cardiac insufficiency, cirrhosis of the liver, excess collagen/fibrosis, benign prostate hypertrophy and depression.

Besides being useful for human treatment, these solid state forms of eplerenone are also useful for veterinary treatment of companion animals, exotic animals and farm animals, including mammals, rodents, and the like. More preferred animals include horses, dogs, and cats.

These solid state forms of eplerenone also may be used (i) in combination therapies partially or completely in place of other conventional aldosterone receptor antagonists, or (ii) in combination therapies with other active drugs. The phrase "combination therapy" is intended to embrace the administration of each agent in a sequential manner in a regimen that will provide beneficial effects of the drug combination, and is intended as well to embrace co-administration of these agents in a substantially simultaneous manner, such as in a single capsule or injection having a fixed ratio of these active agents or in multiple, separate capsules or injections for each agent. Non-limiting examples of combination therapy include the treatment of cardiovascular diseases using a combination of an aldosterone receptor antagonist and an angiotensin II receptor antagonist as described in WO96/24373; the treatment of congestive heart failure using a combination of an aldosterone receptor antagonist and an angiotensin II antagonist as described in WO96/40257; and the treatment of heart failure using a

combination of an aldosterone receptor antagonist, an ACE inhibitor and a diuretic as described in WO96/24372.

Working Examples

The following examples contain detailed descriptions of the methods of preparation of the various solid state forms of eplerenone described in this application. These detailed descriptions fall within the scope, and serve to exemplify the invention. These detailed descriptions are presented for illustrative purposes only and are not intended as a restriction on the scope of the invention. All parts are by weight and temperatures are in degrees Centigrade unless otherwise indicated. The eplerenone starting material used in each of the following examples was prepared in accordance with scheme 1 set forth in Ng et al., WO98/25948.

Example 1: Preparation of (a) methyl ethyl ketone solvate from high purity eplerenone starting material and (b) Form II crystalline eplerenone from resulting solvate

A. Preparation of Methyl Ethyl Ketone Solvate:

High purity eplerenone (437 mg; greater than 99% purity with less than 0.2% diepoxide and 11,12 epoxide present) was dissolved in 10 mL of methyl ethyl ketone by heating to boiling on a hot plate with magnetic stirring at 900 rpm. The resulting solution was allowed to cool to room temperature with continuous magnetic stirring. Once at room temperature, the solution was transferred to a 1°C bath with maintenance of the stirring for one hour. After one hour, the solid methyl ethyl ketone solvate was collected by vacuum filtration.

B. Preparation of Form II crystalline eplerenone:

The solid methyl ethyl ketone solvate prepared in Step A above was dried in an oven at 100°C for four hours at ambient pressure. The dried solid was determined to be pure Form II by DSC and XPRD analysis.

Example 2: Preparation of additional solvates from high purity eplerenone starting material

Additional solvated crystalline forms were prepared by replacing methyl ethyl ketone with one of the following solvents: n-propanol, 2-pentanone, acetic acid, acetone, butyl acetate, chloroform, ethanol, isobutanol, isobutyl acetate, isopropanol, methyl acetate, ethyl propionate, n-butanol, n-octanol, propyl acetate, propylene glycol, t-butanol, tetrahydrofuran, and toluene and carrying out the crystallization substantially as described above in Step A of Example 1.

Example 3: Preparation of Methyl Ethyl Ketone Solvate by Vapor Diffusion Growth

Eplerenone (400 mg; greater than 99.9% purity) was dissolved in 20 mL of methyl ethyl ketone by warming on a hot plate to form a stock solution. An 8 mL amount of the stock solution was transferred to a first 20 mL scintillation vial and diluted to 10 mL with methyl ethyl ketone (80%). A 10 mL amount of the stock solution was transferred to a second 20 mL scintillation vial and diluted to 10 mL with methyl ethyl ketone (40%). The final 2 mL of the stock solution was diluted to 10 mL with methyl ethyl ketone (20%). The four vials containing the dilutions were transferred to a dessicator jar containing a small amount of hexane as an anti-solvent. The dessicator jar was sealed and the hexane

vapor allowed to diffuse into the methyl ethyl ketone solutions. Methyl ethyl ketone solvate crystals grew in the 80% dilution sample by the next day.

Example 4: Preparation of Methyl Ethyl Ketone Solvate by Rotary Evaporation

About 400 mg of eplerenone (greater than 99.9% purity) is weighed into a 250 mL round bottom flask. Solvent (150 mL) is added to the flask and, if necessary, the solution is heated gently until the solid is dissolved. The resulting clear solution is placed on a Buchi rotary evaporator with a bath temperature of about 85°C and the solvent is removed under vacuum. Solvent removal is stopped when approximately 10 mL of solvent remain in the round bottom flask. The resulting solids are analyzed by appropriate method (XPRD, DSC, TGA, microscopy, etc.) for determination of form.

Example 5: Slurry Conversion

Approximately 150 mg of Form II eplerenone and 150 mg of Form I eplerenone were added to 5 mL of ethyl acetate. The resulting slurry was allowed to stir at 300 rpm (magnetic stirring) overnight. The next day a sample of the solid was collected by filtration. Analysis of the sample by XPRD indicated that the sample was entirely composed of Form II eplerenone.

Example 6: Preparation of (a) solvate from low purity eplerenone starting material and (b) Form I crystalline eplerenone from resulting solvate

Samples containing varying amounts of the impurity 7-methyl hydrogen 4α,5α:9α,11α-diepoxy-17-hydroxy-3-oxo-17α-

pregnane-7α,21-dicarboxylate, γ-lactone (the "diepoxide") or the impurity 7-methyl hydrogen 11α,12α-epoxy-17-hydroxy-3-oxo-17α-pregn-4-ene-7α,21-dicarboxylate, γ-lactone (the "11,12-epoxide") were prepared by adding the desired amount of the impurity to a 7 mL scintillation vial together with an amount of eplerenone sufficient to provide a total sample mass of 100 mg. The weight percent of the diepoxide or 11,12-epoxide in each sample is given in Tables X-6A and X-6B, respectively. A micro-flea magnetic stirrer was added to each scintillation vial along with 1 mL of methyl ethyl ketone. The vials were loosely capped and the solid dissolved by heating to reflux on a hot plate with magnetic stirring. Once the solids were dissolved, the solutions were allowed to cool to room temperature on the hot plate. Magnetic stirring was maintained during the cooling period. After the solutions reached room temperature, the solids were collected by vacuum filtration and immediately analyzed by X-ray powder diffraction (XPRD). The solids were then placed in a 100°C oven and dried for one hour at ambient pressure. The dried solids were analyzed by XPRD for Form I content by monitoring the area of the Form I diffraction peak at about 12.1 degrees two theta. All XPRD diffraction patterns were recorded using an Inel Multipurpose Diffractometer.

TABLE X-6A

Weight Percent Diepoxide	Weight Eplerenone (mg)	Weight Diepoxide (mg)
0%	100.44	--
1%	99.08	1.24
2%	98.09	2.24
3%	97.08	3.04
5%	95.09	5.04

TABLE X-6B

Weight Percent 11,12-Epoxide	Weight Eplerenone (mg)	Weight 11,12-Epoxide (mg)
0%	101.38	0
1%	99.23	1.10
5%	94.97	5.36
10%	90.13	10.86

A. Diepoxide Results

Fig. 13 shows the X-ray powder diffraction patterns for the wet cake (methyl ethyl ketone solvate) obtained from the (a) 0%, (b) 1%, (c) 3%, and (d) 5% diepoxide-doped methyl

ethyl ketone crystallizations. The peak intensities have been normalized for ease of comparison. No peaks characteristic of Form I or the diepoxide are present in the diffraction patterns. The patterns are characteristic of the methyl ethyl ketone solvate of eplerenone.

Fig. 14 shows the X-ray powder diffraction patterns for the dried solids obtained from the (a) 0%, (b) 1%, (c) 3%, and (d) 5% diepoxide-doped methyl ethyl ketone crystallizations. The peak intensities have been normalized for ease of comparison. No Form I was detected for the dried samples corresponding to the methyl ethyl ketone crystallizations performed at doping levels of 0 and 1%. Form I was detected in the dried samples corresponding to the methyl ethyl ketone crystallizations performed at doping levels of 3 and 5%. The area for the Form I diffraction peak at about 12.1 degrees two theta and the estimated Form I content for each sample are given in Table X-6C below.

TABLE X-6C

Weight Percent of Diepoxide in Starting Eplerenone Mixture	Weight Percent of Diepoxide in Resulting Crystals (HPLC)	Form I Peak Area 12° Two Theta Peak	Estimated Weight Percent of Form I
0%	---	None Detected	None Detected
1%	0.29%	None Detected	None Detected
3%	0.58%	1168	10%
5%	1.05%	4175	30%

The results reported in Table X-6C confirm that the presence of the diepoxide affects the formation of Form I during the desolvation. These results indicate that the

diepoxide is effective in inducing the formation of Form I eplerenone when it is incorporated into and/or adsorbed onto the methyl ethyl ketone solvate crystals.

The 3% diepoxide doping experiment was repeated to analyze the impact of the route of preparation on the amount of Form I formed during the desolvation. In this experiment, the methyl ethyl ketone solvate obtained from the doped crystallization was divided into two portions. The first portion was left untreated while the second portion was lightly ground in a mortar and pestle to induce a higher level of crystal defects. The two portions were both dried at 100 °C for one hour at ambient pressure. The dried solids were analyzed by XPRD. The XPRD patterns are given in Fig. 15 for the dried solids from the methyl ethyl ketone crystallization with 3% doping of diepoxide (a) without grinding of the solvate prior to drying, and (b) with grinding of the solvate prior to drying. The XPRD patterns indicated a greater amount of Form I in the ground sample relative to the unground sample. These results suggest that the conditions under which the methyl ethyl ketone solvate is isolated and handled can affect the crystal form that results from the desolvation.

B. 11,12-Epoxide Results

Fig. 16 shows the X-ray powder diffraction patterns for the wet cake (methyl ethyl ketone solvate) obtained from the (a) 0%, (b) 1%, (c) 5%, and (d) 10% 11,12-epoxide-doped methyl ethyl ketone crystallizations. The peak intensities have been normalized for ease of comparison. No peaks characteristic of Form I or the 11,12-epoxide are present in the diffraction

patterns. The patterns are characteristic of the methyl ethyl ketone solvate of eplerenone.

Fig. 17 shows the X-ray powder diffraction patterns for the dried solids obtained from the (a) 0%, (b) 1%, (c) 5%, and (d) 10% 11,12-epoxide-doped methyl ethyl ketone crystallizations. The peak intensities have been normalized for ease of comparison. No Form I was detected for the dried samples corresponding to the methyl ethyl ketone crystallizations performed at doping levels of 0, 1% and 5%. Form I was detected in the dried samples corresponding to the methyl ethyl ketone crystallization performed at a doping level of 10%. The area for the Form I diffraction peak at 12.1 degrees two theta and estimated Form I content for each sample are given in Table X-6D.

TABLE X-6D

Weight Percent 11,12-Epoxide in Starting Eplerenone Mixture	Weight Percent 11,12-Epoxide in Resulting Crystals (HPLC)	Form I Peak Area 12° Two Theta Peak	Estimated Weight Percent of Form I
0%	Not Available	None Detected	None Detected
1%	Not Available	None Detected	None Detected
5%	Not Available	None Detected	None Detected
10%	Not Available	1541	10-15%

The results reported in Table X-6D confirm that the presence of the 11,12-epoxide impacts the formation of Form I during the desolvation. The percentage of impurity in the methyl ethyl ketone crystallization required to induce the formation of Form I eplerenone appears to be greater for the 11,12-epoxide than for the diepoxide.

Example 7: Effect of Crystallization and Drying on Final Crystal Form

The following four experiments analyzing the effect of crystallization and drying on the final crystal form were conducted: (i) methyl ethyl ketone crystallization of eplerenone (2^3+3 statistical design of experiment), (ii) crystallization of poor quality mother liquor residue, (iii) crystallization of high purity eplerenone with form I seeding, and (iv) crystallization of low purity eplerenone with form II seeding. Variables in the design of the experiments included cooling rate, starting material purity level, and end point temperature of crystallization. For purposes of this Example, high purity eplerenone was defined as ultra-pure milled eplerenone (HPLC analysis showed this material to be 100.8% pure) and low purity eplerenone was defined as 89% pure eplerenone. To prepare the low purity eplerenone, stripped-down mother liquors from the process for the preparation of eplerenone were analyzed and blended to yield a material that was 61.1% eplerenone, 12.8% diepoxide and 7.6% 11,12-epoxide. This material was then blended with a sufficient amount of high purity eplerenone to yield the 89% eplerenone.

A. Methyl Ethyl Ketone Crystallization

In the methyl ethyl ketone crystallization experiment, all runs were performed using 60 g of high purity eplerenone. High endpoint was defined as 45°C and low endpoint was defined as 5°C. High cooling rate was defined as 3°C/minute cooling and low cooling rate was defined as 0.1°C/minute cooling. Center points were 1.5°C/minute cooling, 94.5% pure eplerenone, and a 25°C endpoint.

After a background reading was taken with the FTIR, 250 mL of methyl ethyl ketone was charged to a 1L Mettler RC-1, MP10 reactor and stirred at 100 rpm. After several scans, eplerenone was charged to the reactor followed by an additional 470 mL of methyl ethyl ketone. Agitation was increased to 500 rpm to suspend solids and the batch temperature was increased to 80°C. The batch temperature was held at 80°C to ensure dissolution of the eplerenone. Black or white specks generally were visible in the resulting transparent solution. The batch temperature was then ramp cooled at the desired rate to the desired endpoint, where it was maintained for one hour before being pulled into a transfer flask and filtered. The vacuum was reactor, transfer flask and cake were then washed with 120 mL methyl ethyl ketone. Once the wash was pulled through the cake, the stopped. About 10 grams of each wet cake were dried in a vacuum oven under nominal conditions of 75°C with a light nitrogen bleed. For the "high, high, high" and "low, low, low" experiments described below, fluid bed drying was operated under high and low conditions. High fluid bed drying was defined as 100°C with a blower setting of "4" while low fluid bed drying was defined as 40°C with a blower setting of "1".

B. Crystallization of Poor Quality Mother Liquor Residue

In the crystallization of poor quality mother liquor residue experiment, 60 g of the 61.1% pure material and 720 mL methyl ethyl ketonewere charged directly to a 1L Mettler RC-1, MP10 reactor. The 61.1% pure material was not blended with high purity eplerenone prior to being charged to the reactor. The resulting mixture was heated to 80°C and was an opaque

slurry at that temperature. The crystallization continued and the mixture was filtered at 45°C under fast cooling conditions.

C. Form I Seeding

In the Form I seeding experiment, 60 g of pure (100.8%) eplerenone and 720 mL of methyl ethyl ketone were charged to a 1L Mettler RC-1, MP10 reactor. The mixture was heated to 80°C and then cooled to 25°C at a rate of 1.5° C/minute. When the solution had cooled to 62°C, it was seeded with 3 g of phase pure Form I crystals to initiate crystallization. The Form I seed crystals were prepared by the digestion process described in Example 9 below.

D. Form II Seeding

In the Form II seeding experiment, 66.6 g of 89.3% eplerenone (prepared by mixing 48.3 g of 100% eplerenone with 18.3 g of 61.1% eplerenone) and 720 mL of methyl ethyl ketone were charged to a 1L Mettler RC-1, MP10 reactor. The mixture was heated to 80°C and then cooled to 25°C at a rate of 1.5° C/minute. When the solution had cooled to 63°C, it was seeded with 3 g of phase pure Form II crystals to initiate crystallization. The Form II seed crystals were prepared by the crystallization and desolvation process described in Example 1 above.

Results from the experiments are reported in Table X-7A. In the n+1 crystallization experiment, Form I was detected only in the experiments employing low purity eplerenone where the product contained the diepoxide. Elevated levels of the diepoxide in the final product were also observed with higher cooling rates.

The crystallization of poor quality mother liquor residue experiment yielded poor quality material that appeared to be a mixture of the diepoxide and Form I when analyzed by X-ray powder diffraction.

The Form I seeding experiment (where high purity eplerenone was seeded with Form I) yielded a product that was 77% Form I based on X-ray powder diffraction analysis, but entirely Form I based on DSC. The X-ray powder diffraction model, however, had not been tested for linearity beyond about 15% Form I. This experiment was the only one of the four experiments of this Example where Form I was created in the absence of the diepoxide.

The Form II seeding experiment (where low purity eplerenone was seeded with Form II) yielded a product that was entirely Form II.

The data obtained for the high fluid bed drying of eplerenone appeared to correspond to the data obtained for the vacuum oven drying. The low fluid bed dryings yielded results that differed from those of the vacuum oven dryings.

TABLE X-7A

Cooling Rate[1]	Cooling Endpoint[2]	Impurity Level[3]	Nucleation Temperature (°C)	Weight Percent 11,12-Epoxide[4]	Weight Percent Diepoxide[4]	Assay For Desolvated Crystal	Percent Yield	Weight Percent Form I (XPRD)
+	+	-	57.0	ND	ND	100.3	66.1	ND
+	-	-	54.9	ND	ND	100.3	98.1	ND
-	+	-	60.9	ND	ND	100.3		ND
-	-	-	63.4	ND	ND	100.5	79.3	ND
+	+	++	N/A	4.8	36.6	43.3	27	100[5]
+	+	+	52.2	0.49	0.88	98.3	62	29
+	-	+	53.3	0.56	1.0	98.1	87	9
0	0	0	59.0	0.18	0.36	99.4	75	5
-	+	+	63.3	0.20	0.44	99.4	36	31
-	-	+	61.4	0.18	0.40	99.5	87	ND
0	0	0	60.6	0.18	0.36	99.5	79.2	ND
0	0	0	55.9	0.38	0.80	98.6	80.5	<3%
0	0	100.8% eplerenone seeded with Form I		0.03	ND	100.4	82.2	77/100[6]
0	0	89.3% eplerenone seeded with Form II		0.33	0.50	97.5	80.2	ND

[1]Cooling Rate: (+) = 3°C/min.; (0) = 1.5 °C/min.; and (-) = 0.1 °C/min.
[2]Cooling Endpoint: (+) = 45°C; (0) = 25 °C; and (-) = 5°C.
[3]Impurity Level: : (+) = 89.3% purity eplerenone starting material; (++) = 61.1% purity eplerenone starting material; (0) = 100.8% purity eplerenone starting material; and (-) = 94.5% purity eplerenone starting material.
[4]Weight percent after drying solvate in a vacuum oven at 75°C.
[5]Appears to be mixture of Form I and diepoxide when analyzed by XPRD.
[6]Appears to be 77% Form I when analyzed by XPRD and 100% Form I when analyzed by DSC.

A. Material Purity

A cube plot of product purity, starting material purity, cooling rate and endpoint temperature based on the data reported in Table X-7A is shown in Fig. 18. The cube plot suggests that the use of a higher purity material at the start of crystallization will yield a higher purity product. The endpoint temperature of crystallization does not appear to greatly affect the product purity. The cooling rate, however, appears to have an effect with slightly less pure product resulting from a faster cooling rate. In fact, the level of diepoxide generally was higher with faster cooling rates.

Fig. 19 shows a half normal plot that was prepared using the results of cube plot to determine which variables, if any, had a statistically significant effect on the product purity. Starting material purity had the greatest statistically significant effect on product purity, although cooling rate and the interaction between cooling rate and starting material purity were also seen as statistically significant effects.

Fig. 20 is an interaction graph based on these results and showing the interaction between starting material purity and cooling rate on product purity. With the high purity eplerenone (100.8% eplerenone starting material) the cooling rate appears to have little or no effect on final purity. With the low purity eplerenone (89.3% eplerenone starting material), however, the product purity decreases as cooling rate increases. This result suggests that more impurities crystallize out in eplerenone crystallizations conducted at higher cooling rates.

B. Form I Content

A cube plot of Form I weight fraction, starting material product purity, cooling rate and endpoint temperature based on the data reported in Table X-7A is shown in Fig. 21. The cube plot suggests that the use of a higher purity eplerenone at the start of crystallization will yield a lower amount of Form I. The endpoint temperature of crystallization also appears to have an effect on the form of the final product. The cooling rate does not appear to greatly affect the formation of Form I although some Form I may result from faster cooling at the low endpoint temperature in the presence of impurities.

Fig. 22 shows a half normal plot that was prepared using the results of the cube plot to determine which variables, if any, had a statistically significant effect on the amount of Form I in the final material. Starting material purity, endpoint temperature of the crystallization and the interaction between the two variables were seen as statistically significant effects.

Fig. 23 is an interaction graph based on these results and showing the interaction between starting material purity and endpoint temperature on final Form I content. With the high purity eplerenone (100.8% eplerenone starting material), endpoint temperature appears to have little effect on Form I content. No Form I resulted in either case with pure eplerenone. With low purity eplerenone (89.3% eplerenone starting material), however, Form I was present in both cases, with significantly more Form I at higher endpoint temperatures.

Table X-7B reports the weight fraction of Form I measured in materials dried using either a fluid bed (LAB-LINE/P.R.L.

Hi-Speed Fluid Bed Dryer, Lab-Line Instruments, Inc.) or a vacuum oven (Baxter Scientific Products Vacuum Drying Oven, Model DP-32). Similar Form I content was observed for comparable materials dried in either the high fluid bed or the vacuum oven. A difference was observed, however, for comparable materials dried in the low fluid bed relative to the vacuum oven.

TABLE X-7B

Cooling Rate	End Point	Impurity Level	Drying Type	Weight Percent Form I
High	High	High	Vacuum Oven	29%
High	High	High	High Fluid Bed	25%
High	High	High	Low Fluid Bed	4.7%
Low	Low	Low	Vacuum Oven	ND
Low	Low	Low	High Fluid Bed	ND
Low	Low	Low	Low Fluid Bed	5.5%

Example 8: Crystallization of a Mixture of Form I and Form II From Methyl Ethyl Ketone To Prepare a Solvate, and (b) Desolvation of the Solvate to Prepare Form II

Form I eplerenone (10 g) was combined with 80 mL of methyl ethyl ketone. The mixture was heated to reflux (79°C) and stirred at this temperature for about 30 minutes. The resulting slurry was then cooled with a stepwise, holdpoint protocol by maintaining the slurry at 65°C, 50°C, 35°C and 25°C for about 90 minutes at each temperature. The slurry was filtered and rinsed with about 20 mL methyl ethyl ketone. The isolated solid was initially dried on the filter and then in a vacuum oven at 40-50°C. The drying was completed in the vacuum oven

at 90-100°C. The desolvated solid was obtained with an 82% recovery. XPRD, MIR and DSC confirmed that the solid had a Form II crystalline structure.

Example 9: Digestion of Low Purity Eplerenone Starting Material With a Solvent to Prepare Form I

A. Digestion With Ethanol Solvent:

Low purity eplerenone (24.6 g; 64% by weight assay via HPLC) was combined with 126 mL of ethanol 3A. The slurry was heated to reflux and the distillate removed. An additional 126 mL of ethanol 3A was simultaneously added as 126 ml of solvent was removed via atmospheric distillation. Upon completion of the solvent turnover, the mixture was cooled to 25 °C and stirred for one hour. The solid was filtered and rinsed with ethanol 3A. The solid was air-dried to give the ethanol solvate. The solvate was further dried in a vacuum oven at 90-100°C for six hours to obtain 14.9 g of Form I eplerenone.

B. Digestion With Methyl Ethyl Ketone Solvent

In an alternative digestion process, 1 gram of low purity eplerenone (about 65% pure) was digested in 4 mL of methyl ethyl ketone for two hours. After the two hours, the mixture was allowed to cool to room temperature. Once cooled, the solid was collected by vacuum filtration and determined to be the methyl ethyl ketone solvate by XPRD analysis. The solid was dried at 100°C for 30 to 60 minutes. The dried solids were determined to be pure Form I by XPRD.

Example 10: Digestion of High Purity Eplerenone Starting Material With a Solvent to Prepare Form II

A. Digestion With Ethanol Solvent:

High purity eplerenone (1 gram) was digested in 8 mL of ethanol for approximately two hours. The solution was then allowed to cool to room temperature and the solids were collected by vacuum filtration. Analysis of the solids by XPRD immediately after filtration indicated that the solids were a solvate (presumably the ethanol solvate). The solids were subsequently dried at 100°C at atmospheric pressure for 30 minutes. The dried solid was analyzed by XPRD and determined to be predominately Form II (no Form I detected).

B. Digestion with Methyl Ethyl Ketone Solvent:

High purity eplerenone (1 gram) was digested in 4 mL of methyl ethyl ketone for two hours. After the two hours, the solution was allowed to cool to room temperature and the solids collected by vacuum filtration. The solid was immediately analyzed by XPRD and determined to be a solvate of eplerenone (presumably the methyl ethyl ketone solvate). The solvate was subsequently dried at 100°C at ambient pressure for 30 to 60 minutes. The dried solids were analyzed by XPRD and determined to be primarily Form II with no diffraction peaks for Form I present.

Example 11: Crystallisation of Form II Directly From Solution

Procedure A: Eplerenone (2.5 g) was dissolved in ethyl acetate by heating to 75°C. Once the eplerenone dissolved,

the solution was held at 75°C for 30 minutes to ensure complete dissolution. The solution was then cooled at 1 °C/min to 13°C. Once at 13°C, the slurry was allowed to stir for two hours at 750 rpm with an overhead stirrer. The crystals were collected by vacuum filtration and dried in a vacuum oven at 40°C for one hour. The XPRD pattern and DSC thermogram of the solid were characteristic of Form II eplerenone. Thermal gravimetric analysis (TGA) of the solid indicated no weight loss from the solid up to 200°C.

Procedure B: In an alternative procedure, 2 g of eplerenone was dissolved in 350 mL of 15/85% acetonitrile/water by heating on a hot plate with magnetic stirring. Once the eplerenone was dissolved, the solution was allowed to cool to room temperature overnight with magnetic stirring. The resulting solid was collected by vacuum filtration. The crystals were birefringent and had a triangular, plate-like crystal habit. The solid had an XPRD and DSC characteristic of Form II eplerenone. TGA indicated no weight loss up to 200°C.

Procedure C: In an alternative procedure, 640 mg of eplerenone was placed in a 50 mL flask with 20 mL of ethyl benzene. The resulting slurry was heated to 116°C and became a clear solution. The clear solution was cooled to 25°C over 30 minutes. Nucleation began at 84°C during the cooling period. The resulting solids were filtered from the solution and air-dried to give 530 mg of solids (83% recovery). Hot-stage microscopy and XPRD confirmed that the solids were Form II crystals.

Procedure D: In an alternative procedure, 1.55 g of eplerenone was added to 2.0 mL of nitrobenzene and heated to 200°C. The resulting slurry was stirred overnight at 200°C. The solution was allowed to cool to room temperature (natural air convection) the following day and the solid was isolated. The solid was determined to be Form II eplerenone by XPRD and polarized light microscopy.

Procedure E: In an alternative procedure, 5.0 g of eplerenone (purity greater than 99%) was added to 82 g of methanol (104 mL). Under stirring action (210 rpm), the solution was heated to 60°C and held at that temperature for 20 minutes to ensure complete dissolution. The solution was then cooled to -5°C at a rate of 0.16°C/minute under stirring. The crystals were collected by filtration and dried in a vacuum oven at 40°C for 20 hours. The dried solids were determined to be pure Form II eplerenone by DSC and XPRD analysis.

Procedure F: In an alternative procedure, 6.0 g of eplerenone (ethanol solvate containing 9% ethanol and having a corrected purity of 95.2%) was added to 82 g of methanol (104 mL). Under stirring action (210 rpm), the solution was heated to 60°C and held at that temperature for 20 minutes to ensure complete dissolution. The solution was then cooled to 50°C at a rate of 0.14°C/minute and then held at that temperature for about 2.5 hours. The solution was then cooled to -5°C at a rate of 0.13°C/minute under stirring. The crystals were collected by filtration and dried in a vacuum

oven at 40°C for 16 hours. The dried solids were determined to be pure Form II eplerenone by DSC and XPRD analysis.

Example 12: Crystallization of Form I Directly From Solution

150.5 mg of the diepoxide and 2.85 g of eplerenone were added to 1.5 mL of nitrobenzene. The mixture was magnetically stirred at 200°C for several hours. The slurry was then allowed to cool to room temperature by natural air convection. The sample was dried and analyzed by polarized light microscopy and XPRD. The XPRD indicated that the sample was a mixture of Form I and Form II. The crystals were translucent by microscopy, indicating that desolvation (and conversion to either Form I or Form II) did not occur.

Example 13: Preparation of Amorphous Eplerenone By Comminution

Approximately one-half of a steel Wig-L-Bug container was filled with about 60 g of eplerenone (greater than 99.9% purity). A steel ball and cap were placed on the sample container and agitated for 30 seconds by the Wig-L-Bug apparatus. The eplerenone was scraped off the surface of the Wig-L-Bug container and the container agitated for an additional 30 seconds. The resulting solid was analyzed by XPRD and DSC and determined to be a mixture of amorphous eplerenone and Form II crystalline eplerenone.

Example 14: Preparation of Amorphous By Lyophilization

Approximately 100 mg of crude eplerenone was weighed into a beaker containing 400 mL of water. The solution was heated slightly for five minutes, and then sonicated and heated with stirring for an additional five minutes.

Approximately 350 mL of the eplerenone solution was filtered into a 1000 mL round bottom flask containing 50 mL of HPLC water. The solution was flashed frozen in a dry ice/acetone bath over a time period of one to two minutes. The flask was attached to a Labconco Freezone 4.5 freeze dryer and dried overnight. The solids in the flask were transferred to a small brown bottle. A small aliquot was observed under polarized light microscopy at 10X, 1.25X optivar in cargille oil (1.404) and observed to be at least 95% amorphous eplerenone. Figures 24 and 25 show the XPRD pattern and DSC thermogram obtained for the amorphous eplerenone. The peak observed at 39 degrees two theta in Figure 24 is attributable to the aluminum sample container.

Example 15: Measurement of Form II Solubility

The aqueous solubility of Form II eplerenone was measured at pH 7 (100 mM phosphate buffer) at 5, 25, and 40°C. Approximately 30 mg of Form II eplerenone was mixed with approximately 10 mL of buffer to form a slurry of eplerenone at both 5 and 25 °C. Approximately 40 mg of Form II eplerenone was mixed with approximately 10 mL of buffer to form a slurry of eplerenone at 40 °C. Samples were prepared in duplicate for each condition. The slurries were allowed to equilibrate in appropriate temperature water shaker baths and the solutions were analyzed for eplerenone content by ultraviolet visible analysis (245 nm) at the 1, 5, 12, 19, 27, and 36 day time intervals. Data from each condition was appropriately averaged to determine the solubility of eplerenone at each condition and is reported in Table X-15. The residual solids from each time point were analyzed by DSC and TGA at the end of the 36 day equilibration and were

determined to be Form II.

TABLE X-15

Temperature (°C)	Form II Solubility
5	0.24 mg/mL
25	0.29 mg/mL
40	0.39 mg/mL

Example 16: Measurement of Intrinsic Dissolution Rates

Intrinsic dissolution rates were measured for the following four eplerenone polymorph samples: (i) Form II eplerenone prepared by direct crystallization from acetonitrile using water as an anti-solvent in the same manner as Example 12, Procedure B, (ii) Form I eplerenone prepared by digestion in ethanol in the same manner as Example 10, Procedure A, (iii) a mixture of Form I (5 weight percent) and Form II (95 weight percent), and (iv) Form II eplerenone that was micronized to provide the following particle size distribution: 10% of the particles under 9 microns, 50% of the particles under 22 microns, and 90% of the particles under 41 microns.

Eplerenone (150 mg) was weighed and placed into a VanKel intrinsic dissolution cavity. The powder was compressed at 1200 psi using a Carver press. The sample was then mounted onto the intrinsic dissolution apparatus. The dissolution media used was 1% SDS in HPLC grade water. All experiments were conducted at 37°C for two hours. Before the start of the experiment, 500 mL of the dissolution medium was equilibrated at 37°C for 30 minutes in the dissolution bathing chamber. An initial sample was taken from each dissolution vessel which served as time zero for the experiment. The eplerenone

tablets were then lowered into the dissolution medium. Samples were drawn at determined intervals for the determination of rate of dissolution. Care was taken to avoid air bubbles forming at the surface of the tablet. The samples were analyzed by UV absorbance detection at 243 nm. Intrinsic dissolution rates were calculated from the slope of the linear portion of the concentration versus time profiles corrected for volume and normalized for the surface area of the dissolution tablet (0.5 cm^2).

Fig. 26 reports the intrinsic dissolution rates measured for the four samples. These studies appear to indicate that Form I eplerenone has faster intrinsic dissolution rate than Form II eplerenone. X-Ray powder diffraction measurements comparing compressed against uncompressed eplerenone confirmed that the polymorphs did not interconvert upon compression or during the course of the dissolution studies.

Example 17: Eplerenone Polymorph Composition

Tablets containing 25 mg, 50 mg, 100 mg and 200 mg doses of Form II eplerenone are prepared and have the following composition:

Ingredient	Weight % of Tablet
Form II Eplerenone	29.41
Form I Eplerenone	Not Detected
Lactose Monohydrate (#310, NF)	42.00
Microcrystalline Cellulose (NF, Avicel PH101)	18.09
Croscarmellose Sodium (NF, Ac-Di-Sol)	5.00
Hydroxypropyl Methylcellulose (#2910, USP, Pharmacoat 603)	3.00
Sodium Lauryl Sulfate (NF)	1.00
Talc (USP)	1.00
Magnesium Stearate (NF)	0.5
Total	100.00

Example 18: Eplerenone Polymorph Composition

Capsules (hard gelatin capsule, #0) are prepared containing a 100 mg dose of eplerenone and have the following composition:

Ingredient	Amount (mg)
Form II Eplerenone	90.0
Form I Eplerenone	10.0
Lactose, Hydrous, NF	231.4
Microcrystalline Cellulose, NF	45.4
Talc, USP	10.0
Croscarmellose Sodium, NF	8.0
Sodium Lauryl Sulfate, NF	2.0
Colloidal Silicon Dioxide, NF	2.0
Magnesium Stearate, NF	1.2
Total Capsule Fill Weight	400.0

Example 19: Eplerenone Polymorph Composition

Capsules (hard gelatin capsule, size #0) are prepared containing a 200 mg dose of eplerenone and have the following composition:

Ingredient	Amount (mg)
Form II Eplerenone	190.0
Form I Eplerenone	10.0
Lactose, Hydrous, NF	147.8
Microcrystalline Cellulose, NF	29.0
Talc, USP	10.0
Croscarmellose Sodium, NF	8.0
Sodium Lauryl Sulfate, NF	2.0
Colloidal Silicon Dioxide, NF	2.0
Magnesium Stearate, NF	1.2
Total Capsule Fill Weight	400.0

Example 20: Preparation of Milled Eplerenone

Dried methyl ethyl ketone solvate is first delumped by passing the solvate through a 20 mesh screen on a Fitzmill. The delumped solid is then pin milled using an Alpine Hosakawa stud disk pin mill operating under liquid nitrogen cooling at a feed rate of approximately 250 kilograms/hour. Pin milling produces milled eplerenone with a D_{90} size of approximately 65-100 microns.

Example 21: Effect of Eplerenone Particle Size (Dog Study)

The effect of the particle size of Form II eplerenone on eplerenone plasma concentrations and relative bioavailability was studied in a dog model. Four heathy female beagle dogs

weighing between 8 to 12 kg were intragastrically administered one immediate release capsule containing the formulation described in Table X-21A below followed by about 10 mL of water.

Table X-21A

INGREDIENT	WEIGHT % OF TABLET	Amount (mg)
Eplerenone	50.00	200.00
Lactose, Fast-Flo, Hydrous	36.95	147.80
Microcrystalline Cellulose (Avicel PH102)	7.25	29.00
Sodium Lauryl Sulfate	0.50	2.00
Croscarmellose Sodium	2.00	8.00
Talc	2.50	10.00
Colloidal Silicon Dioxide	0.50	2.00
Magnesium Stearate	0.30	1.20
Total	100.00	400.00
Capsules, Size #0, White Opaque	1 Capsule	

The dogs were fasted for 15 to 20 hours prior to administration of the capsule and were not fed again until at least 4 hours after dose administration. Blood samples (approximately 3 mL) were collected by venipuncture in chilled tubes containing heparin at 0, 0.5, 1, 2, 3, 4, 6, 8 and 24 hours after dose administration. The blood samples were immediately placed on ice. Separation of plasma from the blood samples was complete after about 15 minutes of centrifugation. The resulting plasma samples were frozen at about -20°C and stored until analyzed. Analysis was performed using an LC/MS/MS procedure.

This experiment was conducted using the same four dogs for three formulations that were identical except for the particle size of the eplerenone starting material. The three

formulations tested used eplerenone starting materials having a D_{90} particle size (that is, at least 90% of the particles were) less than about 212 microns, less than about 86 microns, and less than about 36 microns, respectively. A minimum of a five day wash-out period was allowed between administration of each of the formulations. Reducing the D_{90} particle size of the eplerenone starting material from about 212 microns to above 86 microns increased relative bioavailability by almost 100%. The mean results are reported in Tables X-21B and X-21C below.

Table X-21B

Time (hours)	Blood Serum Eplerenone Concentration (μg/mL)		
	D_{90} = 212 microns	D_{90} = 86 microns	D_{90} = 36 microns
0	0	0	0
0.5	1.83	3.65	1.99
1.0	2.40	6.18	5.86
2.0	3.77	6.89	6.77
3.0	2.85	5.70	6.60
4.0	2.61	4.39	5.56
6.0	1.63	3.11	3.31
8.0	1.10	1.90	2.09
24.0	0.0252	0.032	0.0706

Table X-21C

Pharmacokinetic Parameter	Pharmacokinetic Parameter Value		
	D_{90} = 212 microns	D_{90} = 86 microns	D_{90} = 36 microns
C_{max} (μg/mL)	3.98	7.02	7.39
T_{max} (hours)	1.50	1.75	2.25
AUC ((μg/mL)hr)	26.6	49.2	53.1
Relative Bioavailability (%)	53.25	100	107.9

Example 22: Effect of Eplerenone Particle Size (Human Study)

The effect of the particle size of a Form II eplerenone starting material used in a pharmaceutical composition on eplerenone plasma concentrations and relative bioavailability was studied in a human model. Subjects received single doses of Form II eplerenone medication on Days 1, 8, 15, 22 and 29 according to a randomization schedule. All medication was administered with 180 mL of water at 0800 hours. Blood samples for eplerenone pharmacokinetic analyses were collected at -0.5 (predose), 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 16, 24, 36 and 48 hours postdose. Urine samples were collected and pooled between the hours of 0-24 and 24-48 hour postdose.

Plasma and urinary concentrations of eplerenone were determined using a validated high performance liquid chromatography method with MS/MS Detection. Pharmacokinetic data obtained is reported in Table X-23A. The particle size distributions of the eplerenone used in the preparation of the medications was determined in the dry powder state using laser light scattering.

Table X-23A

Pharmacokinetic Parameter	Pharmacokinetic Parameter Value		
	100 mg Capsule A (D_{90}=40 microns)	100 mg Tablet (D_{90}=82 microns)	100 mg Capsule B (D_{90}=96 microns)
C_{max} (ng/mL)	1747	1704	1669
T_{max} (hours)	1.8	1.8	1.3
AUC ((ng/mL)hr)	11349	11945	11981

The specific composition of each medication is reported in Table X-23B below.

Table X-23B

INGREDIENT	WEIGHT % OF TABLET/CAPSULE		
	Capsule A	Tablet A	Capsule B
Form II Eplerenone	25	30	25
Lactose Monohydrate	–	42	57.86 (fast flo lactose)
Lactose, Hydrous NF	57.8	–	–
Microcrystalline Cellulose (Avicel PH101)	11.4	17.5 (7.5% intragranular plus 10% extragranular)	11.34 (Avicel PH 102)
Croscarmellose Sodium (Ac-Di-Sol)	2	5	2
Hydroxypropyl Methylcellulose (Pharmacoat 603)	–	3	–
Sodium Lauryl Sulfate	0.5	1	0.5
Talc	2.5	1	2.5
Magnesium Stearate	0.3	0.5	0.3
Colloidal Silicon Dioxide	0.5	–	0.5
Total	100	100	100

All mentioned references are expressly incorporated by reference herein as if fully set forth at length. In addition, U.S. Provisional Application Serial No. 60/111,646 filed December 9, 1998, and the corresponding nonprovisional application, U.S. Application Serial No. 09/_____ filed December 8, 1999, are expressly incorporated by reference herein as if fully set forth at length.

When introducing elements of the present invention or the preferred embodiment(s) thereof, the articles "a", "an", "the" and "said" are intended to mean that there are one or more of the elements. The terms "comprising", "including" and "having" are intended to be inclusive and mean that there may be additional elements other than the listed elements.

Although this invention has been described with respect to specific embodiments, the details of these embodiments are not to be construed as limitations.

WHAT IS CLAIMED IS:

1. A combination comprising a first solid state form of eplerenone and a second solid state form of eplerenone, wherein said solid state forms of eplerenone are selected from:

the Form I crystalline form of eplerenone;

the Form II crystalline form of eplerenone;

a crystalline form of eplerenone comprising a solvent; and

amorphous eplerenone.

2. A combination of Claim 1 wherein said solid state forms of eplerenone are selected from:

the Form I crystalline form of eplerenone;

the Form II crystalline form of eplerenone;

a crystalline form of eplerenone comprising a solvent, wherein said form does not comprise methylene chloride, ethyl ether or isopropanol; and

amorphous eplerenone.

3. A combination of Claim 1 wherein said solid state forms of eplerenone are selected from:

the Form I crystalline form of eplerenone;

the Form II crystalline form of eplerenone;

a crystalline form of eplerenone comprising a solvent, wherein said form does not comprise methylene chloride, ethyl ether, isopropanol, methyl ethyl ketone or ethanol; and

amorphous eplerenone.

4. The combination of Claim 1 wherein the weight ratio

of said first solid state form to said second solid state form is at least about 1:1.

5. The combination of Claim 4 wherein the weight ratio is at least about 2:1.

6. The combination of Claim 4 wherein the weight ratio is at least about 5:1.

7. The combination of Claim 4 wherein the weight ratio is at least about 9:1.

8. The combination of Claim 1 wherein the first solid state form is the Form II crystalline form of eplerenone, and the second solid state form is the Form I crystalline form of eplerenone.

9. The combination of Claim 8 wherein the weight ratio of said first solid state form to said second solid state form is at least about 1:9.

10. The combination of Claim 8 wherein the weight ratio is at least about 1:1.

11. The combination of Claim 8 wherein the weight ratio is at least about 2:1.

12. The combination of Claim 8 wherein the weight ratio is at least about 5:1.

13. The combination of Claim 8 wherein the weight ratio

is at least about 9:1.

14. The combination of Claim 1 wherein said first solid state form and said second solid state form are each present in the form of particles, wherein at least 90% of said particles have a particle size less than about 400 microns.

15. The combination of Claim 14 wherein at least 90% of said particles have a particle size between about 25 microns to about 400 microns.

16. The combination of Claim 14 wherein at least 90% of said particles have a particle size between about 50 microns to about 150 microns.

17. The combination of Claim 14 wherein at least 90% of said particles have a particle size between about 75 microns to about 125 microns.

18. The combination of Claim 14 wherein at least 90% of said particles have a particle size between about 40 microns to about 100 microns.

19. The combination of Claim 14 wherein at least 90% of said particles have a particle size between about 30 microns to about 50 microns.

20. A pharmaceutical composition comprising a first solid state form of eplerenone and a second solid state form of eplerenone in association with at least one pharmaceutically-acceptable carrier, adjuvant or diluent;

wherein said solid state forms of eplerenone are selected from:

the Form I crystalline form of eplerenone;

the Form II crystalline form of eplerenone;

a crystalline form of eplerenone comprising a solvent; and

amorphous eplerenone; and

wherein said first solid state form and a second solid state form in combination comprise a therapeutically-effective amount of eplerenone.

21. A pharmaceutical composition of Claim 20 wherein said solid state forms of eplerenone are selected from:

the Form I crystalline form of eplerenone;

the Form II crystalline form of eplerenone;

a crystalline form of eplerenone comprising a solvent, wherein said form does not comprise methylene chloride, ethyl ether or isopropanol; and

amorphous eplerenone.

22. A pharmaceutical composition of Claim 20 wherein said solid state forms of eplerenone are selected from:

the Form I crystalline form of eplerenone;

the Form II crystalline form of eplerenone;

a crystalline form of eplerenone comprising a solvent, wherein said form does not comprise methylene chloride, ethyl ether, isopropanol, methyl ethyl ketone or ethanol; and

amorphous eplerenone.

23. The pharmaceutical composition of Claim 20 wherein the weight ratio of said first solid state form to said

second solid state form is at least about 1:9.

24. The pharmaceutical composition of Claim 23 wherein the weight ratio is at least about 1:1.

25. The pharmaceutical composition of Claim 23 wherein the weight ratio is at least about 9:1.

26. The pharmaceutical composition of Claim 20 wherein the first solid state form is the Form II crystalline form of eplerenone, and the second solid state form is Form I crystalline form of eplerenone.

27. The pharmaceutical composition of Claim 26 wherein the weight ratio of said first solid state form to said second solid state form is at least about 1:9.

28. The pharmaceutical composition of Claim 27 wherein the weight ratio is at least about 1:1.

29. The pharmaceutical composition of Claim 27 wherein the weight ratio is at least about 2:1.

30. The pharmaceutical composition of Claim 27 wherein the weight ratio is at least about 5:1.

31. The pharmaceutical composition of Claim 27 wherein the weight ratio is at least about 9:1.

32. The pharmaceutical composition of Claim 1 wherein said first solid state form and said second solid state form

are each present in the form of particles, wherein at least 90% of said particles have a particle size less than about 400 microns.

33. The pharmaceutical composition of Claim 32 wherein at least 90% of said particles have a particle size between about 25 microns to about 400 microns.

34. The pharmaceutical composition of Claim 32 wherein at least 90% of said particles have a particle size between about 50 microns to about 150 microns.

35. The pharmaceutical composition of Claim 32 wherein at least 90% of said particles have a particle size between about 75 microns to about 125 microns.

36. The pharmaceutical composition of Claim 32 wherein at least 90% of said particles have a particle size between about 40 microns to about 100 microns.

37. The pharmaceutical composition of Claim 32 wherein at least 90% of said particles have a particle size between about 30 microns to about 50 microns.

38. A method of treating or preventing an aldosterone-mediated condition or disorder in a subject, said method comprising administering to a subject having or susceptible to said condition or disorder a first solid state form of eplerenone and a second solid state form of eplerenone wherein said solid state forms of eplerenone are selected from:

the Form I crystalline form of eplerenone;

the Form II crystalline form of eplerenone;

a crystalline form of eplerenone comprising a solvent; and

amorphous eplerenone; and

wherein said first solid state form and said second solid state form in combination comprise a therapeutically-effective amount of eplerenone.

39. A method of Claim 38 comprising administering to a subject having or susceptible to said condition or disorder a first solid state form of eplerenone and a second solid state form of eplerenone in association with at least one pharmaceutically-acceptable carrier, adjuvant or diluent;

wherein said solid state forms of eplerenone are selected from:

the Form I crystalline form of eplerenone;

the Form II crystalline form of eplerenone;

a crystalline form of eplerenone comprising a solvent; and

amorphous eplerenone; and

wherein said first solid state form and a second solid state form in combination comprise a therapeutically-effective amount of eplerenone.

40. The method of Claim 39 for use in treatment of hyperaldosteronism.

41. The method of Claim 39 for use in treatment of hypertension.

42. The method of Claim 39 for use in treatment of heart failure.

43. The method of Claim 39 for use in treatment of cirrhosis of the liver.

44. The method of Claim 39 for use in treatment of a condition selected from the group consisting of excess collagen/fibrosis, benign prostate hypertrophy and depression.

45. An amorphous form of eplerenone having an X-ray powder diffraction pattern substantially lacking any characteristic peaks.

46. A form of Claim 45 having a differential scanning calorimetric thermogram substantially as shown in Figure 27.

47. Particles of a form of Claim 45 wherein at least 90% of said particles have a particle size less than about 400 microns.

48. The particles of Claim 45 wherein at least 90% of said particles have a particle size between about 25 microns to about 400 microns.

49. The particles of Claim 45 wherein at least 90% of said particles have a particle size between about 50 microns to about 150 microns.

50. The particles of Claim 45 wherein at least 90% of

said particles have a particle size between about 75 microns to about 125 microns.

51. The particles of Claim 45 wherein at least 90% of said particles have a particle size between about 50 microns to about 100 microns.

52. The particles of Claim 45 wherein at least 90% of said particles have a particle size between about 30 microns to about 50 microns.

53. A method for the preparation of eplerenone comprising lyophilizing a solution comprising eplerenone.

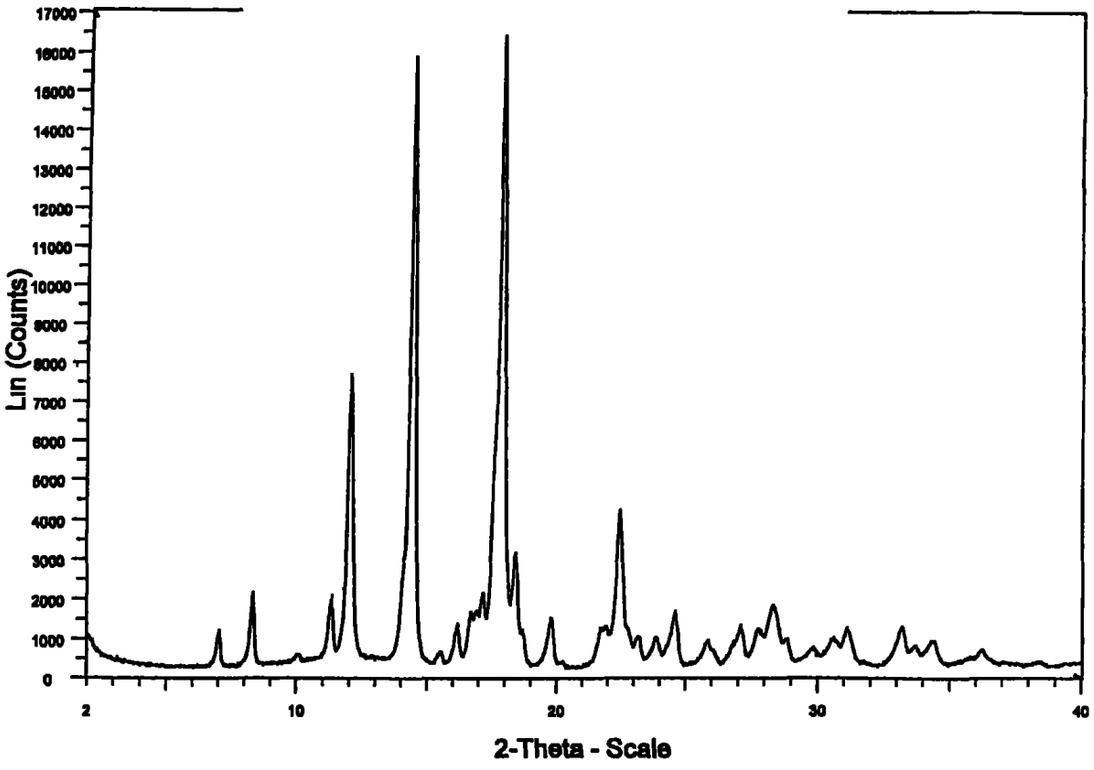
54. The method of Claim 53 wherein the solution is an aqueous solution.

55. A method for the preparation of eplerenone comprising comminuting solid eplerenone.

ABSTRACT OF THE DISCLOSURE

Novel combinations of crystalline and amorphous solid state forms of eplerenone are described. These forms of eplerenone each have unique chemical and physical properties relative to one another and are characterized by their x-ray powder diffraction patterns, infrared spectra, nuclear magnetic resonance spectra, differential scanning calorimetric thermograms, and other physical characterizations.

Figure [illegible]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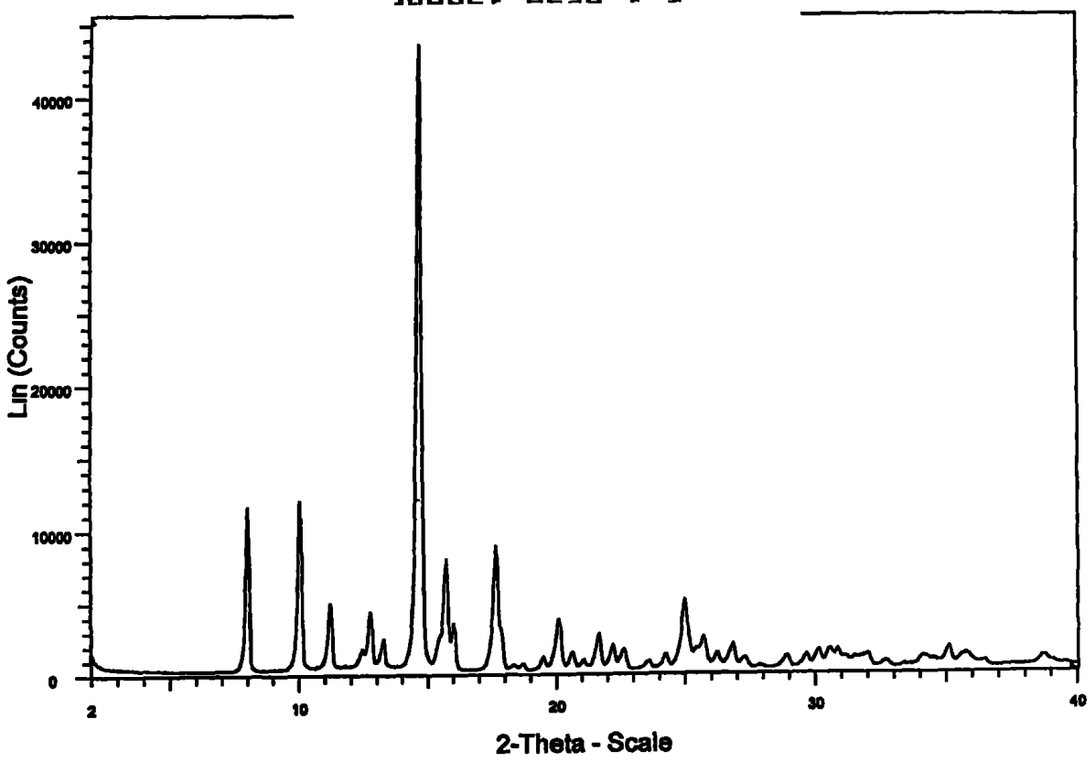
16000
15000
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
2
10
20
30
40
2-Theta - Scale

Figure 1-B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30000
20000
10000
0
Lin (Counts)
2
10
20
30
40
2-Theta - Scale

Figure 6C

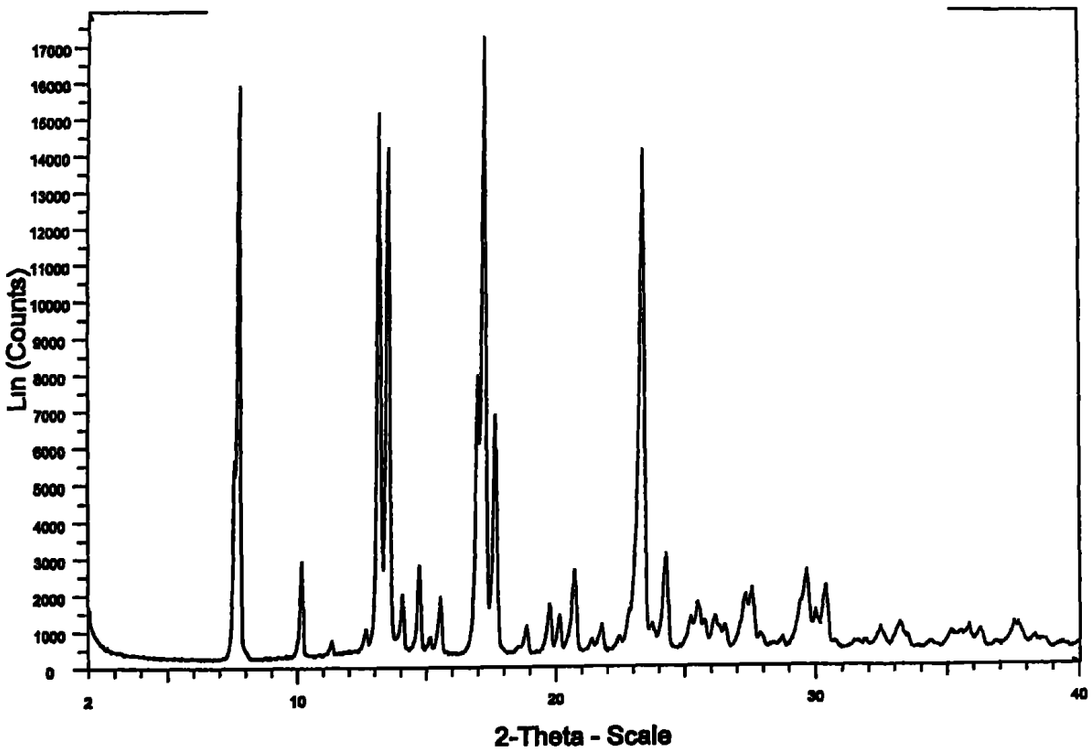

Lin (Counts)
17000
16000
15000
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
2
10
20
30
40
2-Theta - Scale

Figure 1-D

[redacted] n-propyl alcohol solvate[redacted]

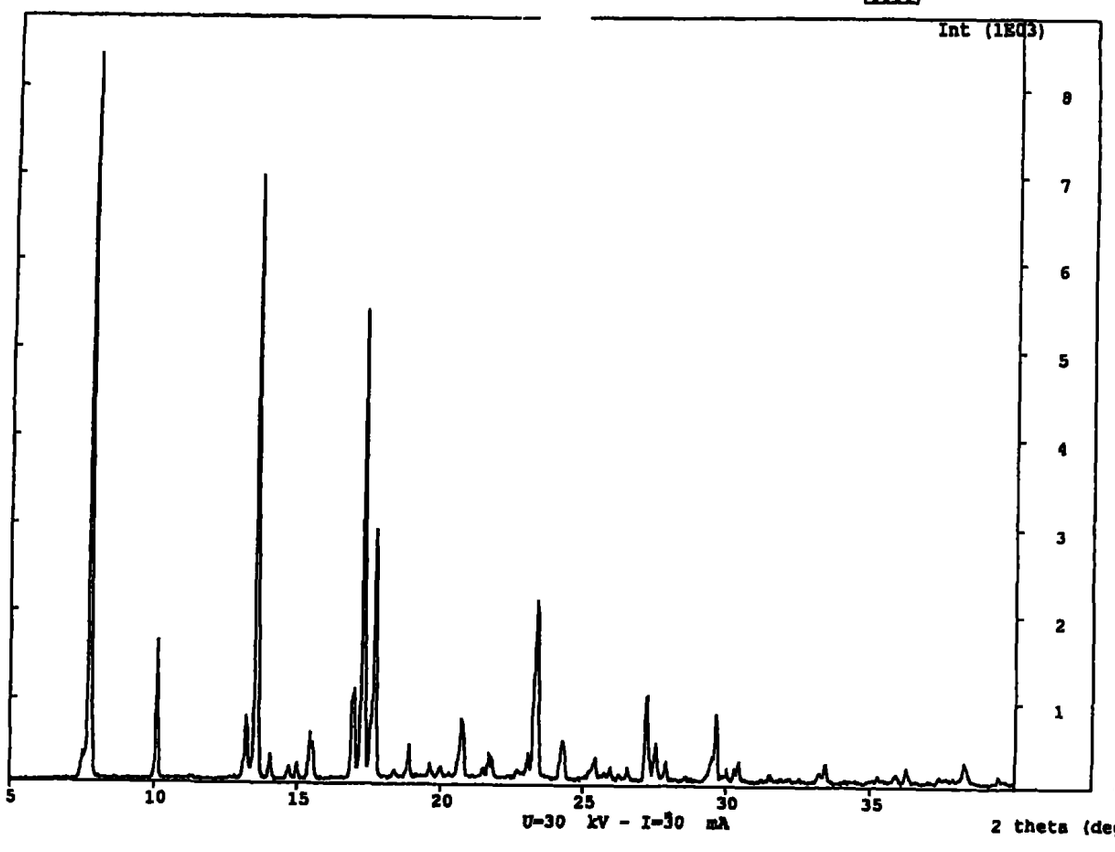


Figure 1-E

[redacted] THF solvate

INEL

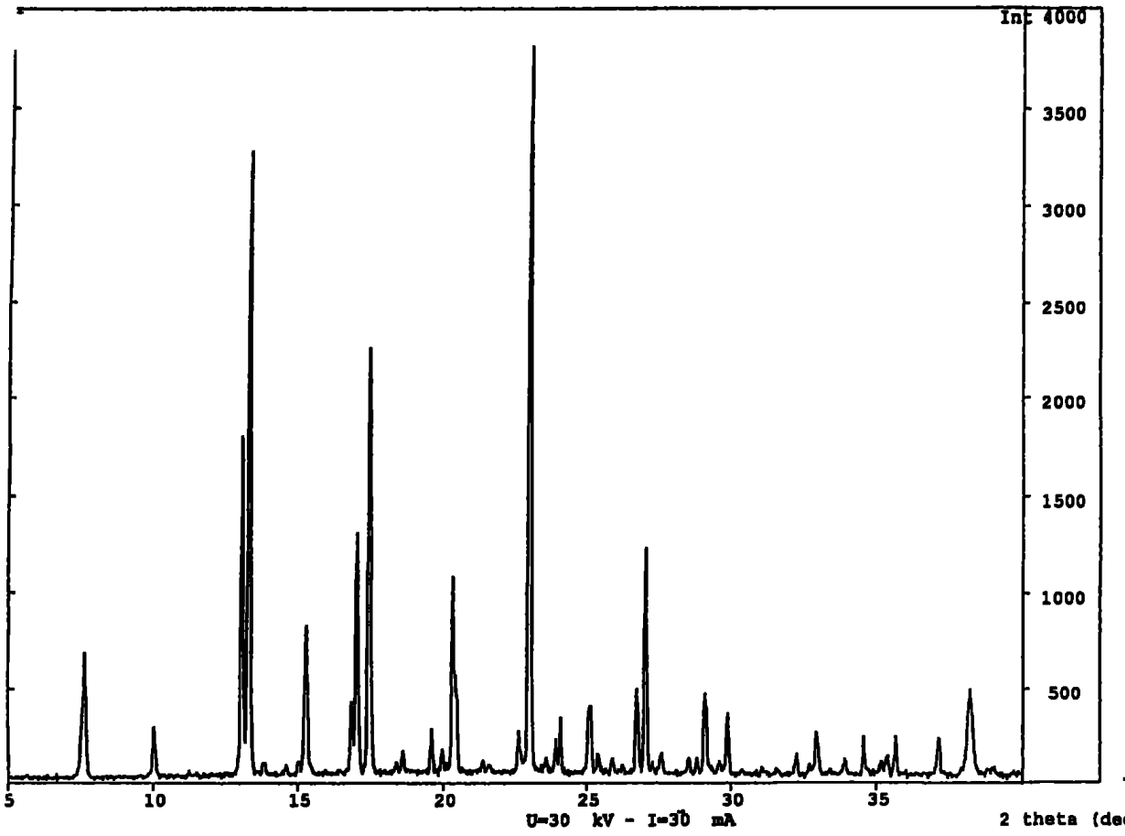


Figure 1-F

[illegible] Ethyl Propionate solvate

INEL

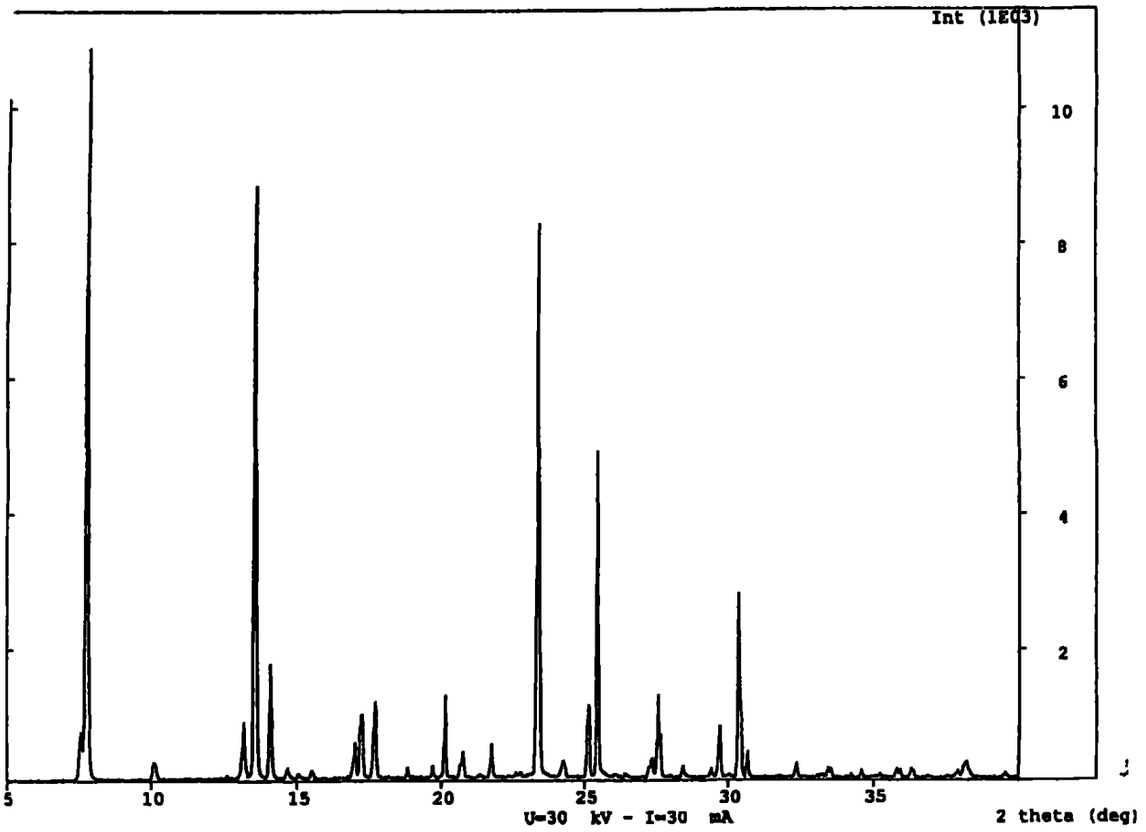


Figure [redacted]
[redacted] Acetic Acid solvate[redacted] INEL

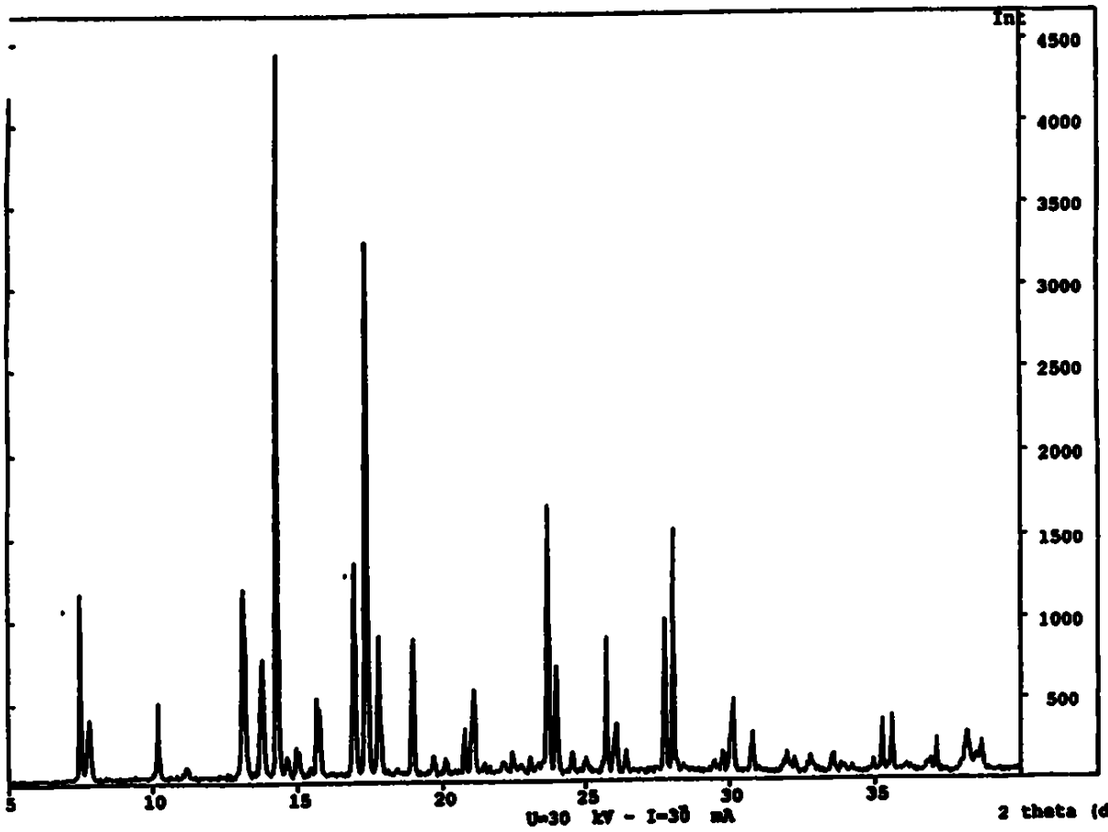


Figure [illegible]
[illegible] Lithothamnium

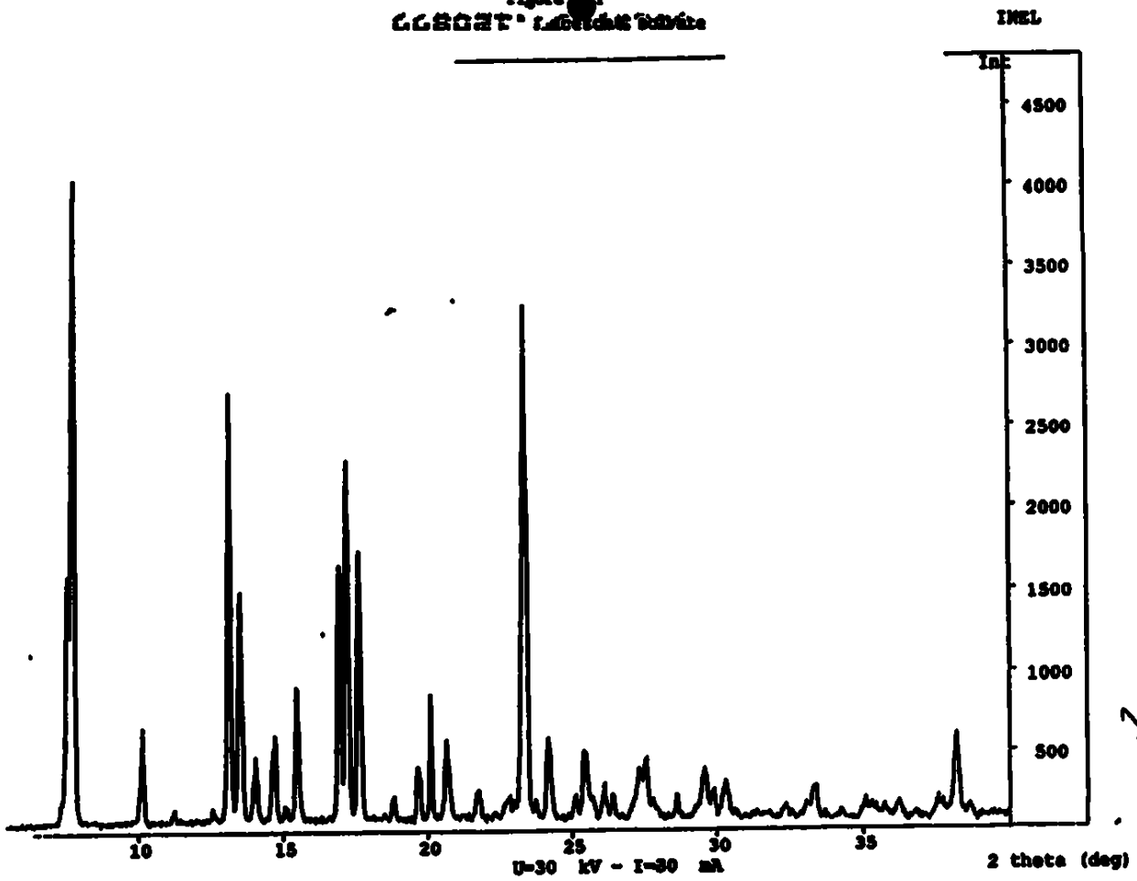


Figure 1-I

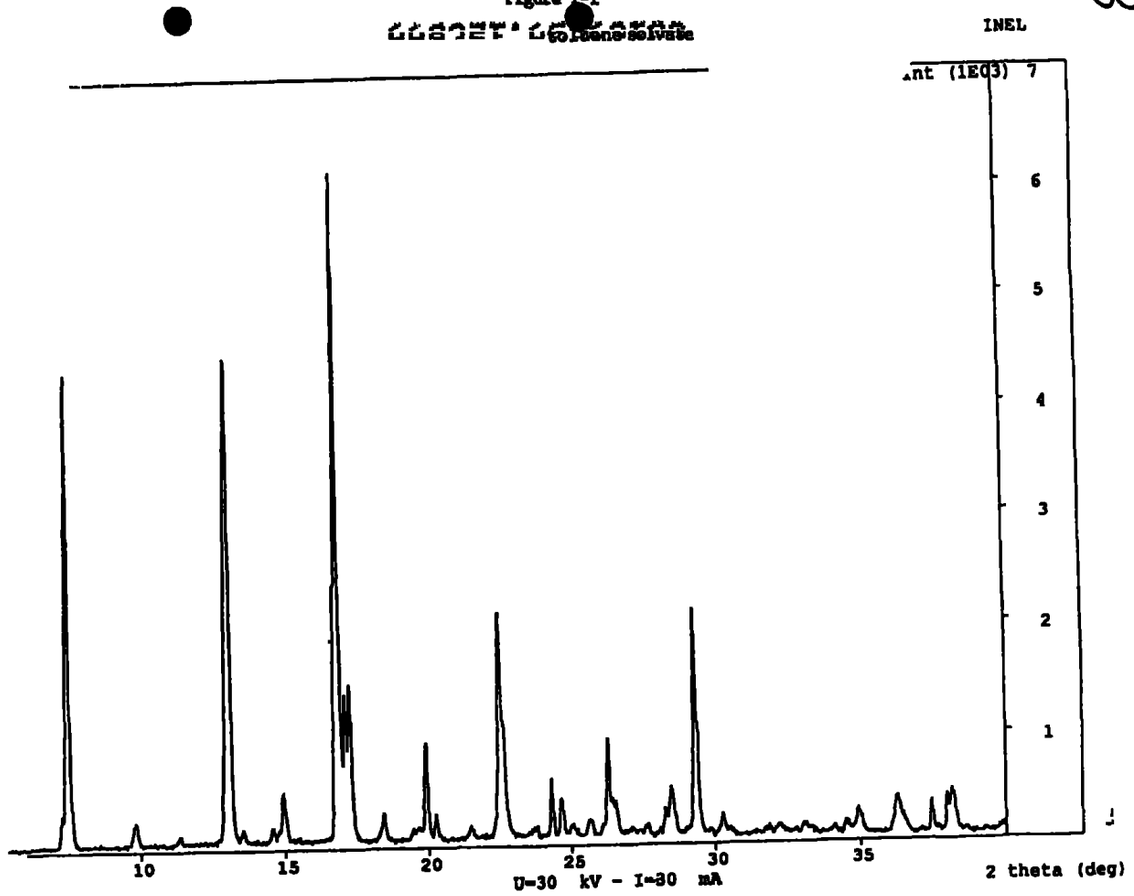


Figure 1-J

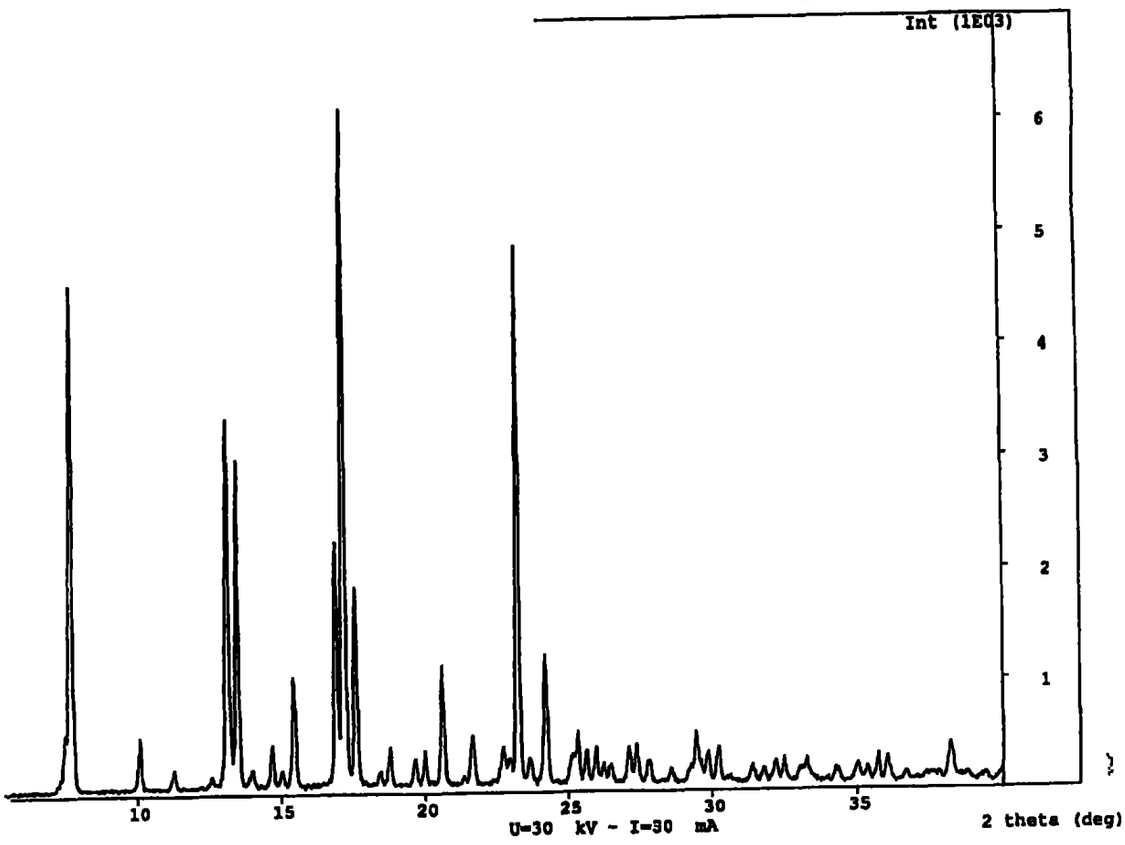


Figure 1 of
ethanol solvate

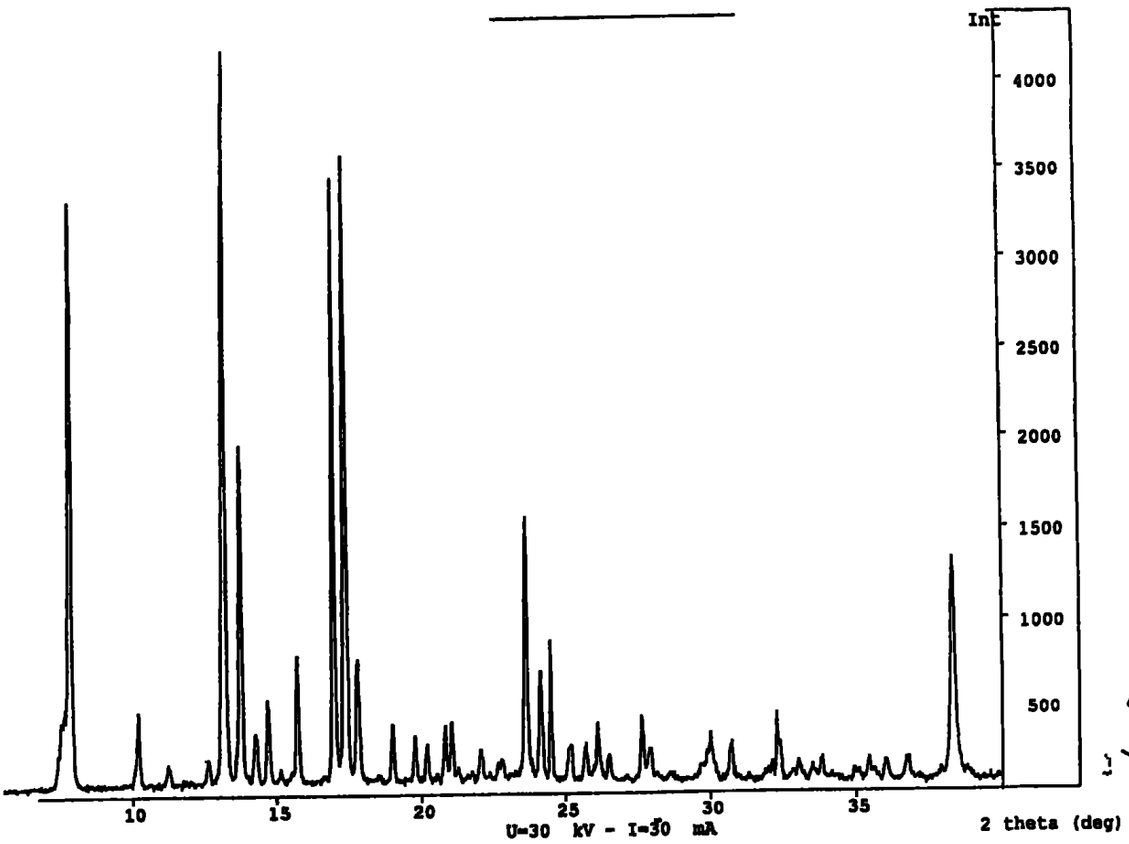


Figure 1-L

[illegible] solvate

INEL

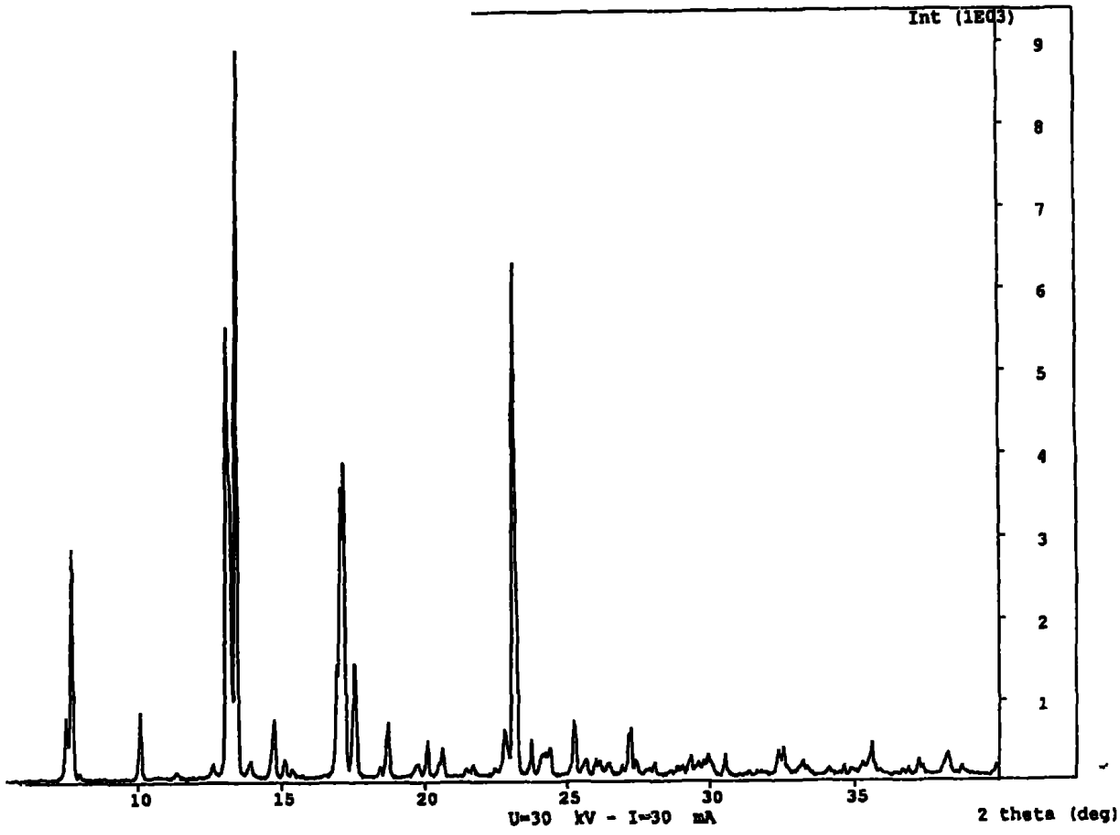


Figure 1-M

804

[redacted] Butyl acetate Solvate

INEL

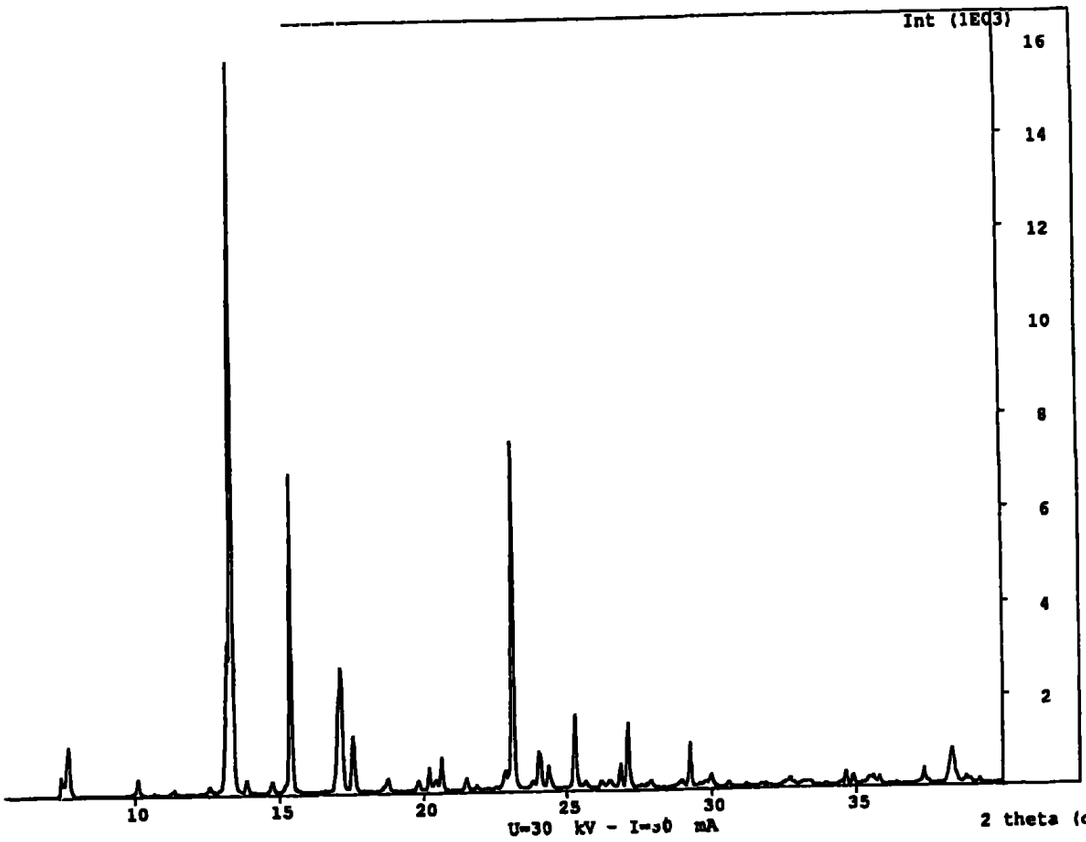


Figure 1-N

INEL

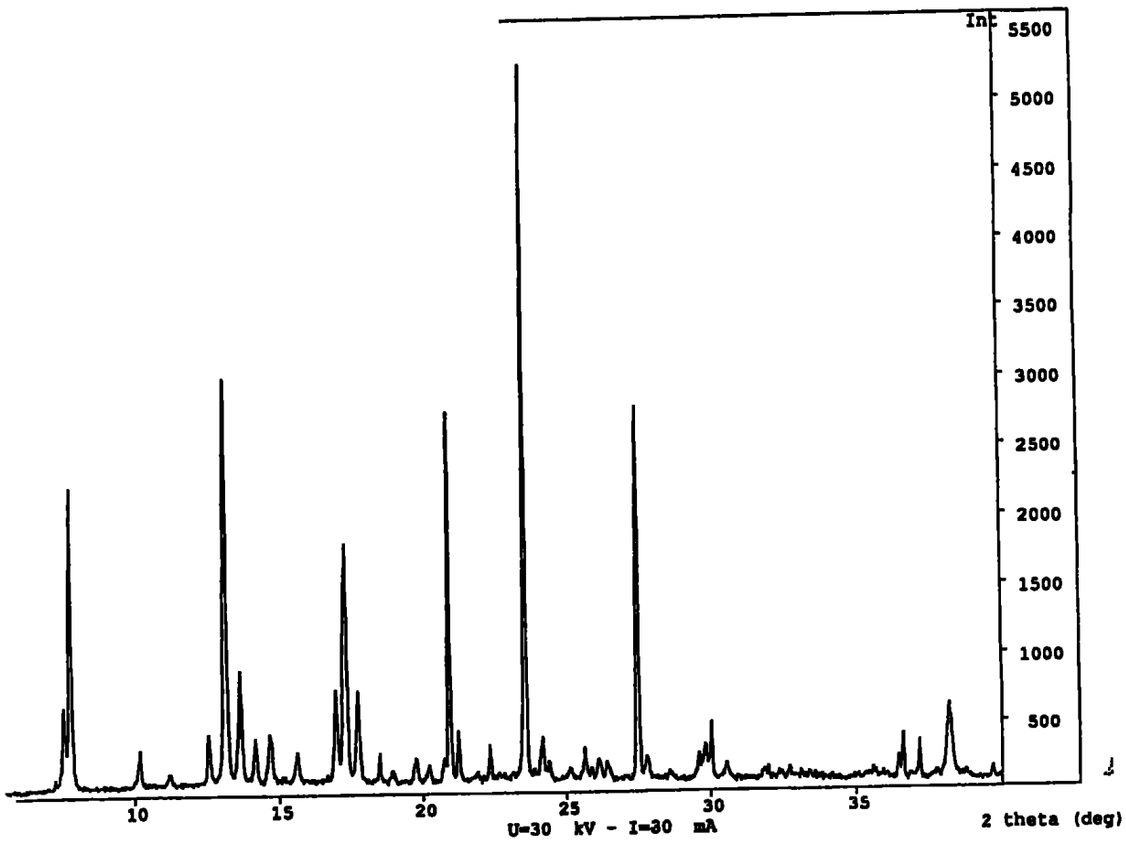
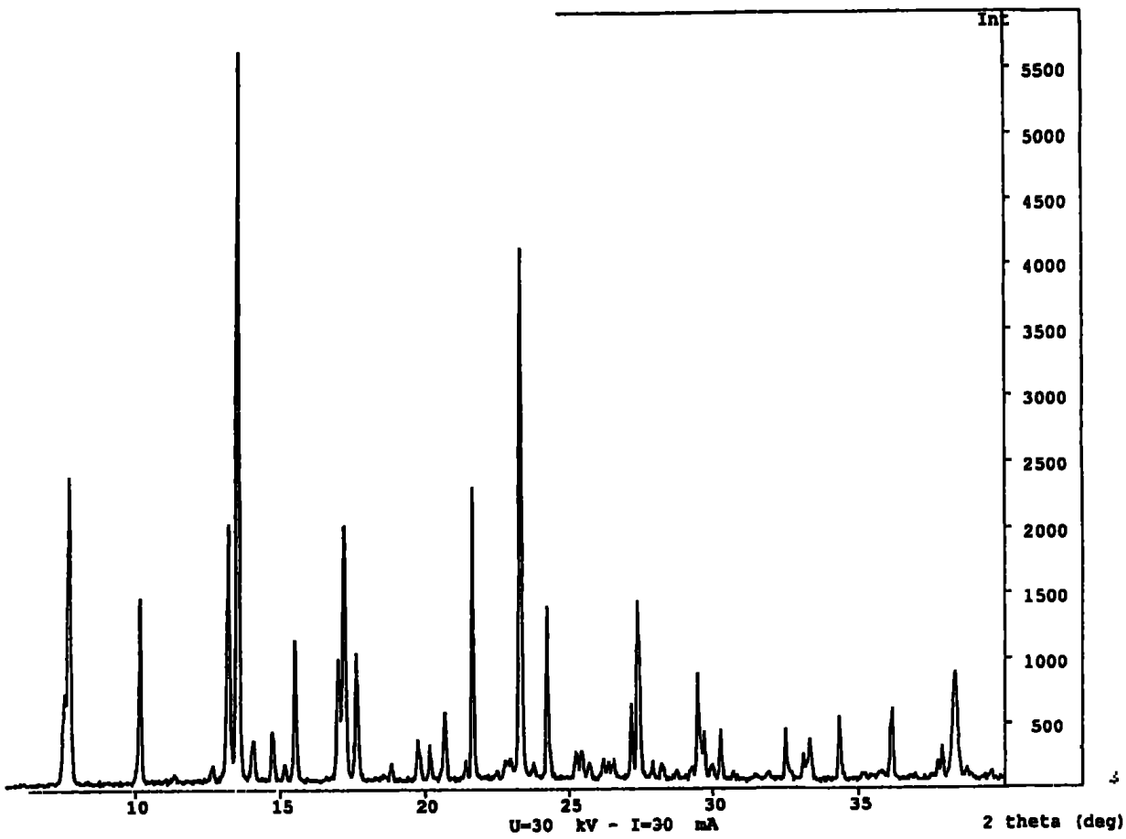


Figure 1-10

[illegible] solvate [illegible] INEL



243

Figure 2-A

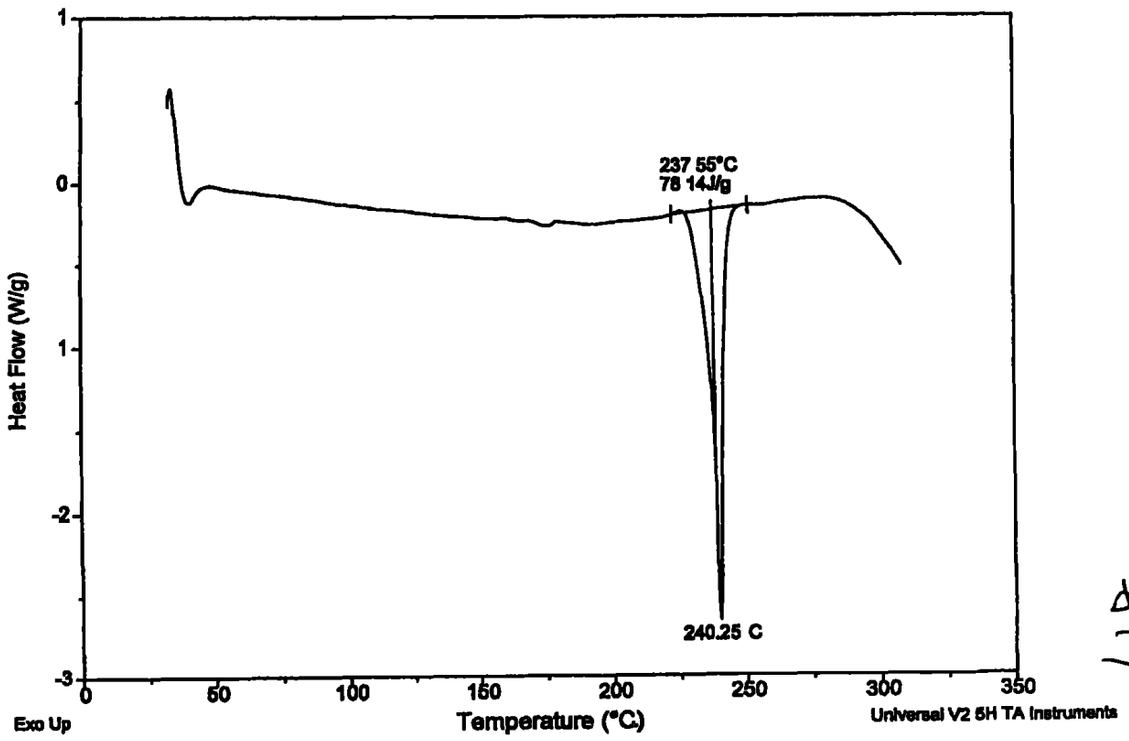

Size 0 6380 mg
Method 10 DEG C/MIN AMB TO 300
Comment: SEALED PAN
237.55°C
78.14J/g
240.25 C
Heat Flow (W/g)
Temperature (°C)
Exo Up
Universal V2.5H TA Instruments

Figure 2-B

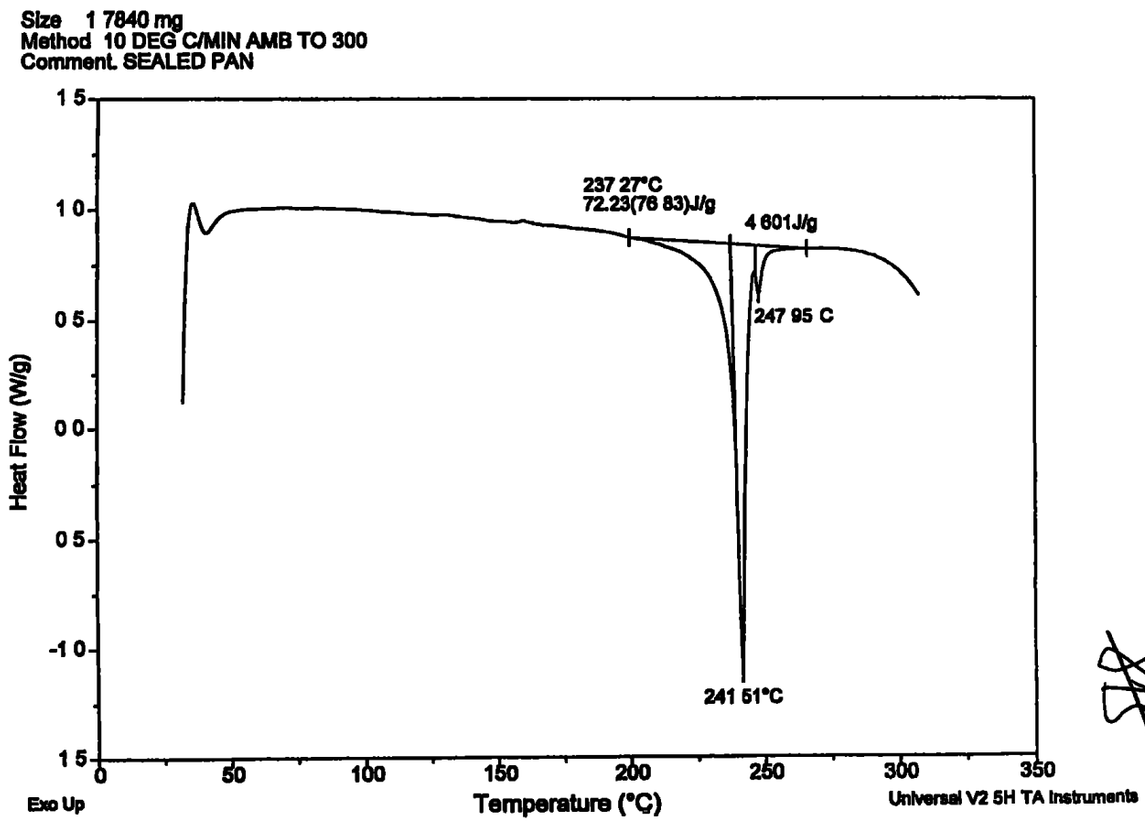

Size 1 7840 mg
Method 10 DEG C/MIN AMB TO 300
Comment. SEALED PAN
237 27°C
72.23(76 83)J/g
4 601J/g
247 95 C
241 51°C
Heat Flow (W/g)
Exo Up
Temperature (°C)
Universal V2 5H TA Instruments

Figure 2-C

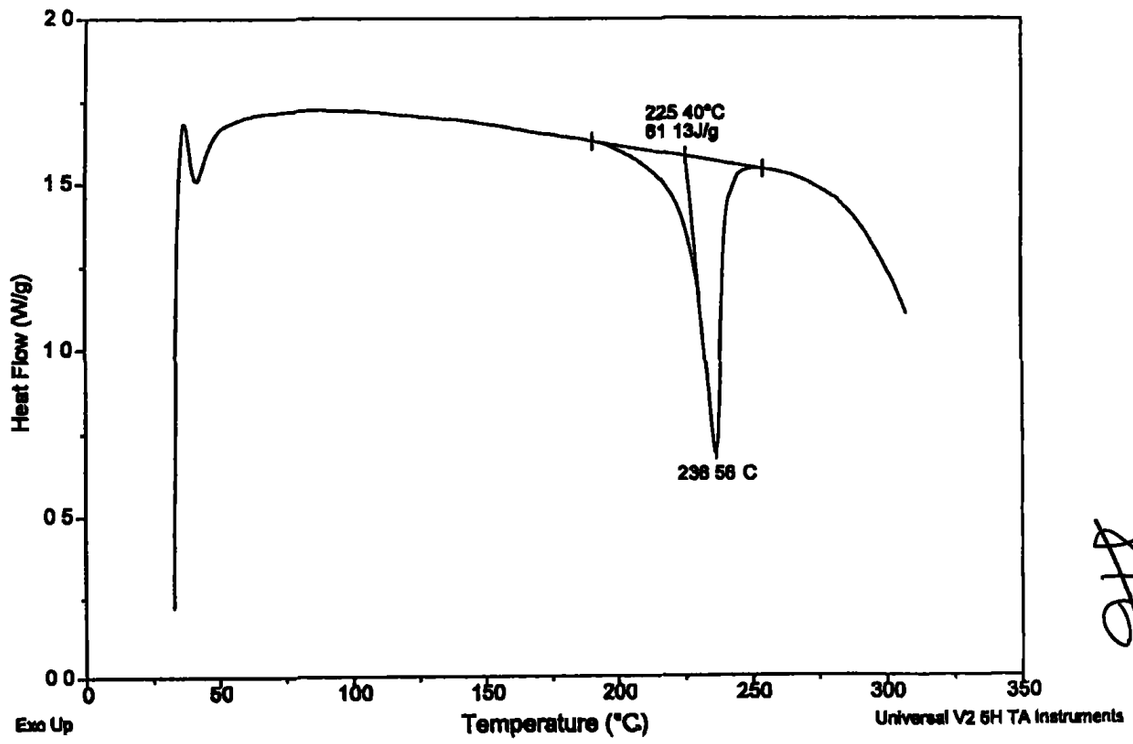

Size 1 4230 mg
Method 10 DEG C/MIN AMB TO 300
Comment SEALED PAN
225 40°C
61 13J/g
238 56 C
Heat Flow (W/g)
Temperature (°C)
Exo Up
Universal V2 5H TA Instruments

Figure 2-D

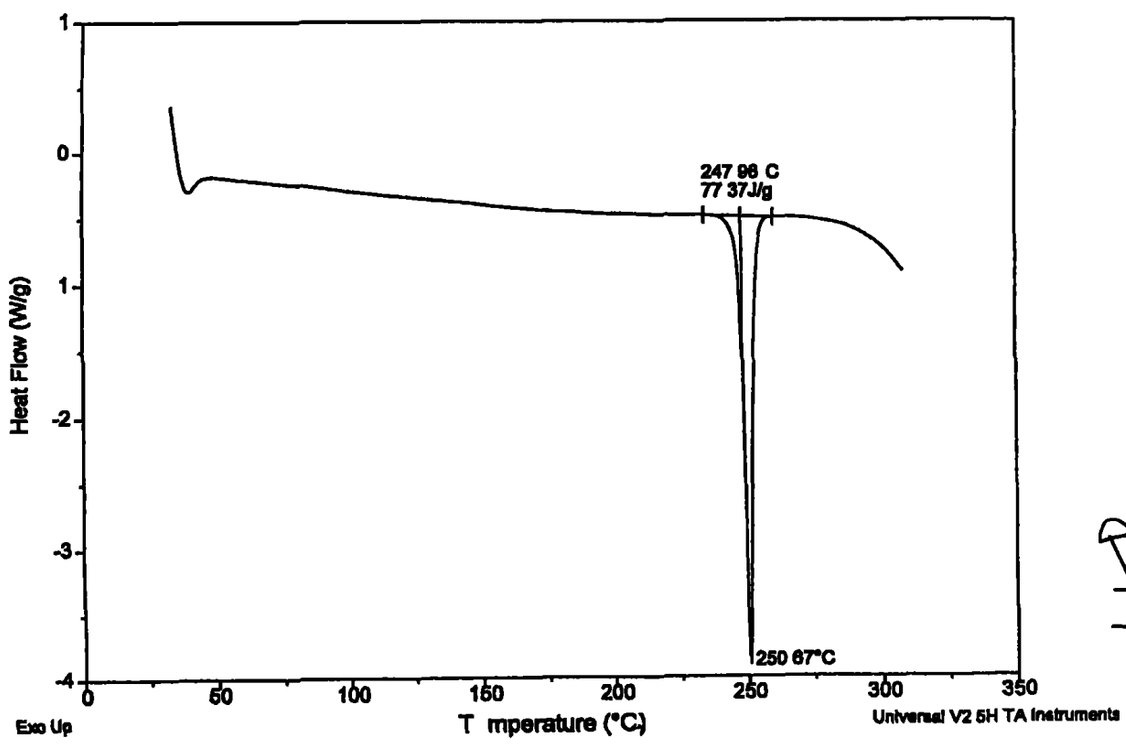

Size 1 0400 mg
Method 10 DEG C/MIN AMB TO 300
Comment SEALED PAN
247 96 C
77 37J/g
250 67°C
Heat Flow (W/g)
Exo Up
T mperature (°C)
Universal V2 5H TA Instruments

Figure 2 E

811

Sample Weight: 16.300 mg
Comment: N-Propyl Alcohol solvate 1 unsealed pan

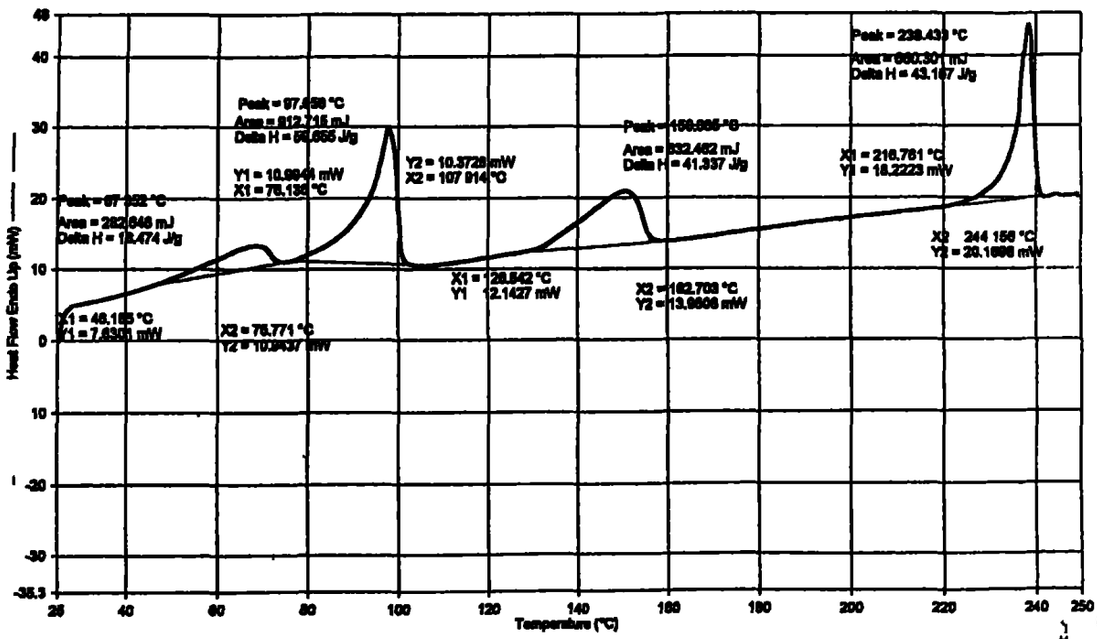


Figure 2 F

Sample Weight: 10.400 mg
Comment: THF activate 1 unsealed pan

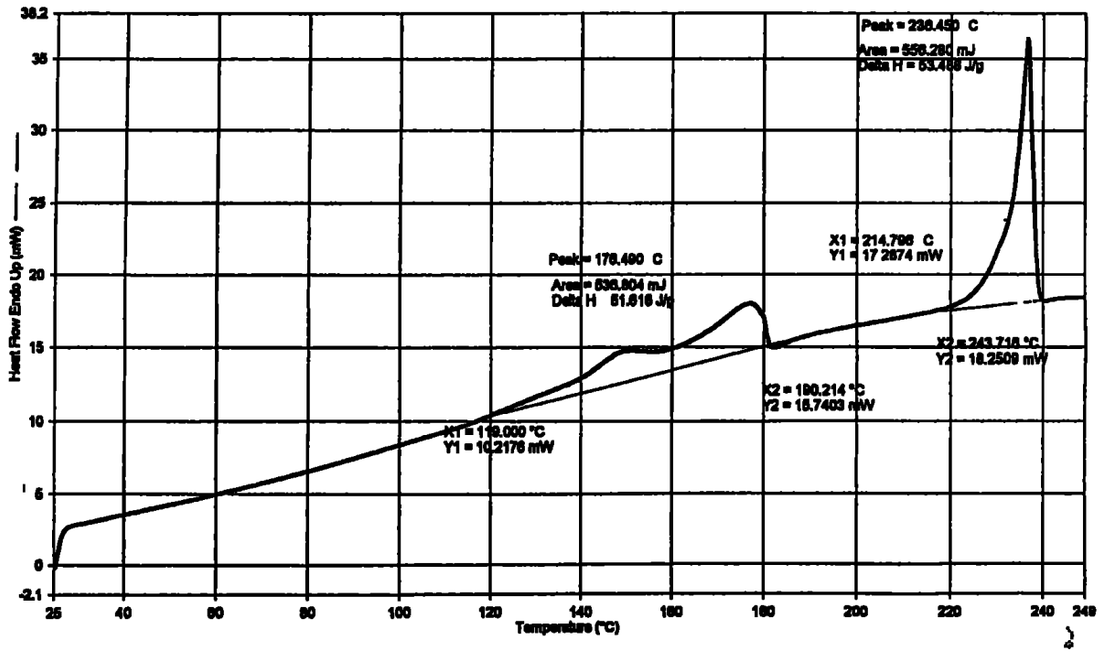


Figure 2C

Sample Weight:	7.200 mg
Comment:	Lot 66K020-F3b
	Ethyl propionate solvate
	unsealed pan

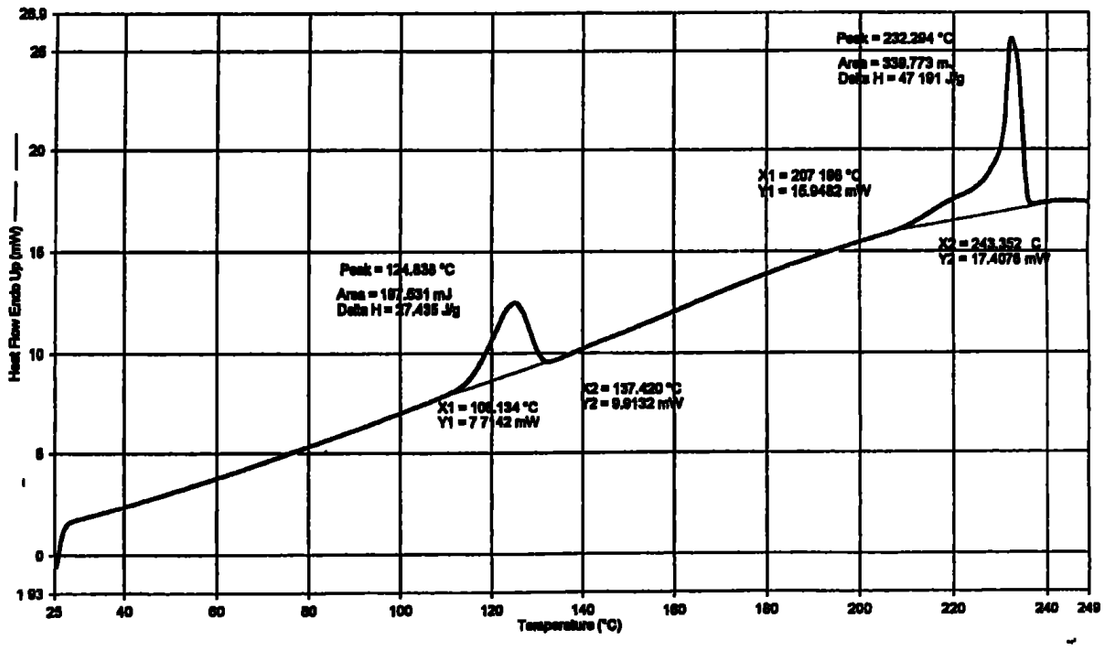
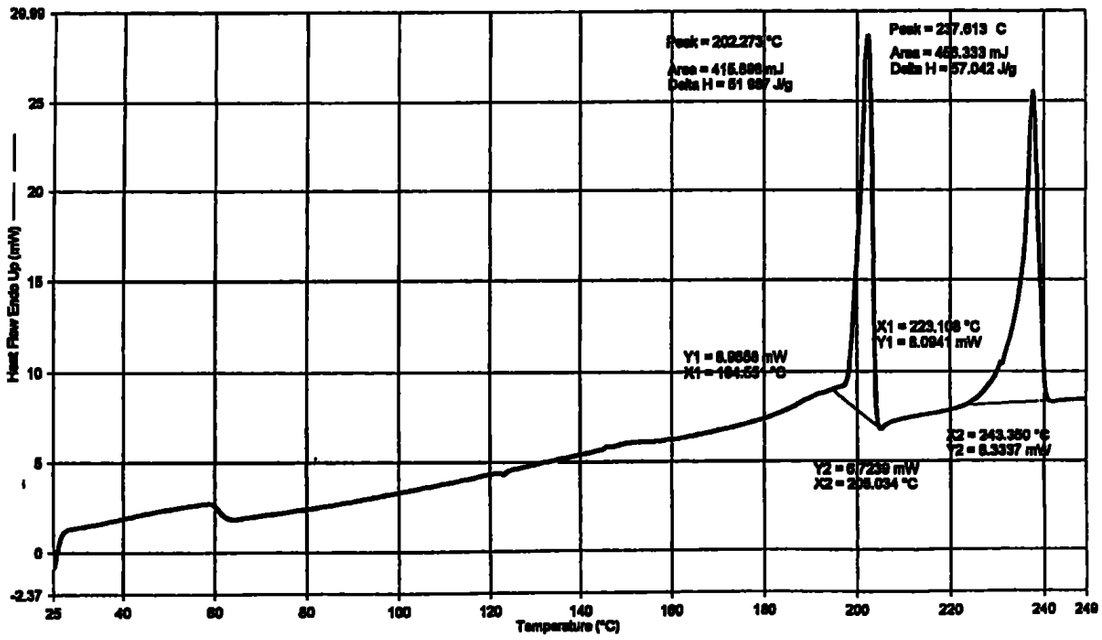


Figure 2 H

Sample Weight: 8.000 mg
Comment: Acetic ACid solvate 1
unsealed pan



824

Figure 2-I

Sample Weight: 12.700 mg
Comment: Chloroform solvate 1
unsealed pan

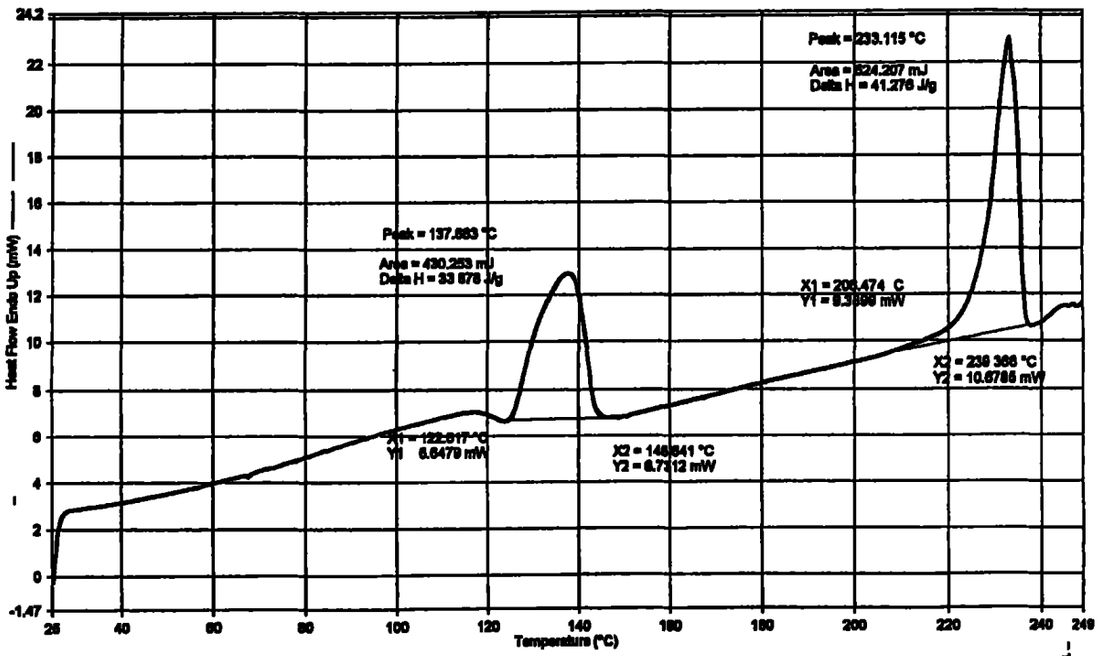


Figure 2-J

Sample Weight: 6.704 mg
Comment: Acetone Solvate

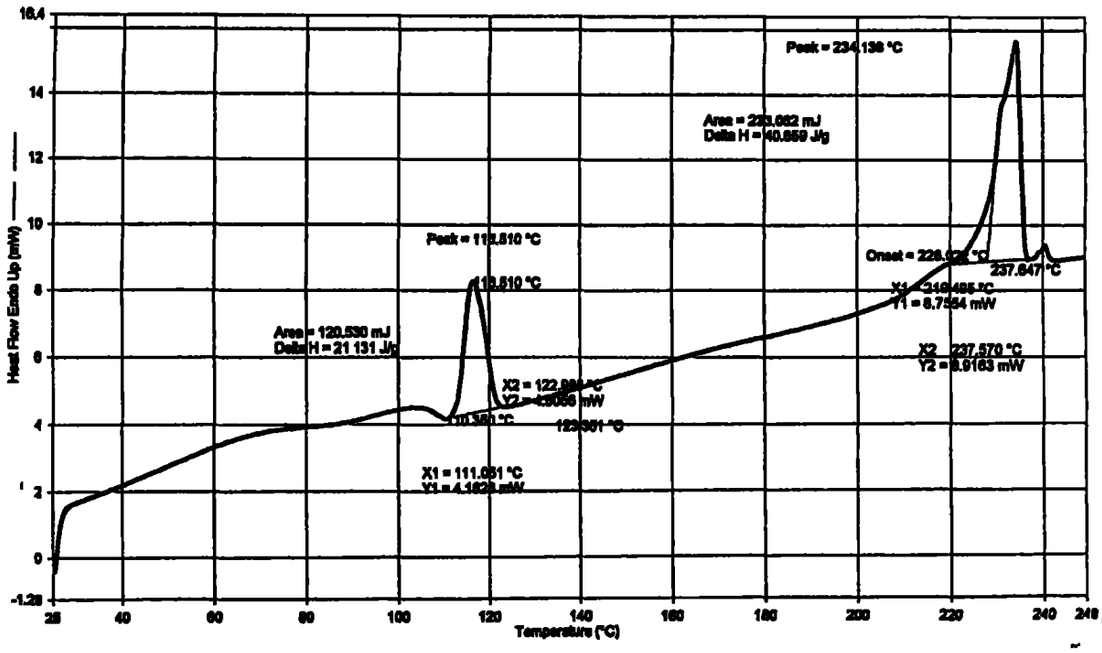


Figure 2-K

DSC021 DESC5T03

Sample Weight: 8.947 mg
Comment: toluene Solvate

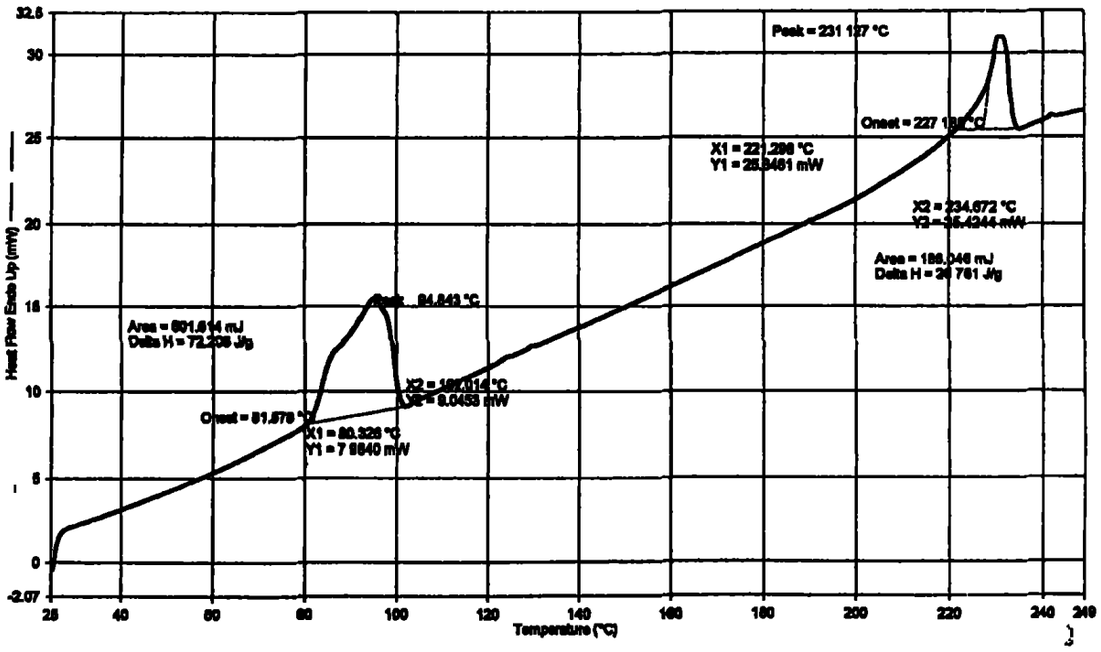


Figure 2-L

B18

Sample Weight: 8.174 mg
Comment: Isopropanol Solvate

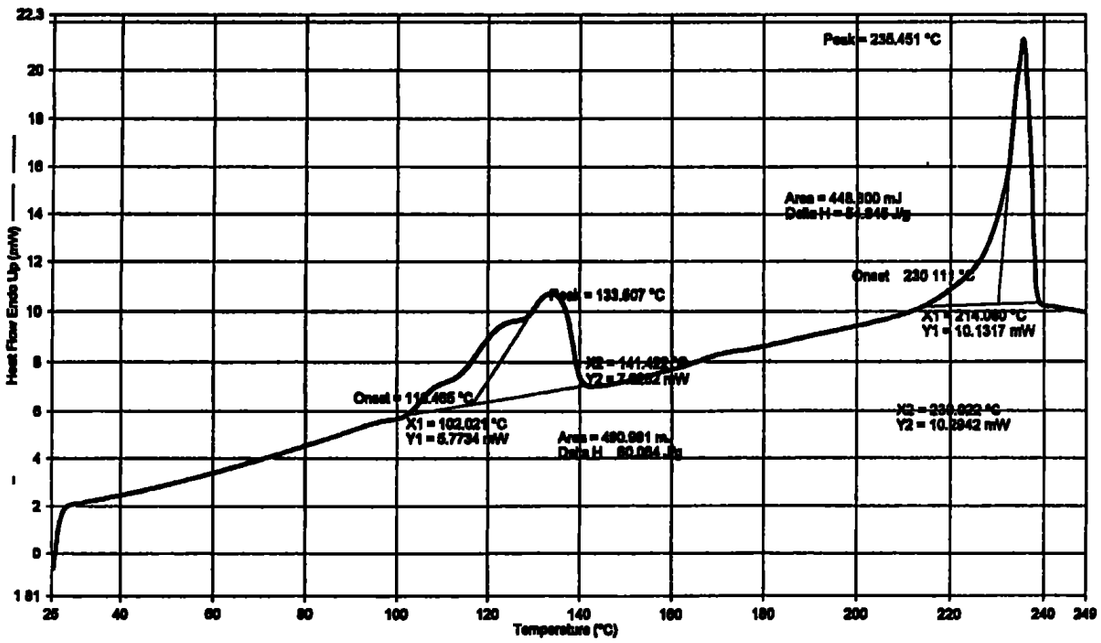


Figure 2 H

Sample Weight: 6.122 mg
Comment: ethanol solvate
10 °C/min sealed pan

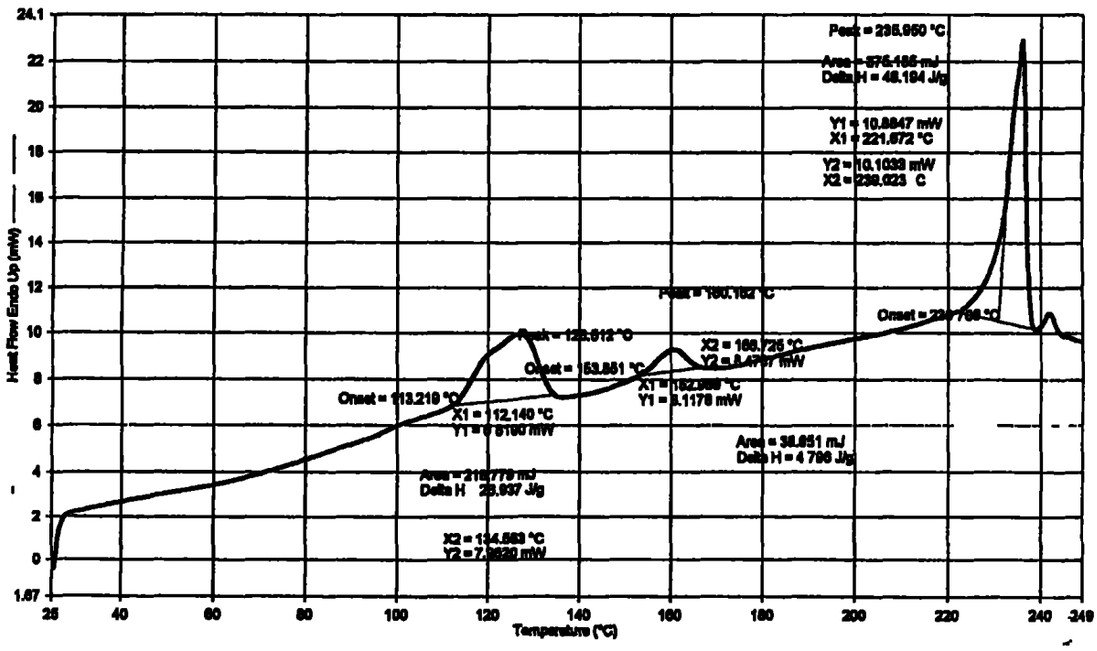


Figure 2-N

[redacted]

Sample Weight: 6.840 mg
Comment:
i-butyl acetate solvate
10 C/min sealed pan

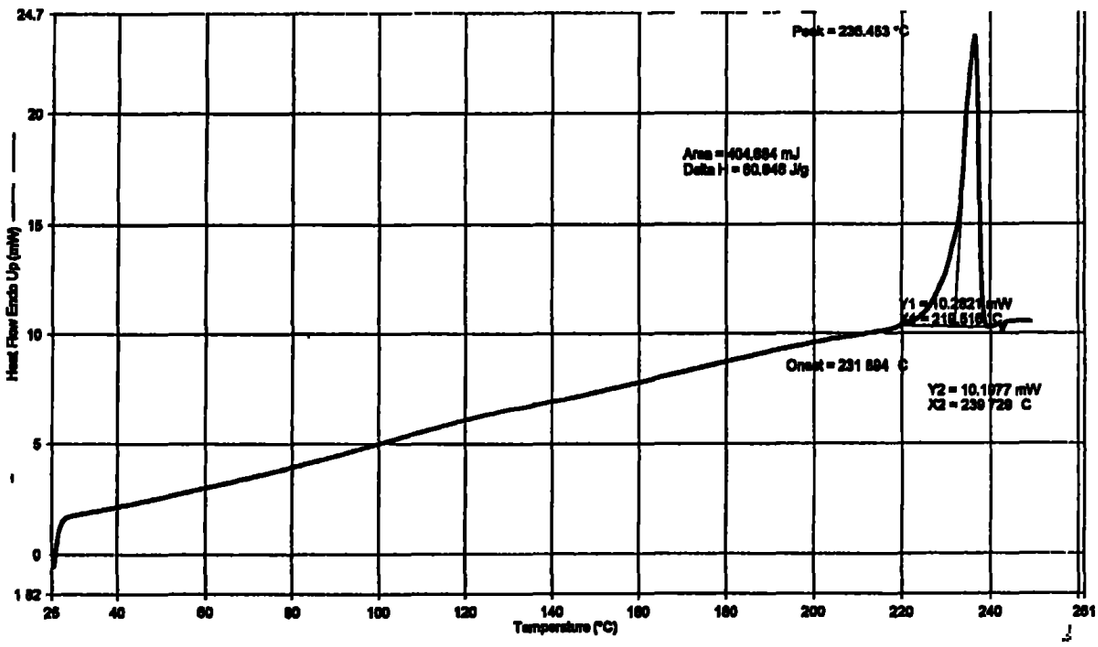


Figure 2-0

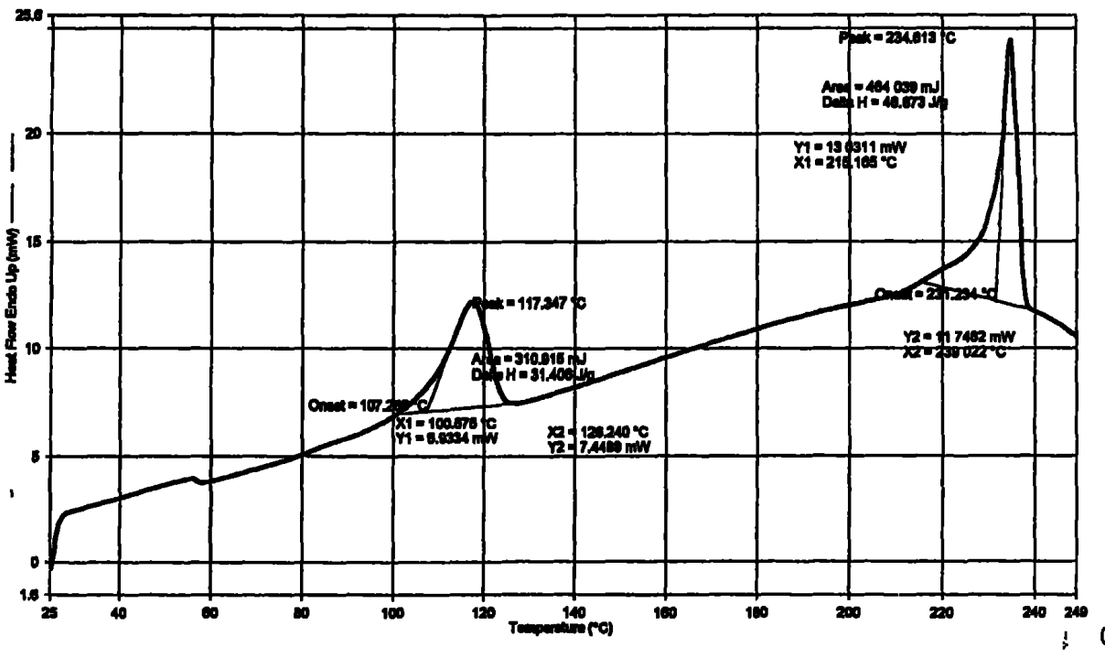
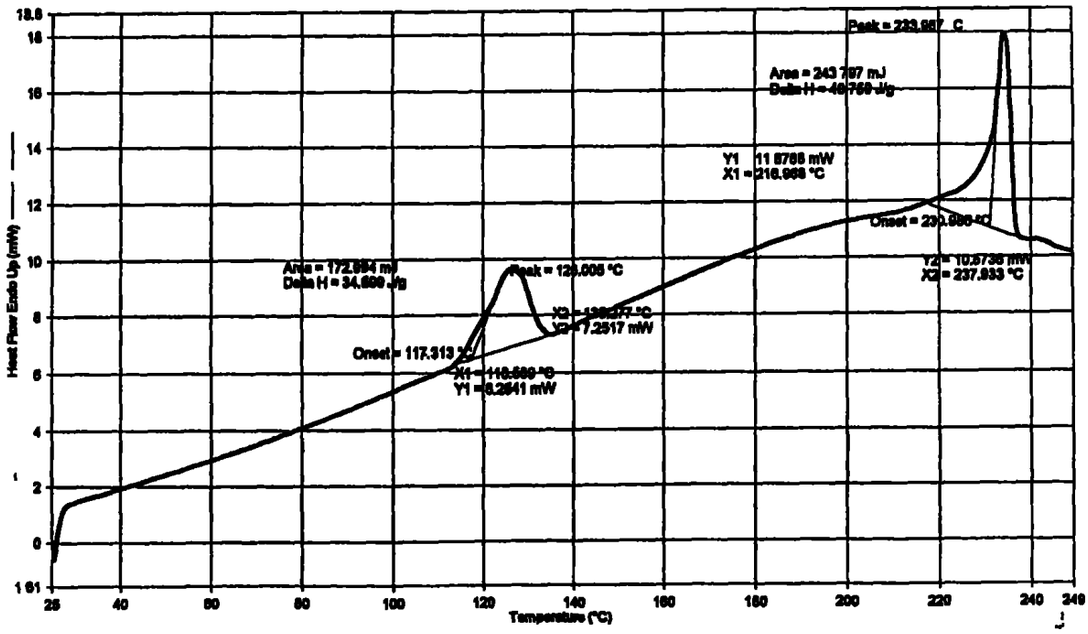

Sample Weight: 6.900 mg
Comment:
isobutyl acetate solvate
10 °C/min sealed pan
Peak = 234.613 °C
Area = 464.039 mJ
Delta H = 46.673 J/g
Y1 = 13.0311 mW
X1 = 215.165 °C
Peak = 117.347 °C
Area = 310.815 mJ
Delta H = 31.406 J/g
Onset = 107.209 °C
X1 = 100.876 °C
Y1 = 6.9334 mW
X2 = 126.240 °C
Y2 = 7.4486 mW
Y2 = 11.7462 mW
X2 = 239.022 °C
Heat Flow Endo Up (mW)
Temperature (°C)

Figure 2-P

Sample Weight: 5.UUU mg
Comment:
butyl acetate solvate
10 °C/min sealed pan



228

Figure 2-Q

823

Sample Weight: 5.538 mg
Comment: methyl acetate solvate 1
10 °C/min sealed pan

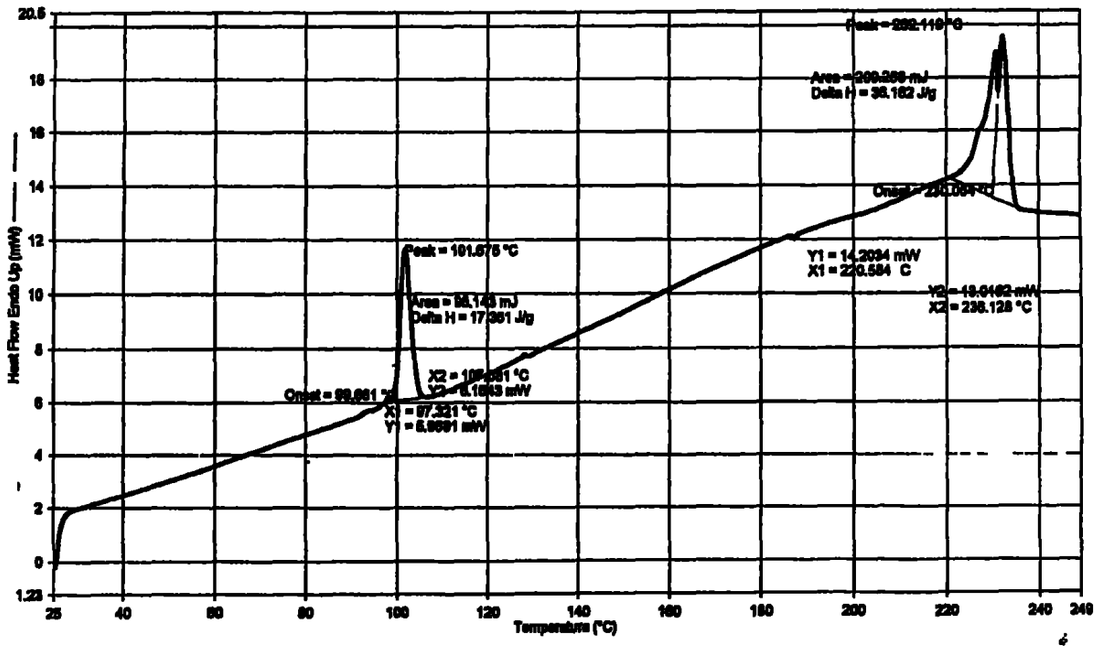


Figure 2-R

Sample Weight: 6.074 mg
Comment:
propyl acetate solvate 1
10 °C/min sealed pan

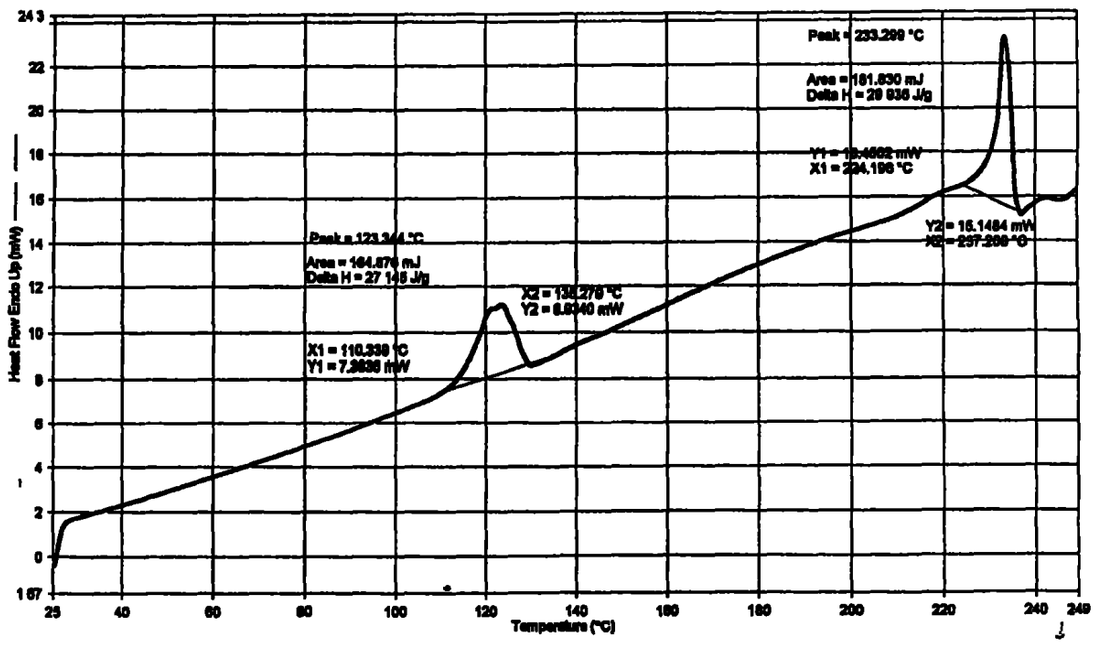


Figure 2-S

n-butanol solvate

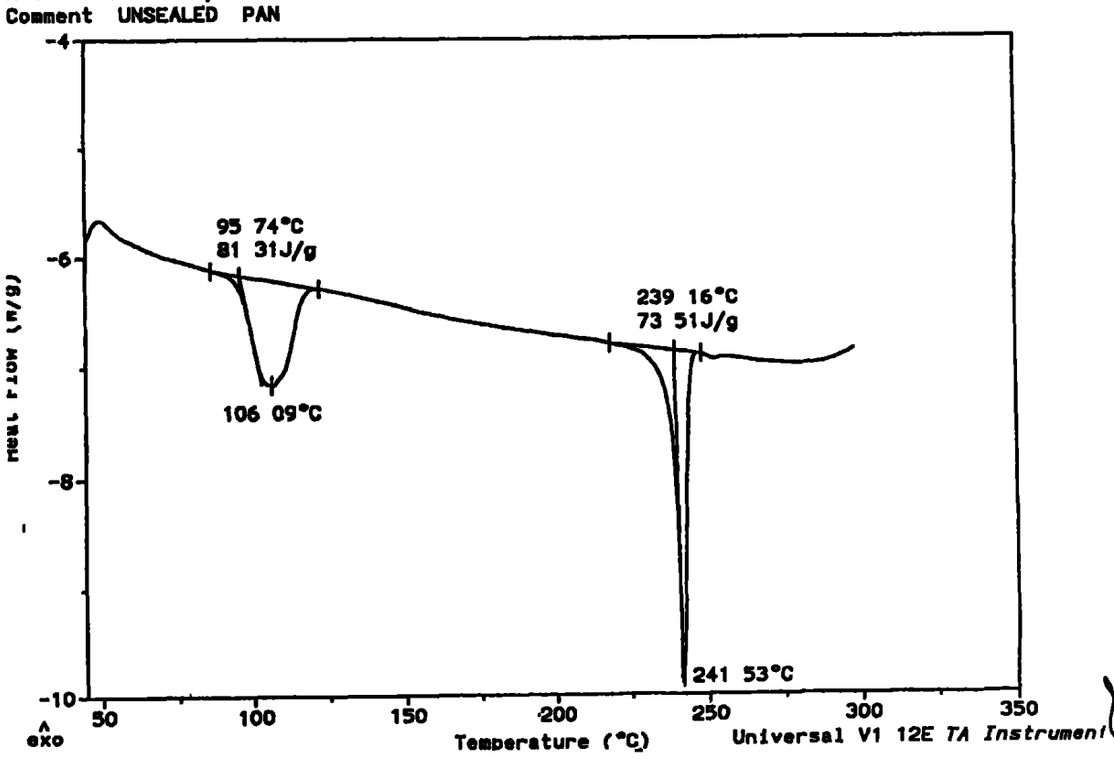


Figure 2-T

n-octanol solvate

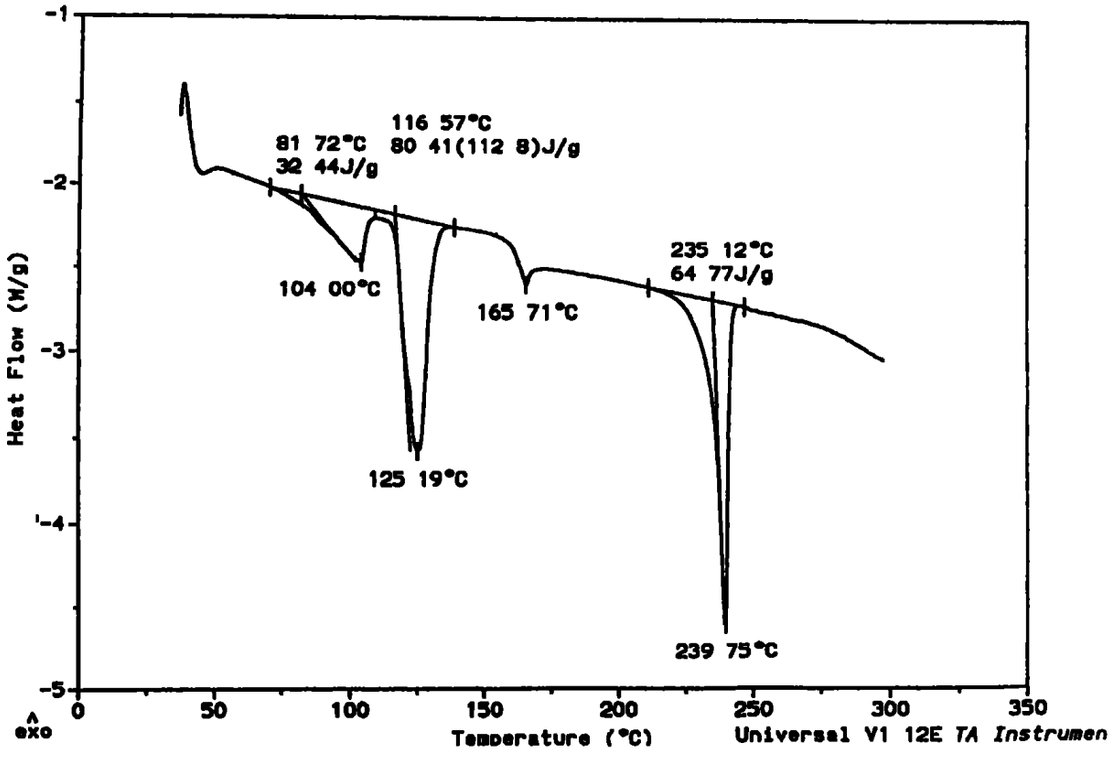


Figure A

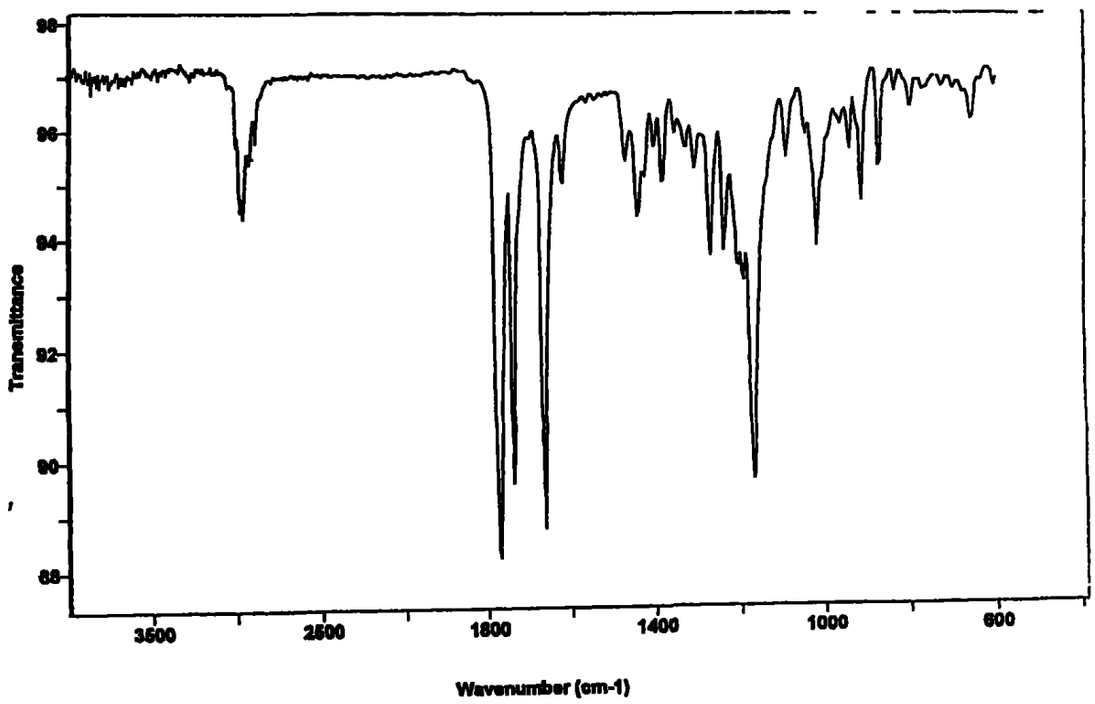

Transmittance
98
96
94
92
90
88
3500
2500
1800
1400
1000
600
Wavenumber (cm-1)

Figure 3B

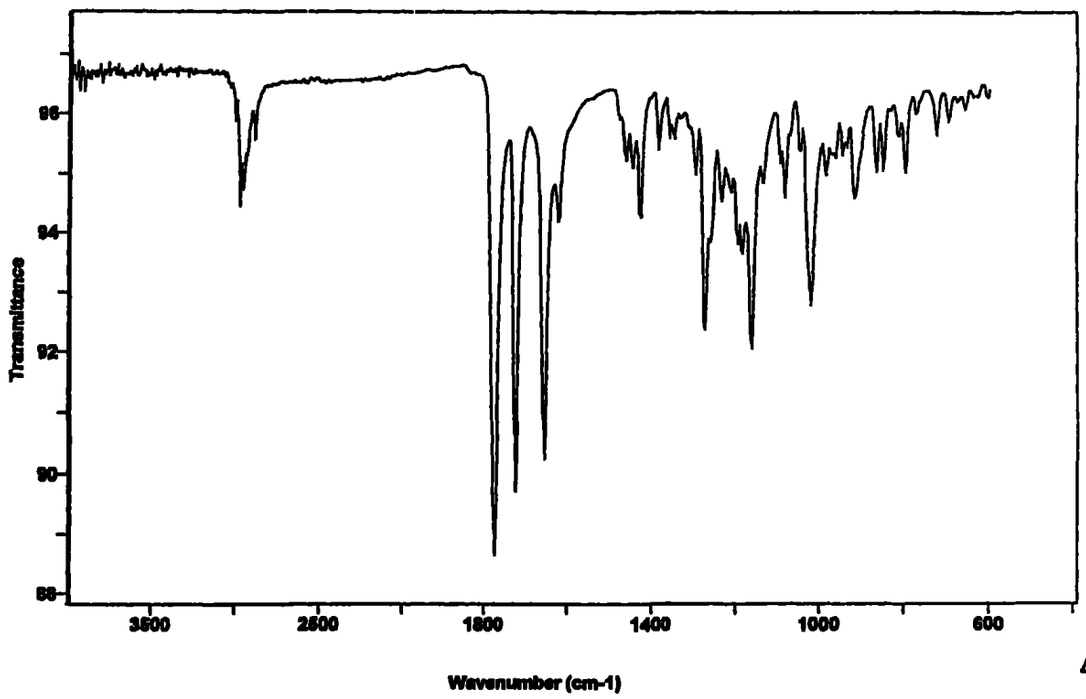

Transmittance
96
94
92
90
88
3500
2500
1800
1400
1000
600
Wavenumber (cm-1)

Figure 2-C

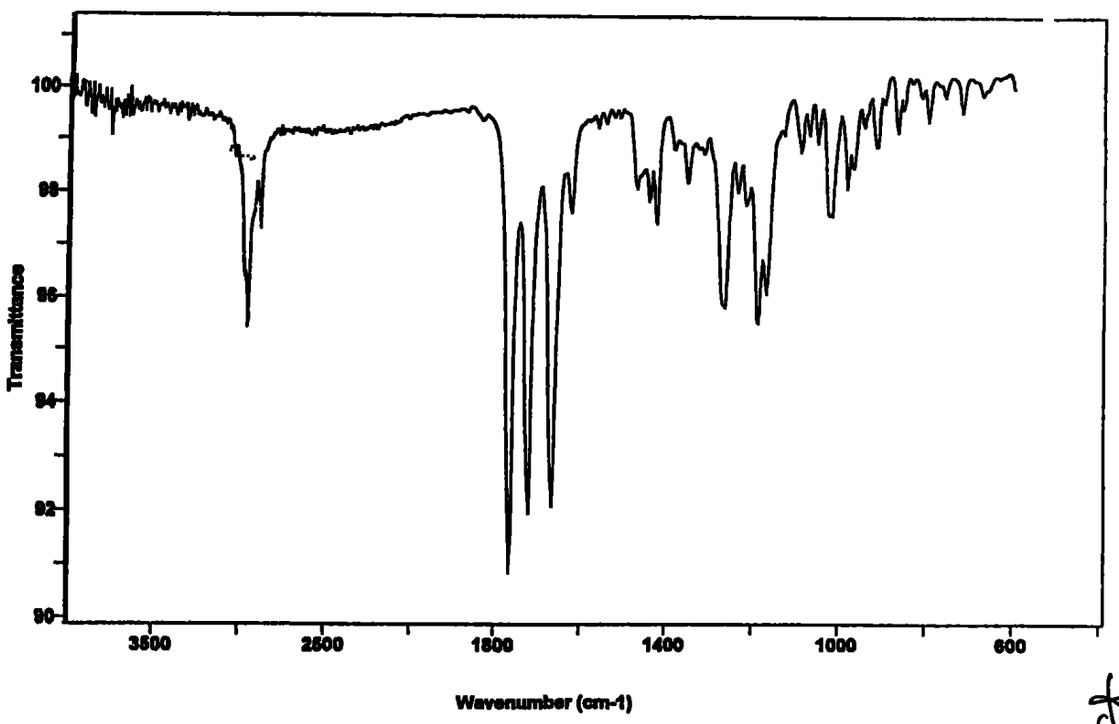

100
98
96
94
92
90
Transmittance
3500
2500
1500
1400
1000
600
Wavenumber (cm-1)

Figure 3-D

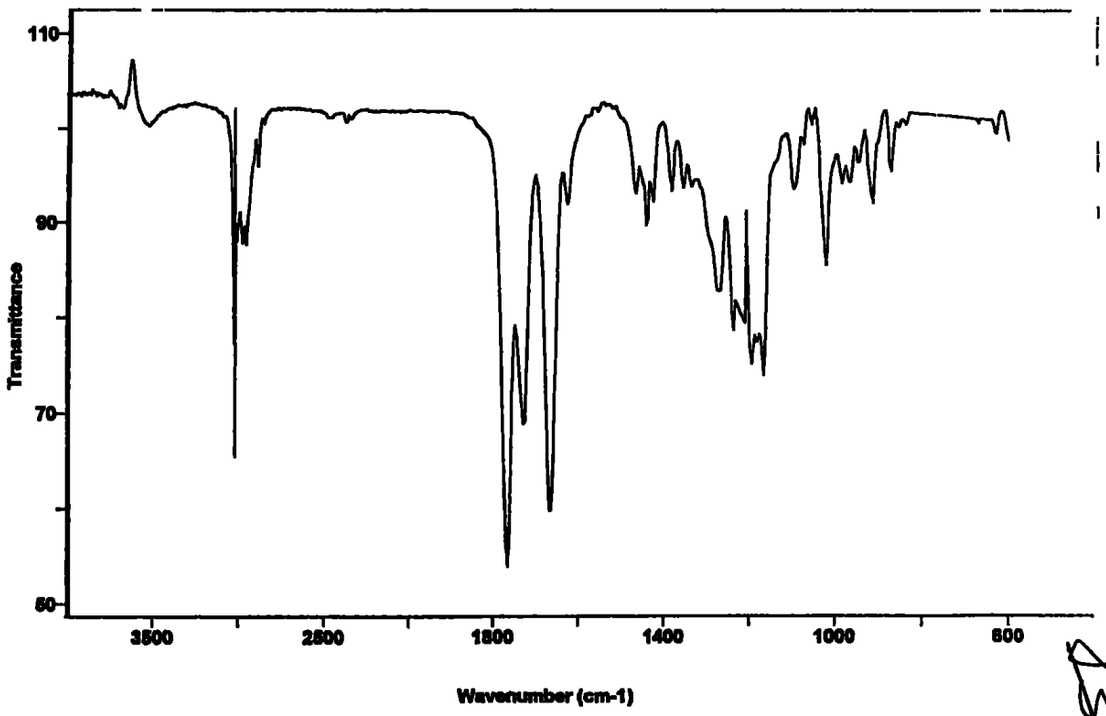

110
90
70
50
Transmittance
3500
2500
1800
1400
1000
800
Wavenumber (cm-1)

Figure 3-E

n-propyl alcohol solvate

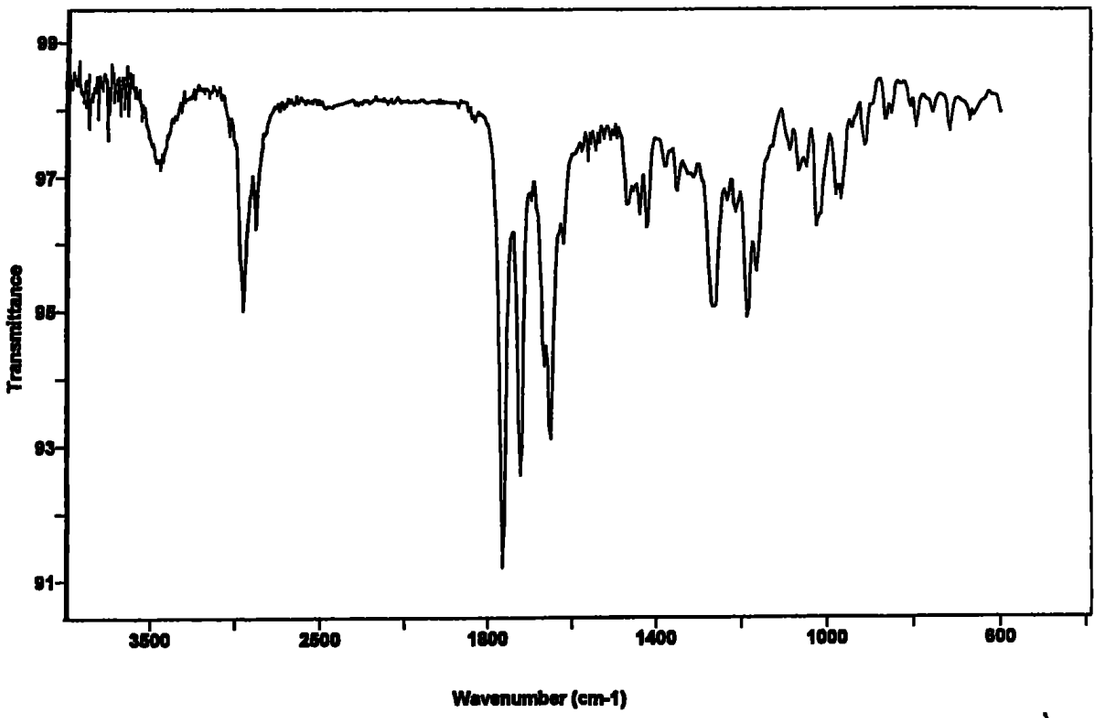


Figure 3-F

tetrahydrofuran solvate

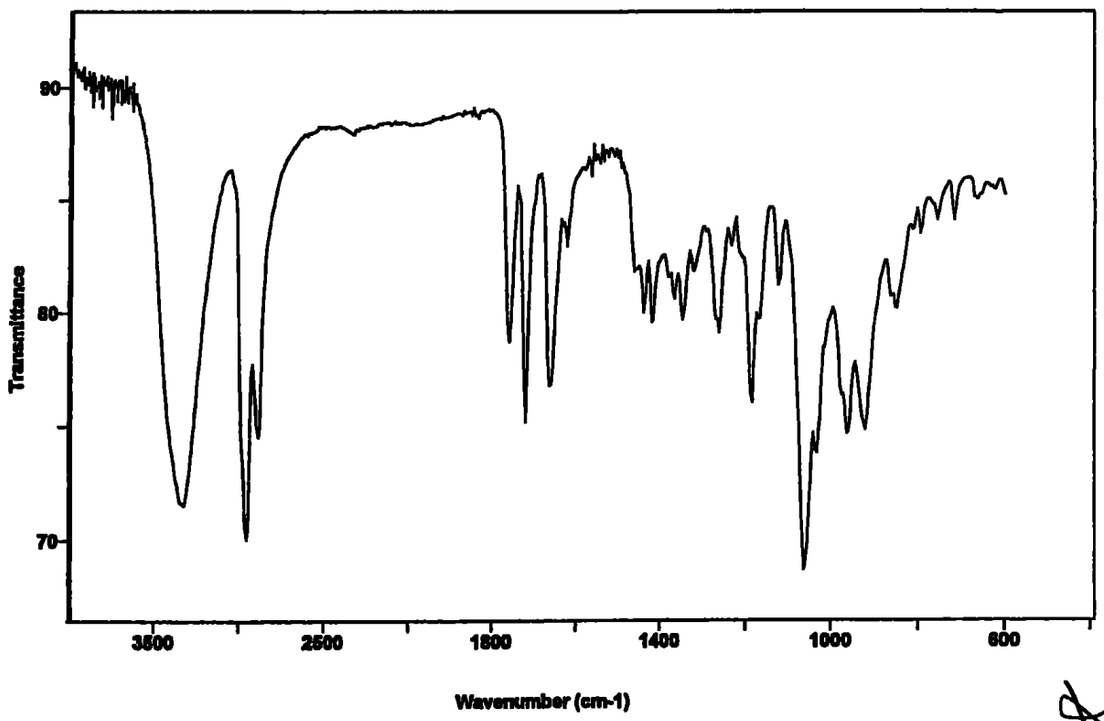


Figure 1-G

ethyl propionate solvate

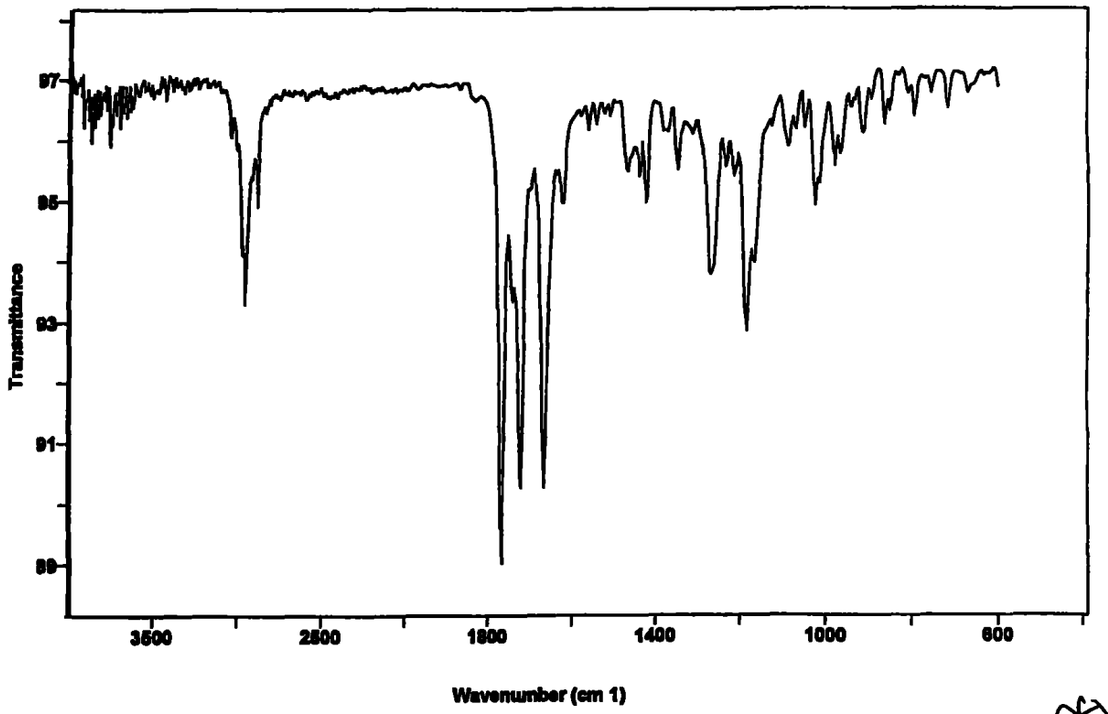


Figure 2-H

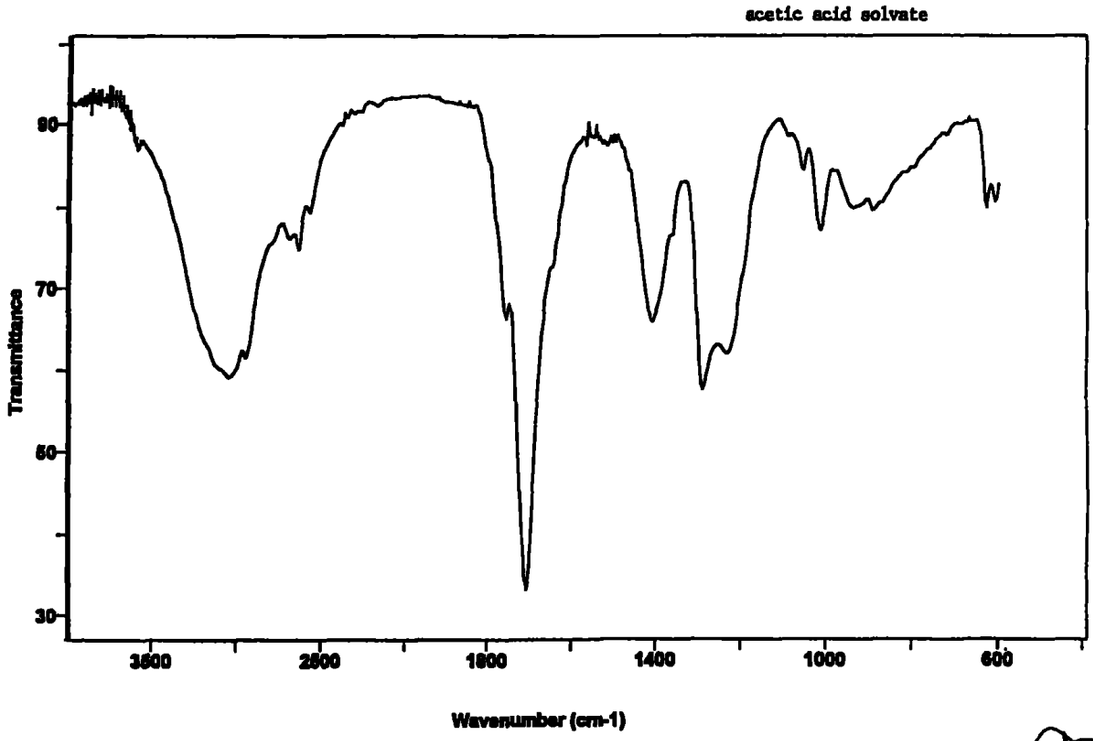

acetic acid solvate
90
70
50
30
Transmittance
3600
2600
1800
1400
1000
600
Wavenumber (cm-1)

Figure 1-I

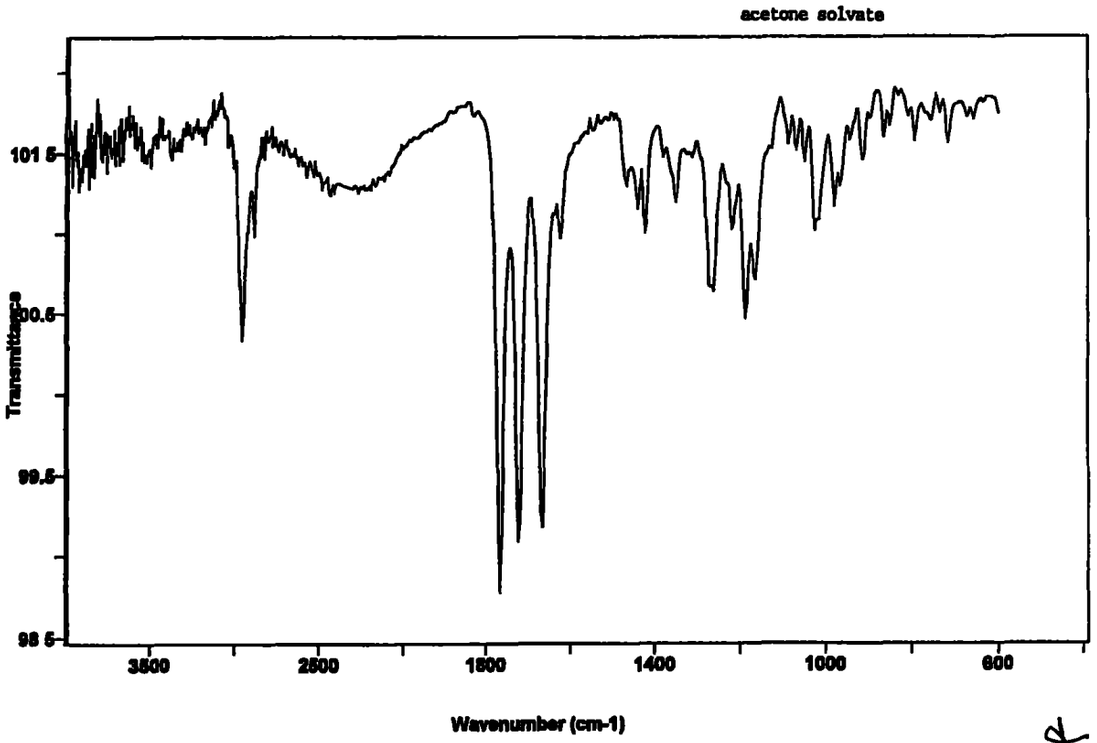

acetone solvate
101.5
100.5
99.5
98.5
Transmittance
3800
2800
1800
1400
1000
600
Wavenumber (cm-1)

Figure 3-J

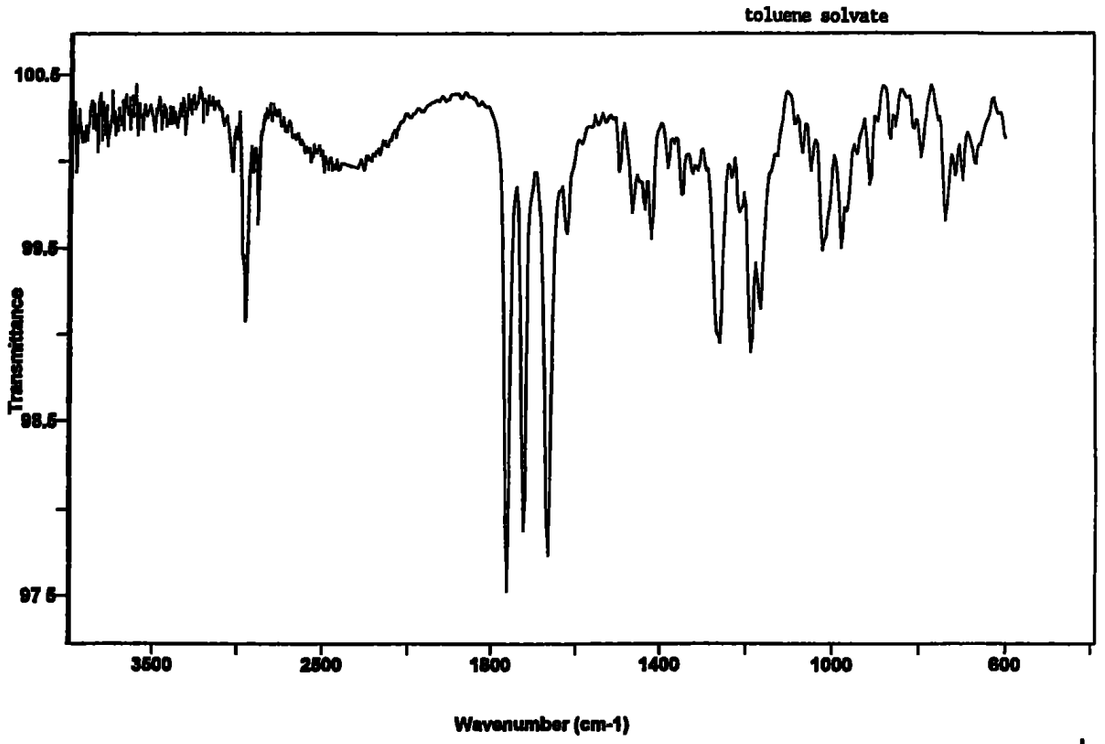

toluene solvate
100.5
99.5
98.5
97.5
Transmittance
3500
2500
1800
1400
1000
600
Wavenumber (cm-1)

Figure 3-K

isopropanol solvate

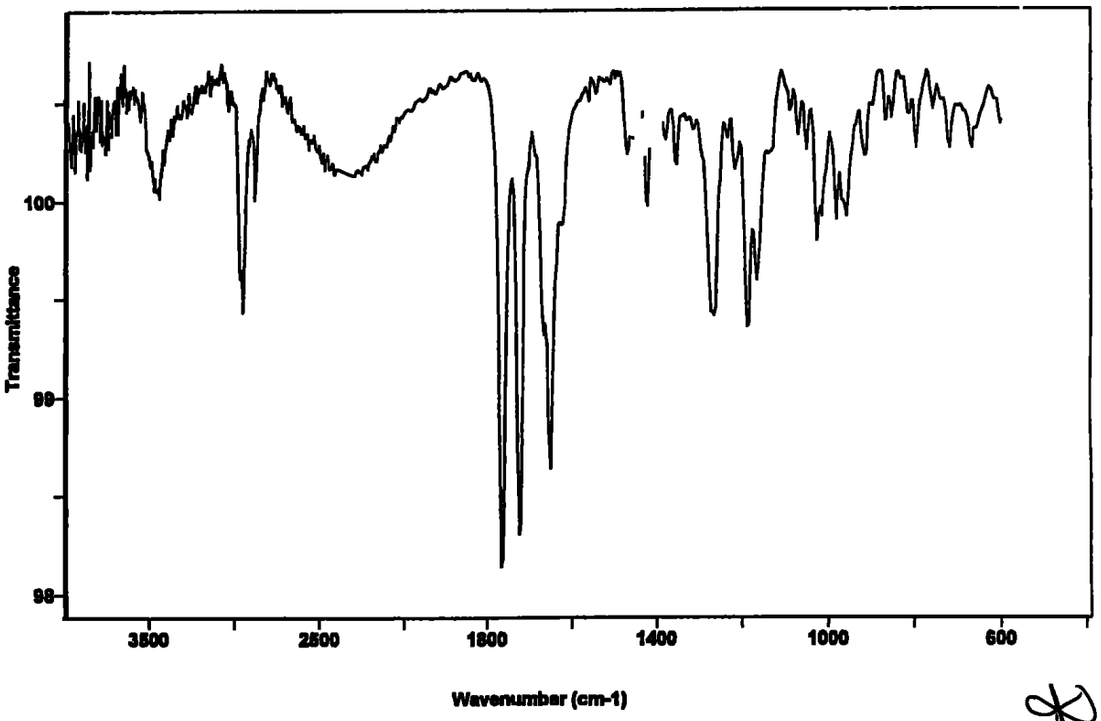
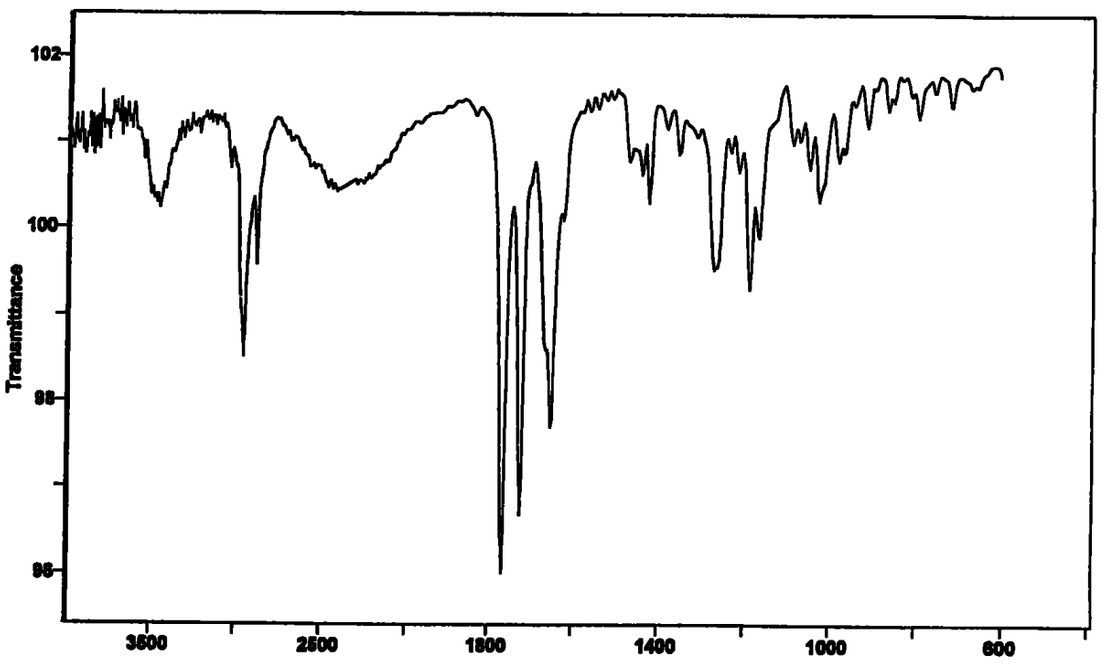


Figure 3-L

ethanol solvate



File # 1 = P4829A02

Wavenumber (cm-1)

X-Zoom CURSOR

Figure 3-M

839

isobutyl acetate solvate

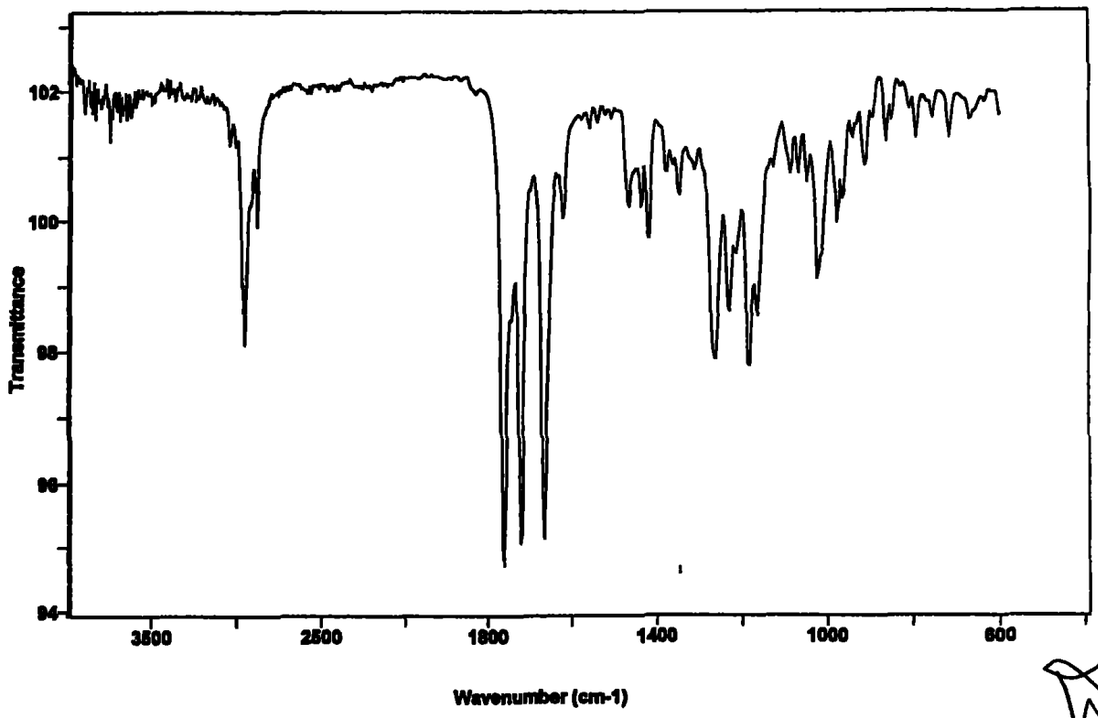


Figure 2-N

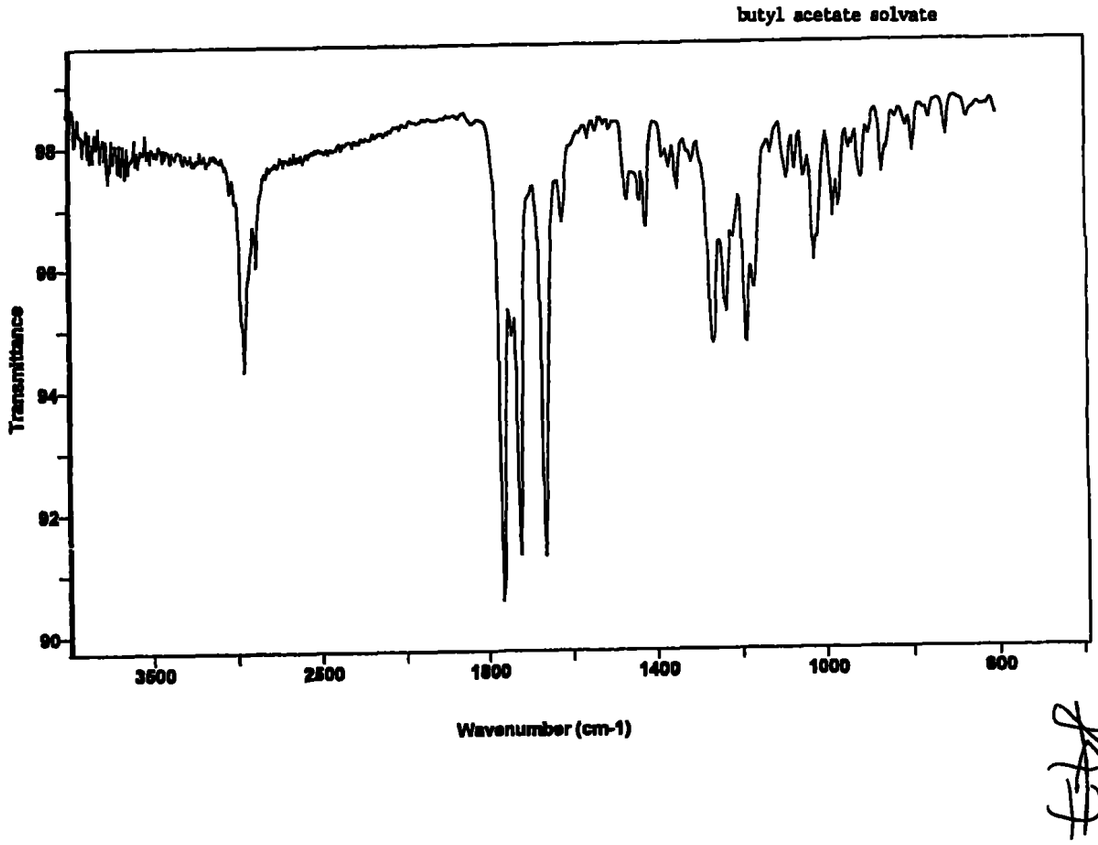

butyl acetate solvate
Transmittance
98
96
94
92
90
3500
2500
1800
1400
1000
800
Wavenumber (cm-1)

Figure 3-0

propyl acetate solvate

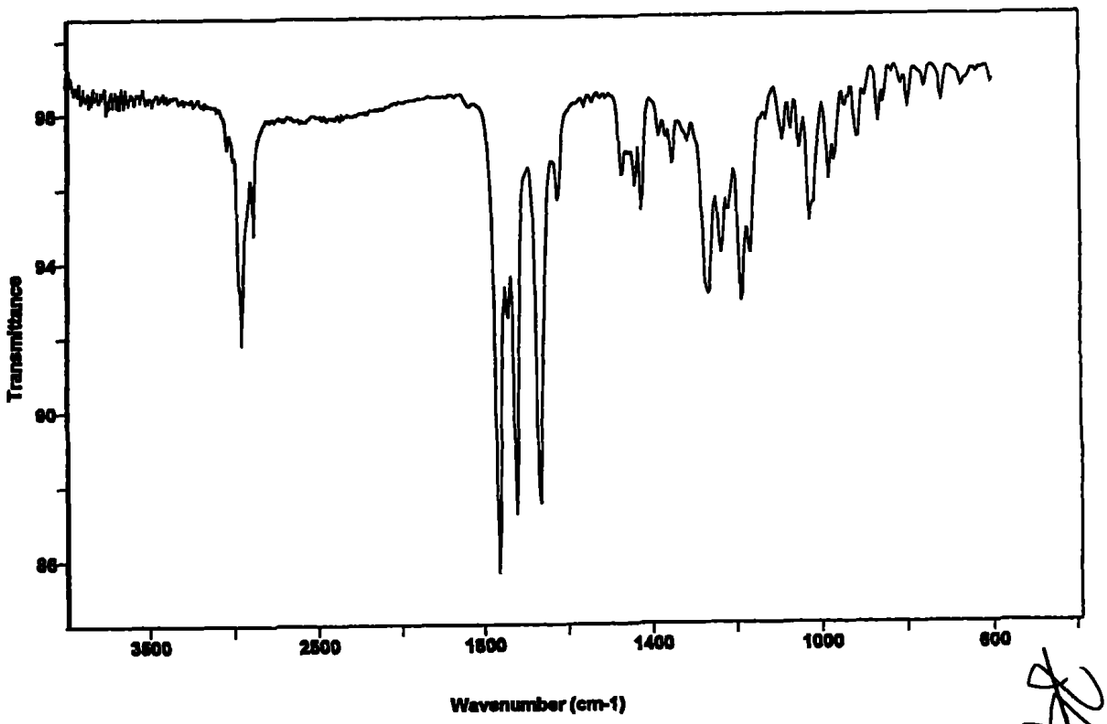


Figure 2P

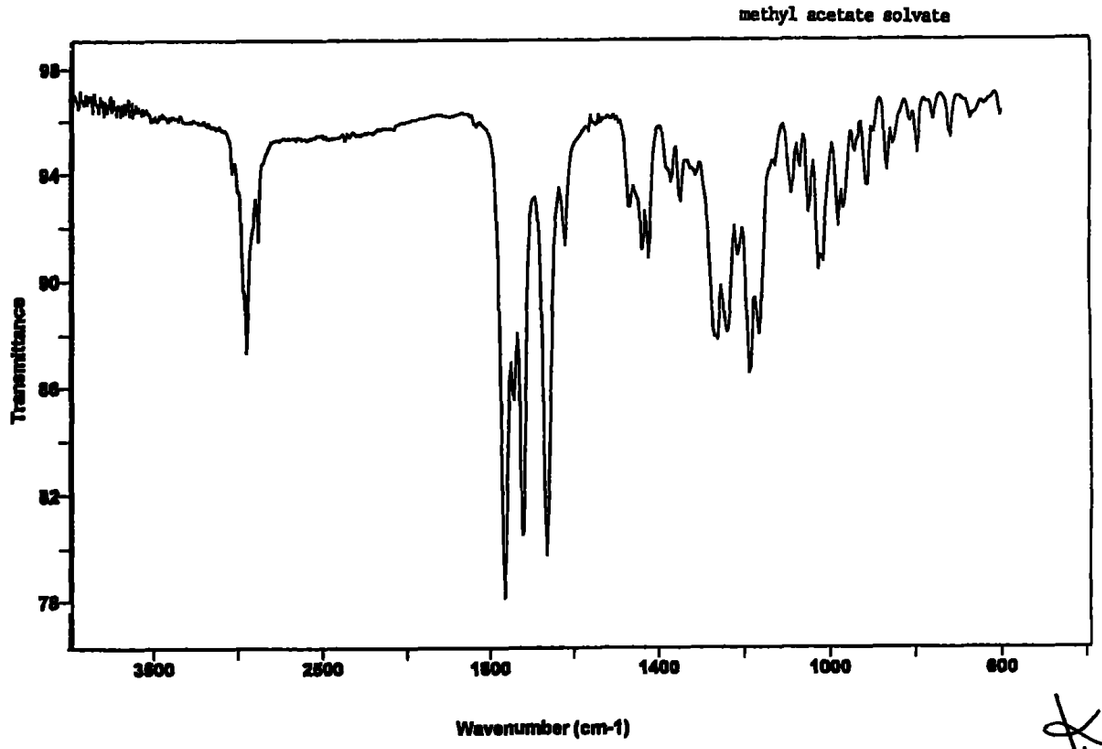

methyl acetate solvate
98
94
90
86
82
78
Transmittance
3500
2500
1500
1400
1000
800
Wavenumber (cm-1)

Figure 2-Q

propylene glycol solvate

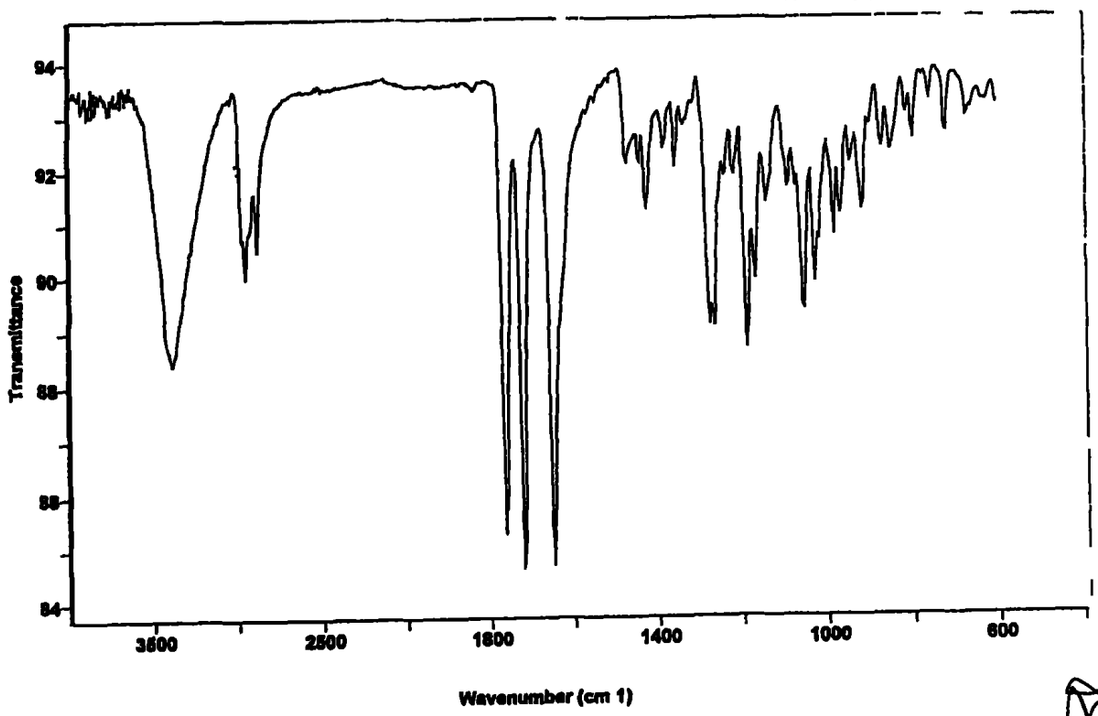


Figure 2aR

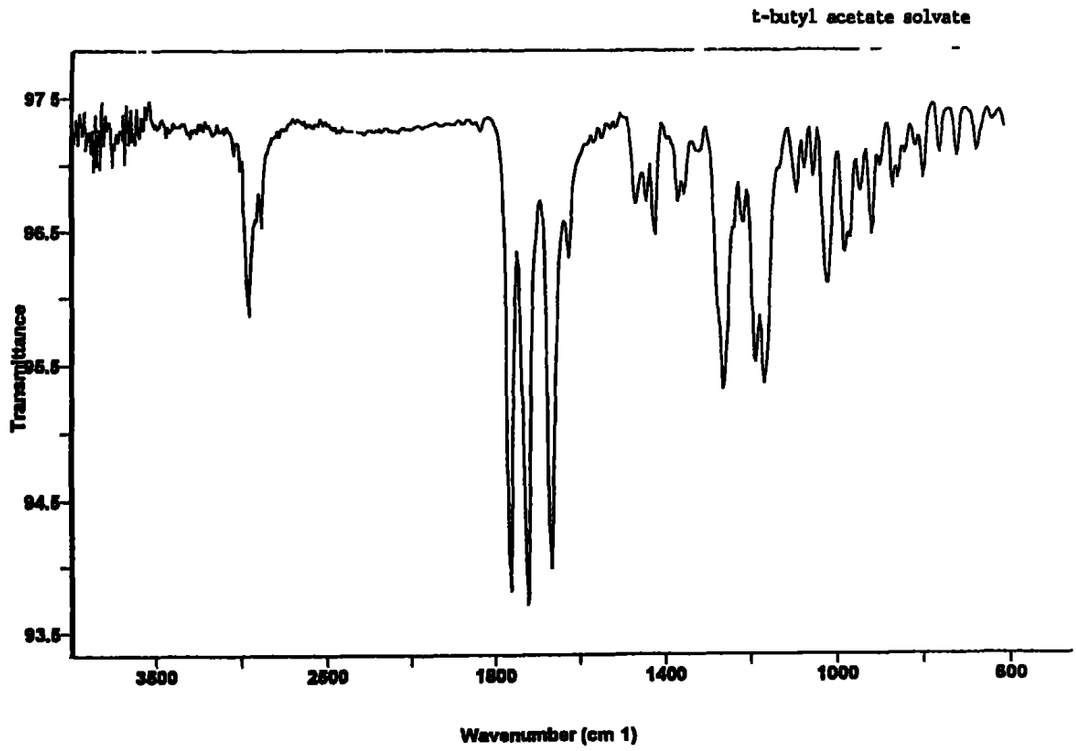
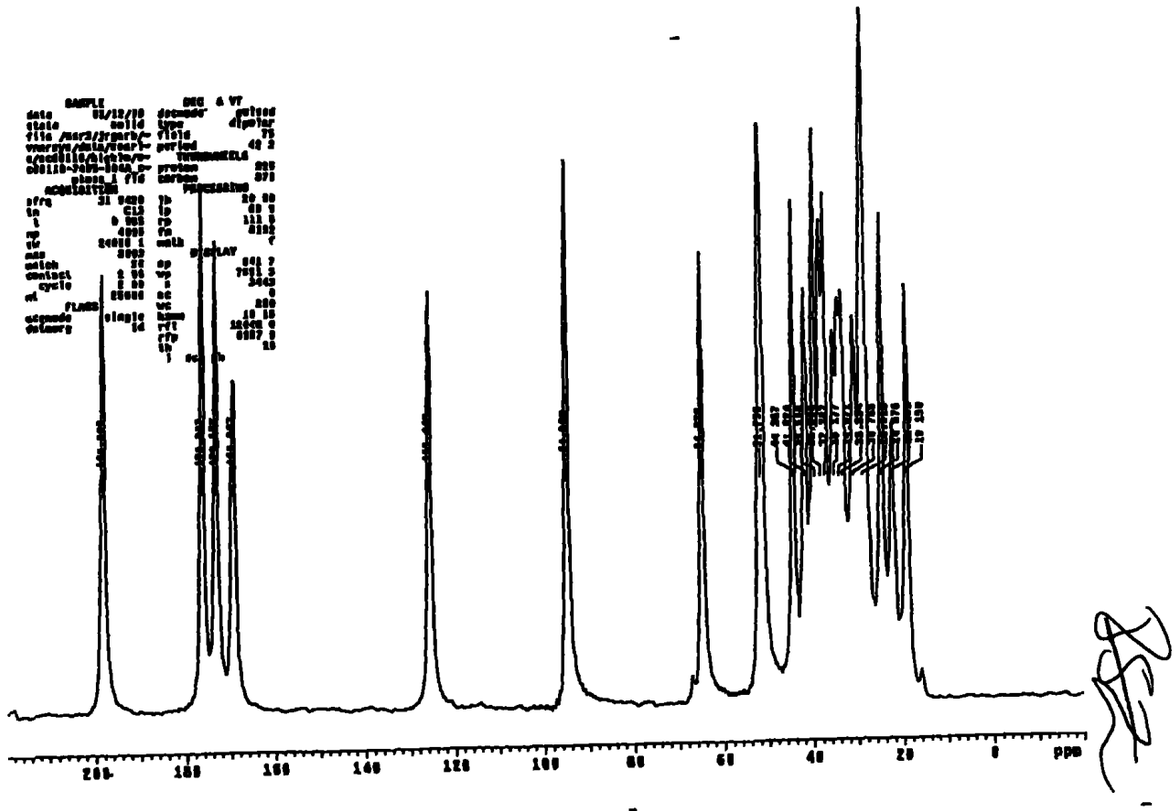

t-butyl acetate solvate
97.5
96.5
95.5
94.5
93.5
Transmittance
3500
2500
1500
1400
1000
600
Wavenumber (cm 1)

Figure 4



Figure

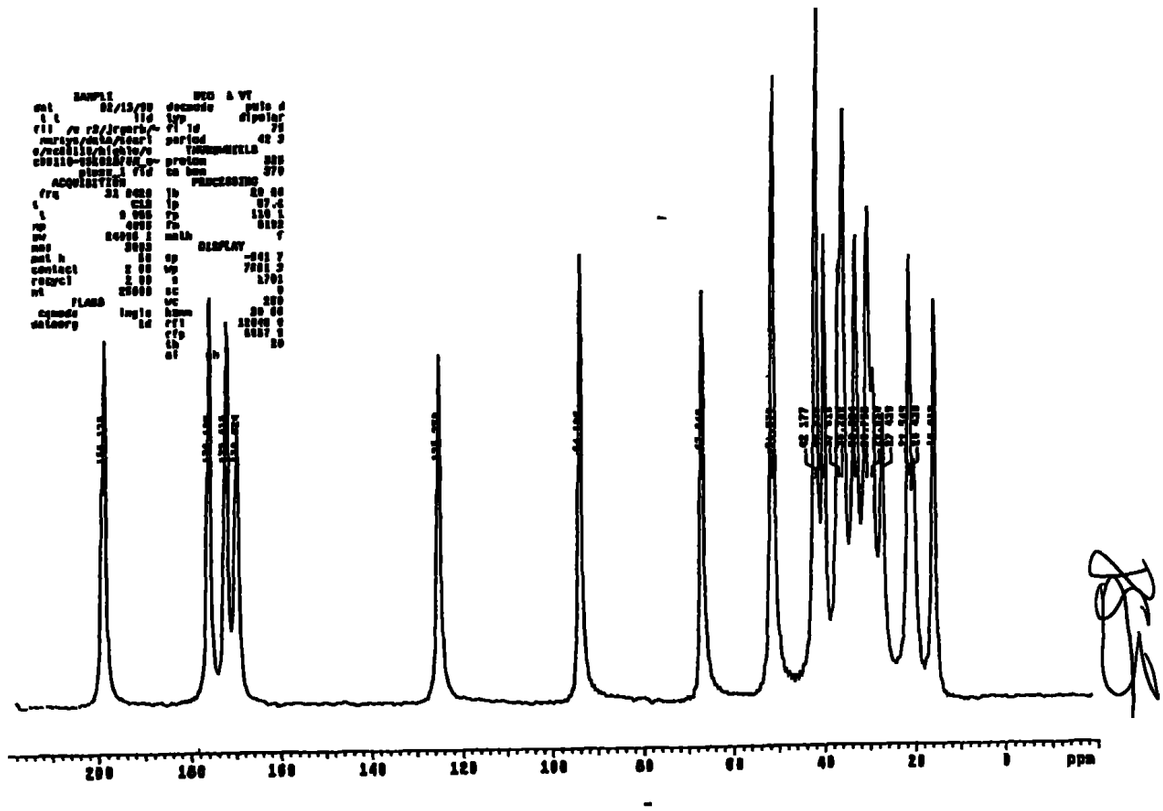


Figure 6-A

Size 3 3010 mg
Method 10 deg ramp to 300 deg

methyl ethyl ketone solvate

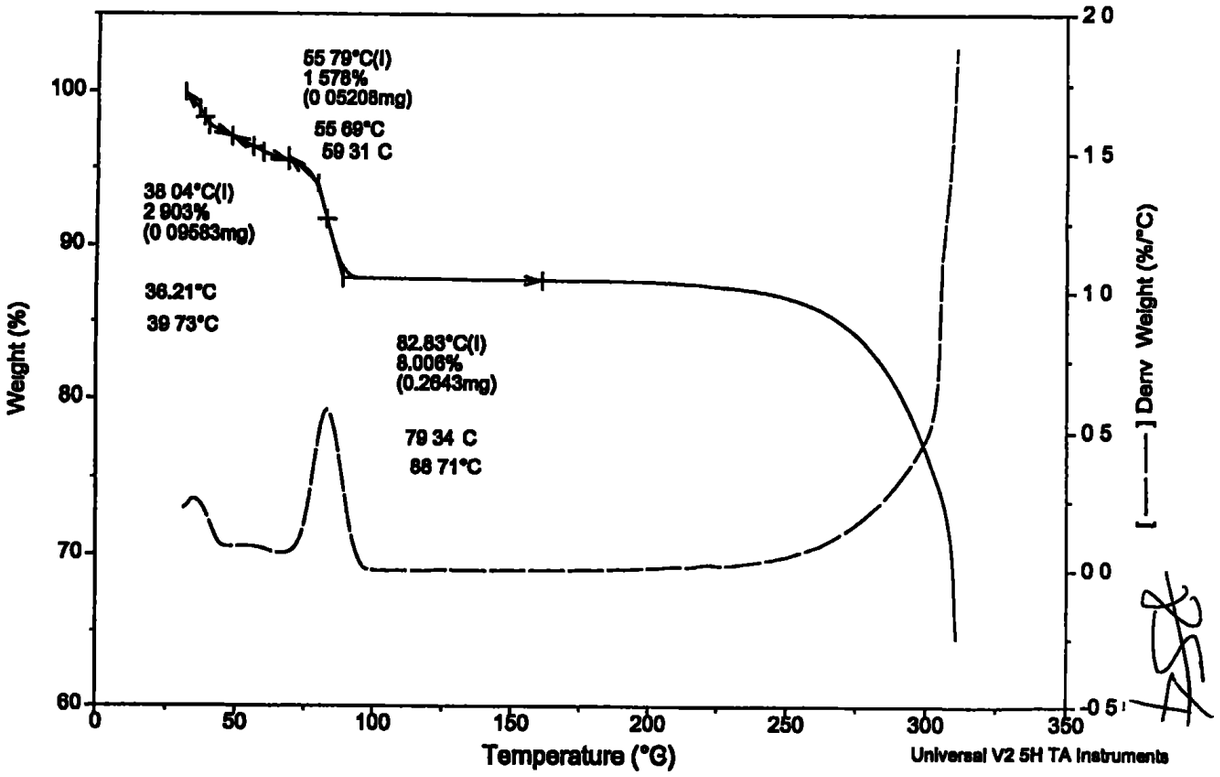


Figure 3B

Size 13.4190 mg
Method 10 deg ramp to 300 deg

n-propyl alcohol solvate

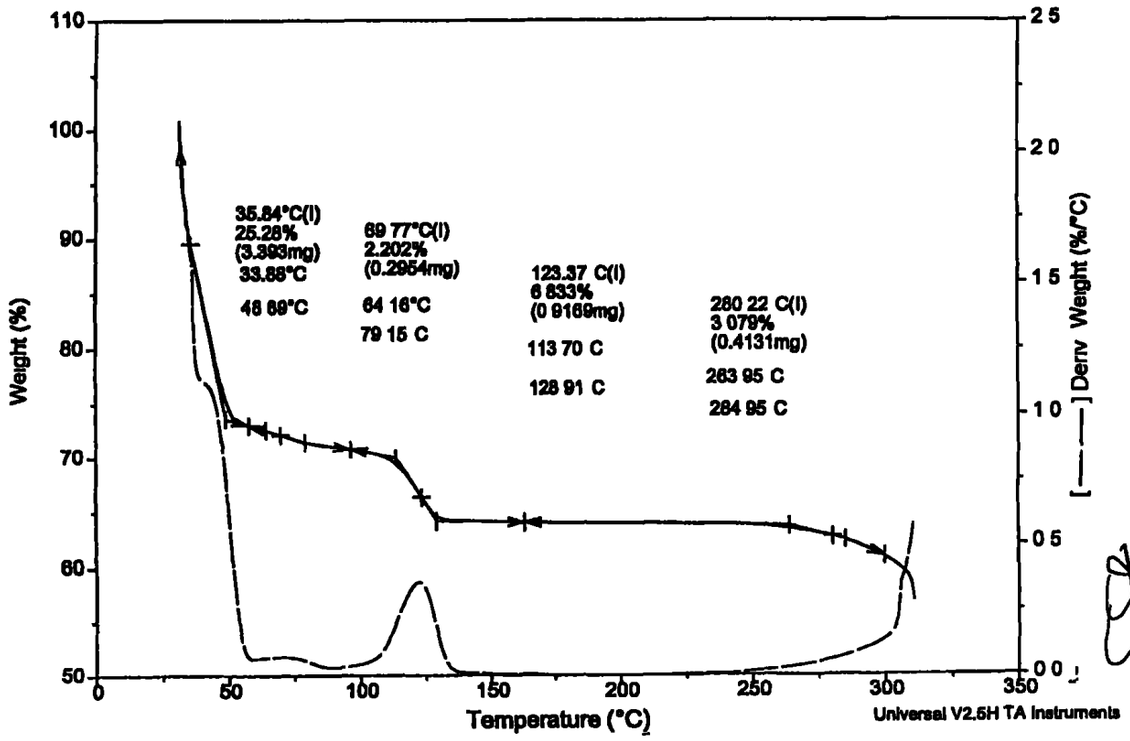


Figure [illegible]C

Size 14 9110 mg
Method 10 deg ramp to 300 deg

tetrahydrofuran solvate

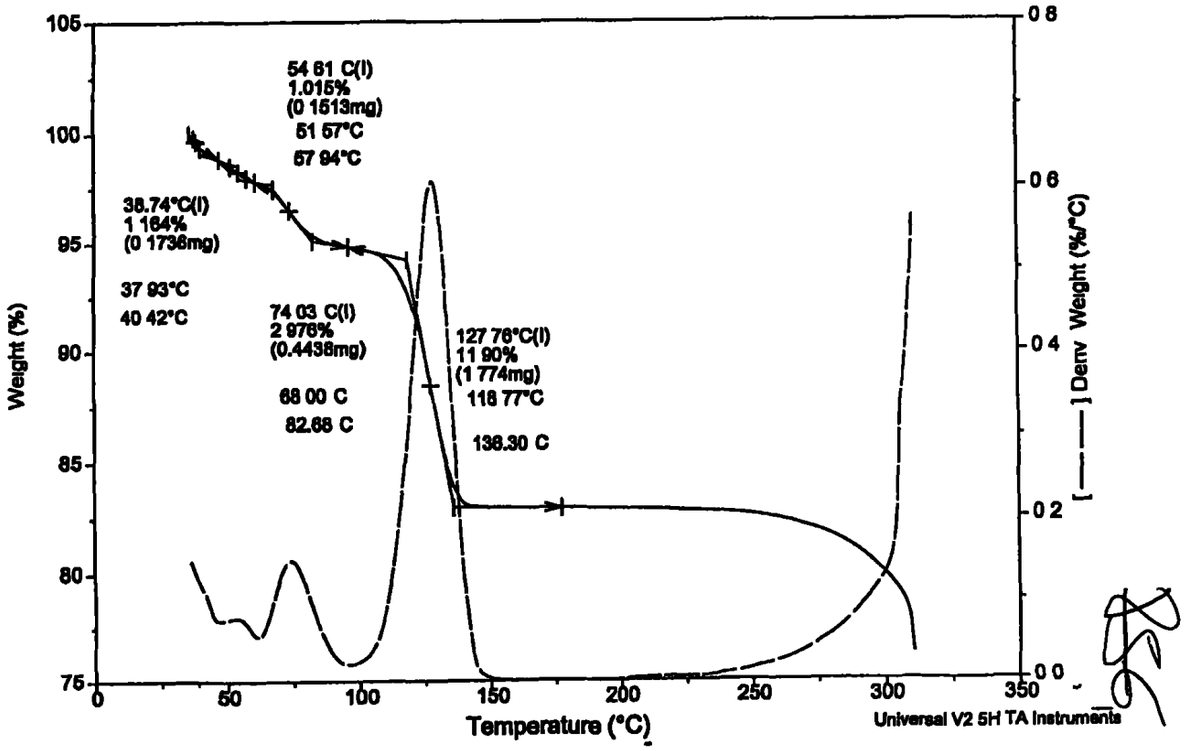


Figure D

Size 9 5030 mg
Method 10 deg ramp to 300 deg

ethyl propionate solvate

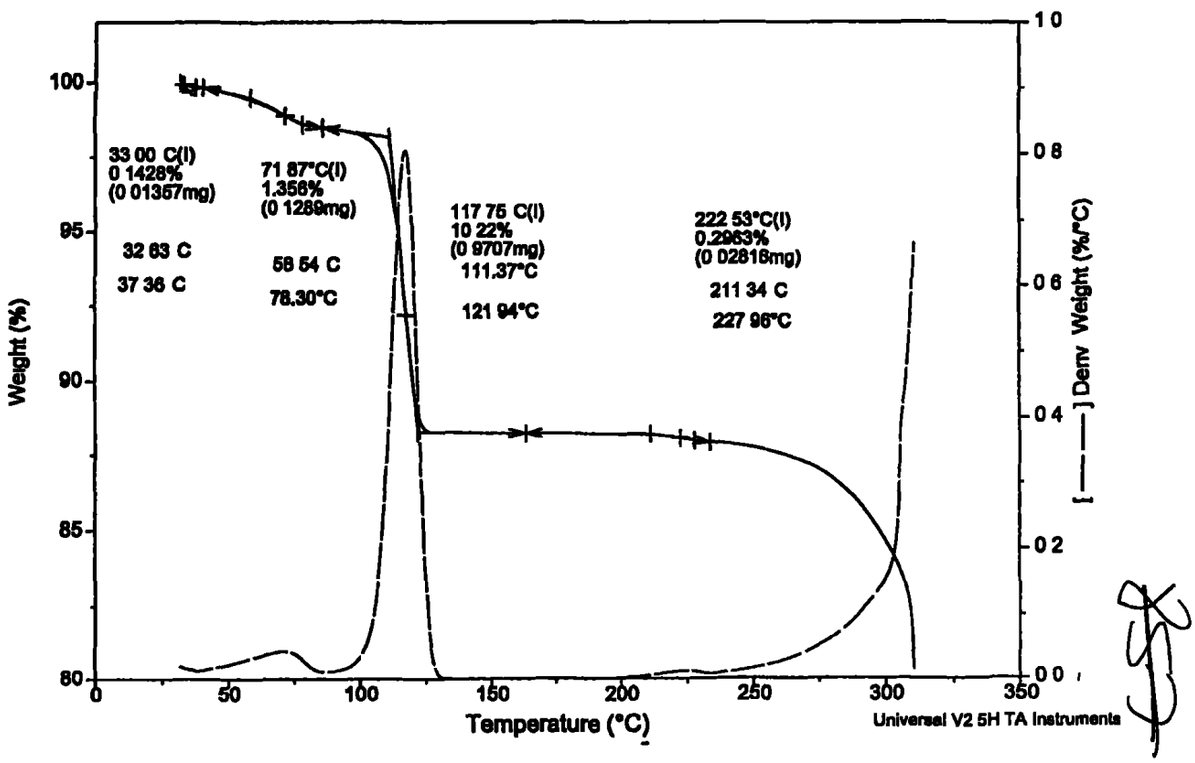


Figure 8g

Size 8 4270 mg
Method 10 deg ramp to 300 deg

acetic acid solvate

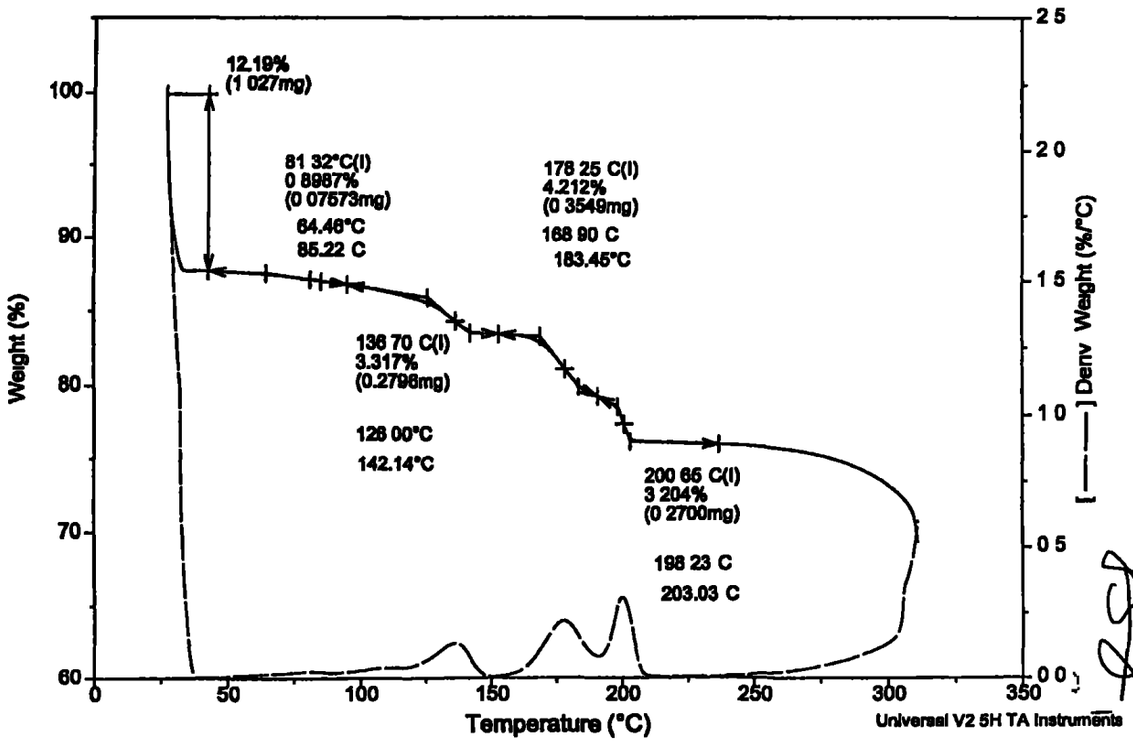


Figure 6 F

Size 3 9730 mg
Method 10 deg ramp to 300 deg

chloroform solvate

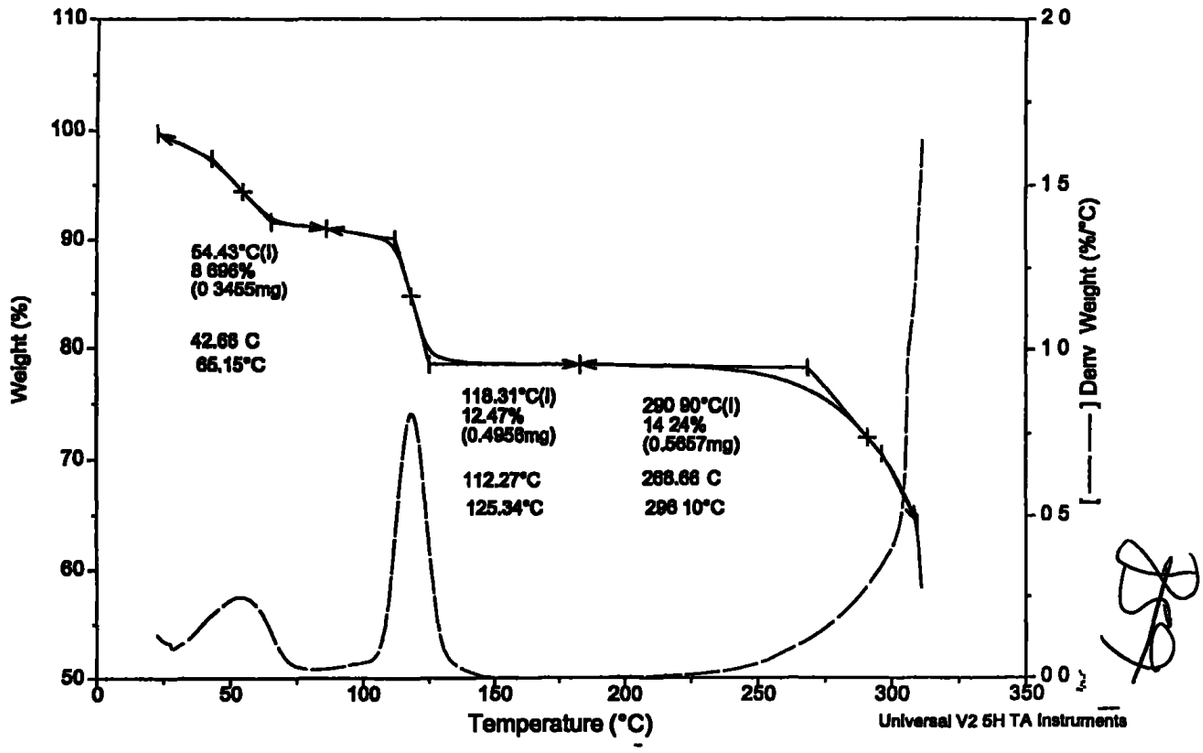


Figure 6

Size 2 2040 mg
Method 10 deg ramp to 300 deg

acetone solvate

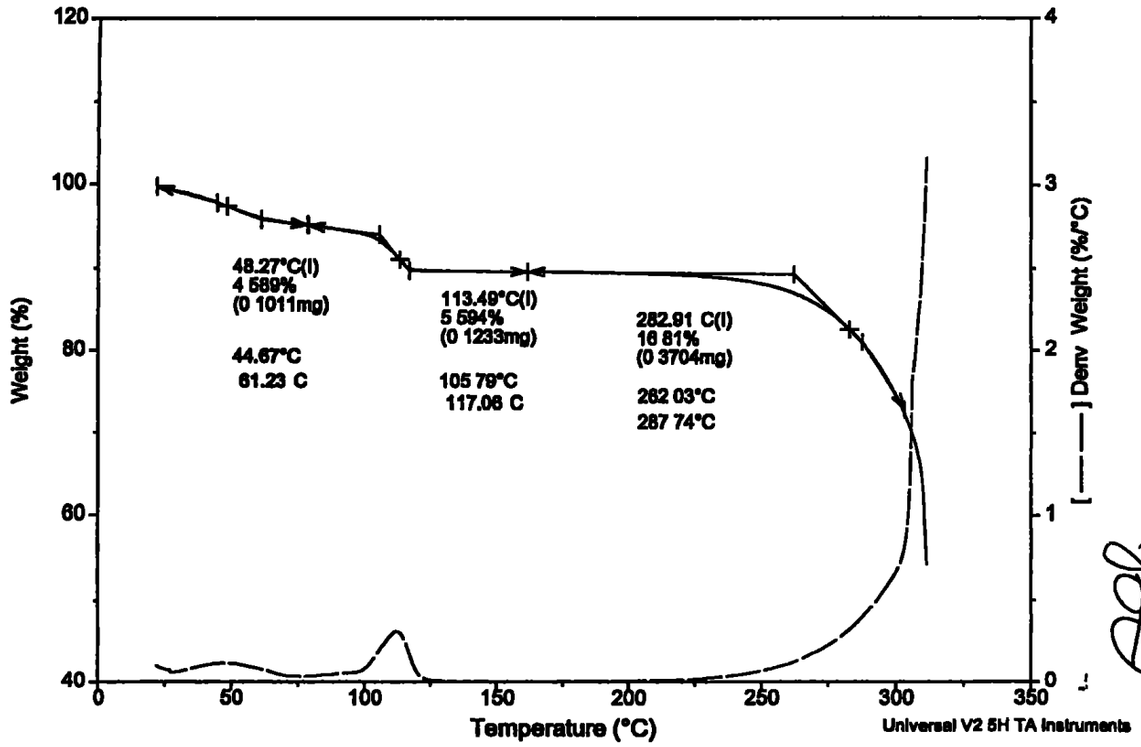


Figure H

Size 1 7480 mg
Method 10 deg ramp to 300 deg

toluene solvate

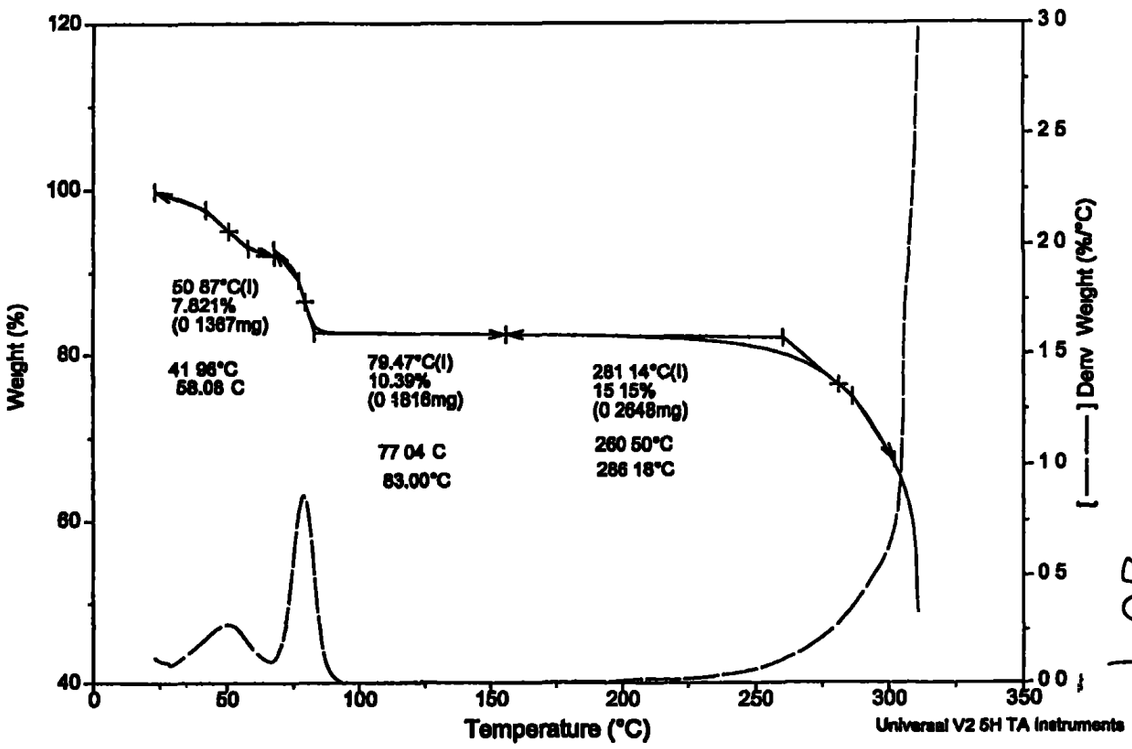


Figure 1

Size 6 4350 mg
Method 10 deg ramp to 300 deg

isopropanol solvate

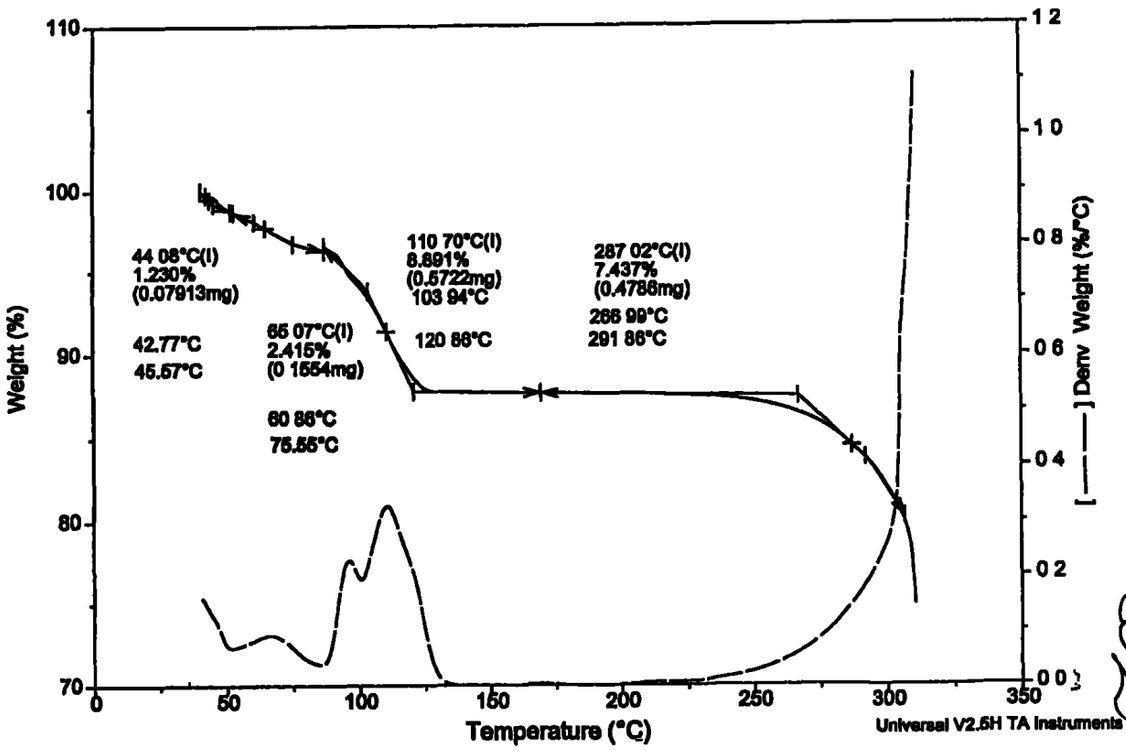


Figure 3

Size 6 1160 mg
Method 10 deg ramp to 300 deg

ethanol solvate

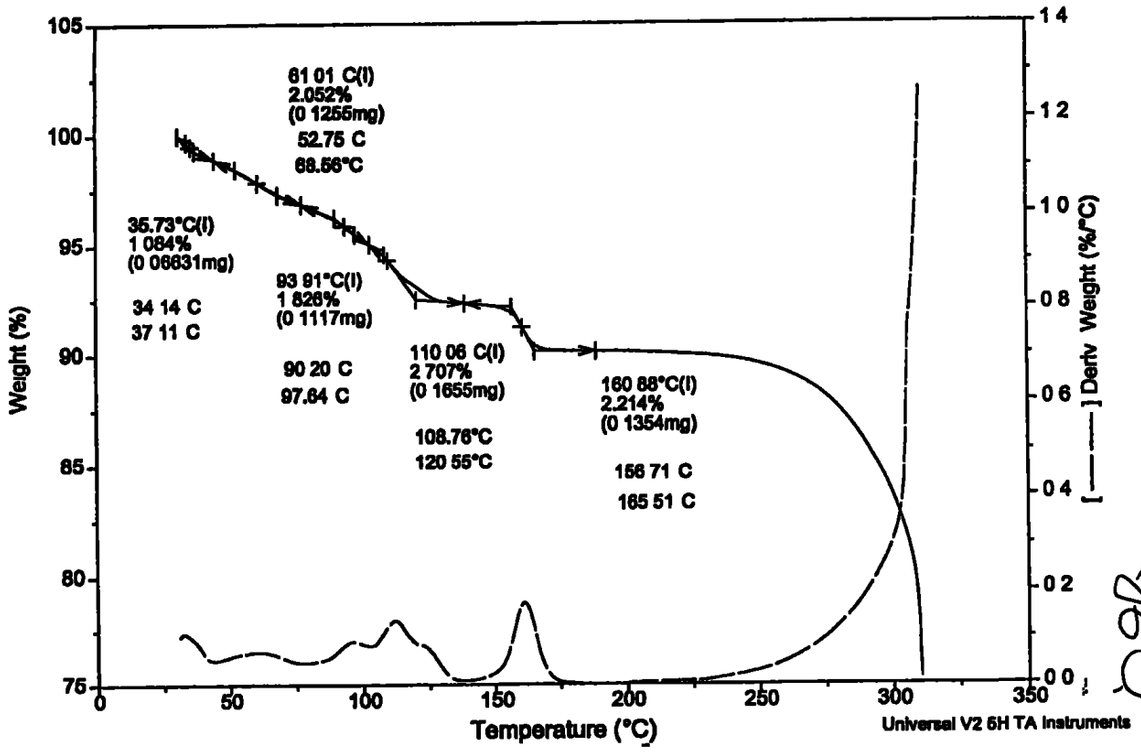


Figure K

Size 4 9710 mg
Method 10 deg ramp to 300 deg

isobutyl acetate solvate

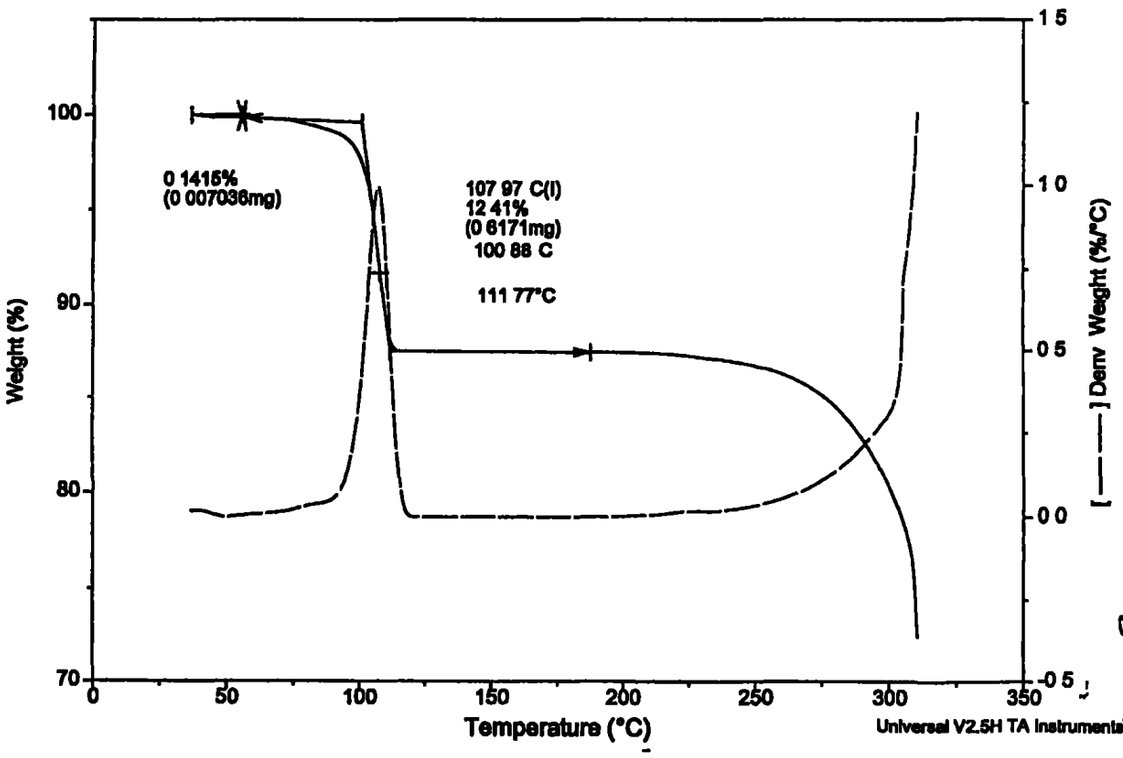


Figure 1L

Size 2 3240 mg
Method 10 deg ramp to 300 deg

butyl acetate solvate

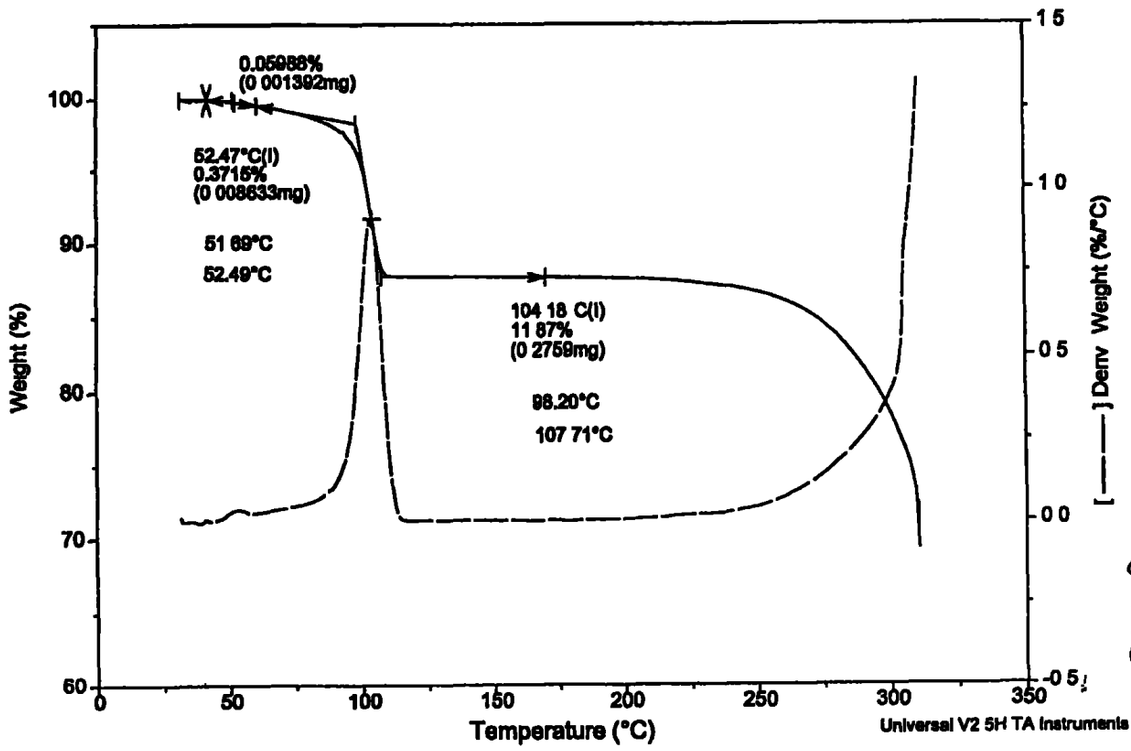


Figure 6-M

Size 2.7750 mg
Method 10 deg ramp to 300 deg

methyl acetate solvate

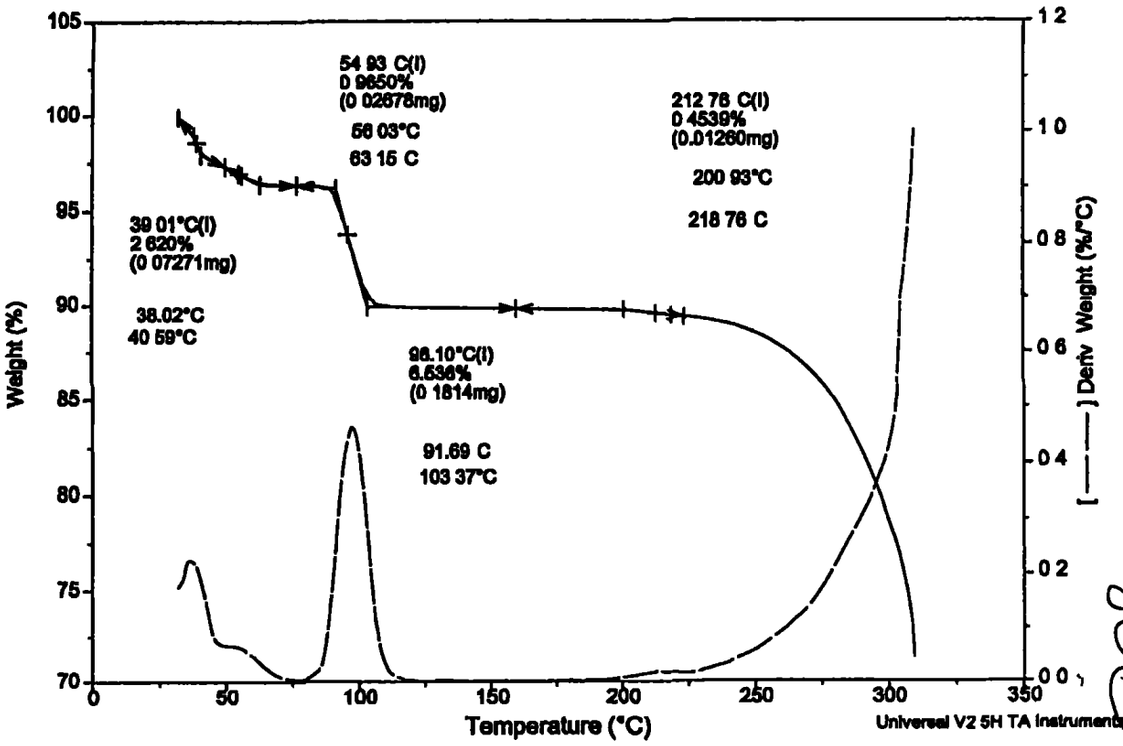


Figure 6-N

Size 4.4780 mg
Method TG 10D/M TO 300

propyl acetate solvate

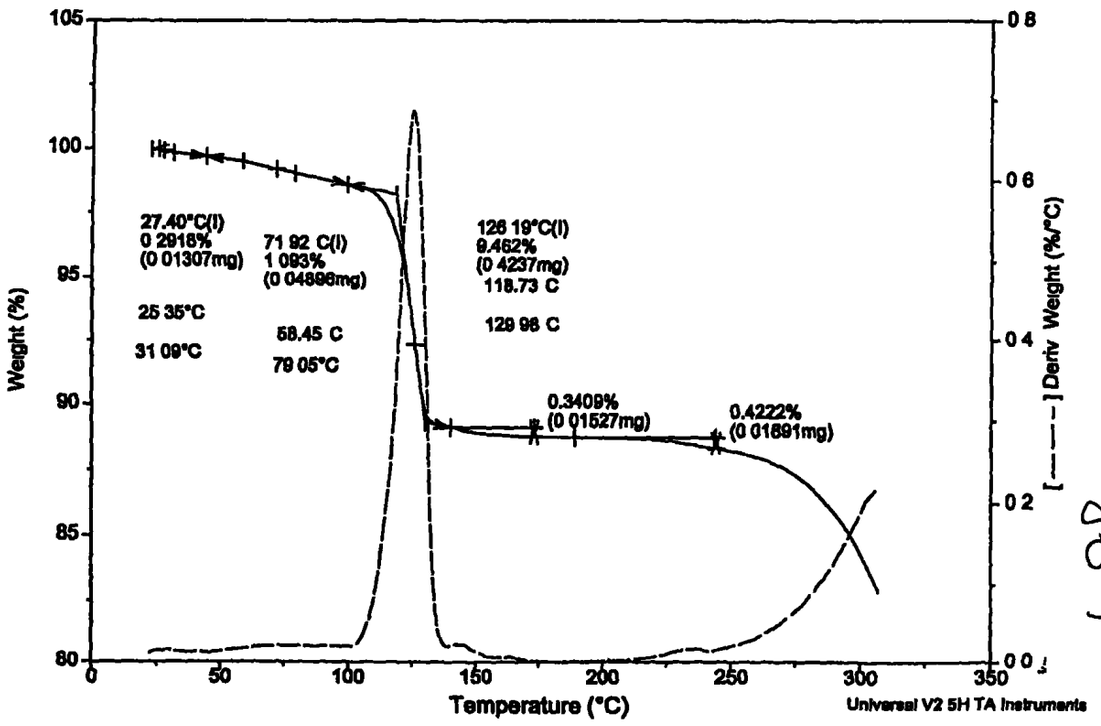


Figure 10

propylene glycol solvate

Size 2 9590 mg
Method TG 10D/M TO 300

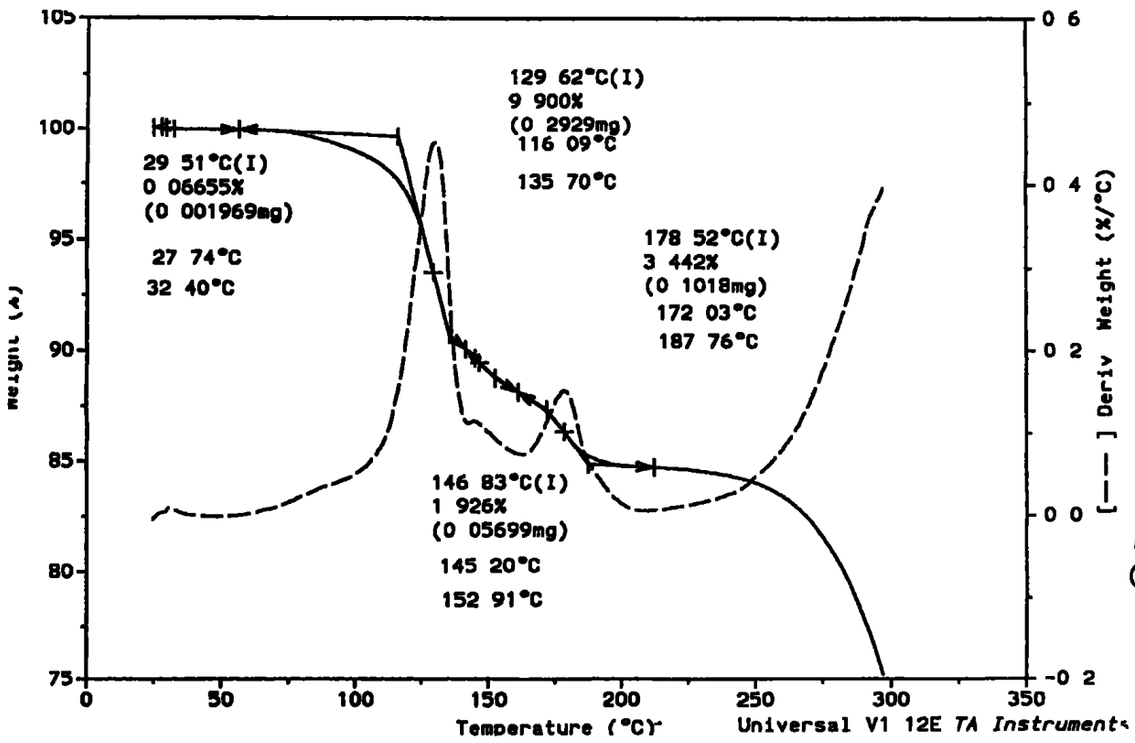


Figure [illegible]

[illegible]

n butanol solvate

Size 2 7050 mg
Method TG 10D/M TO 300

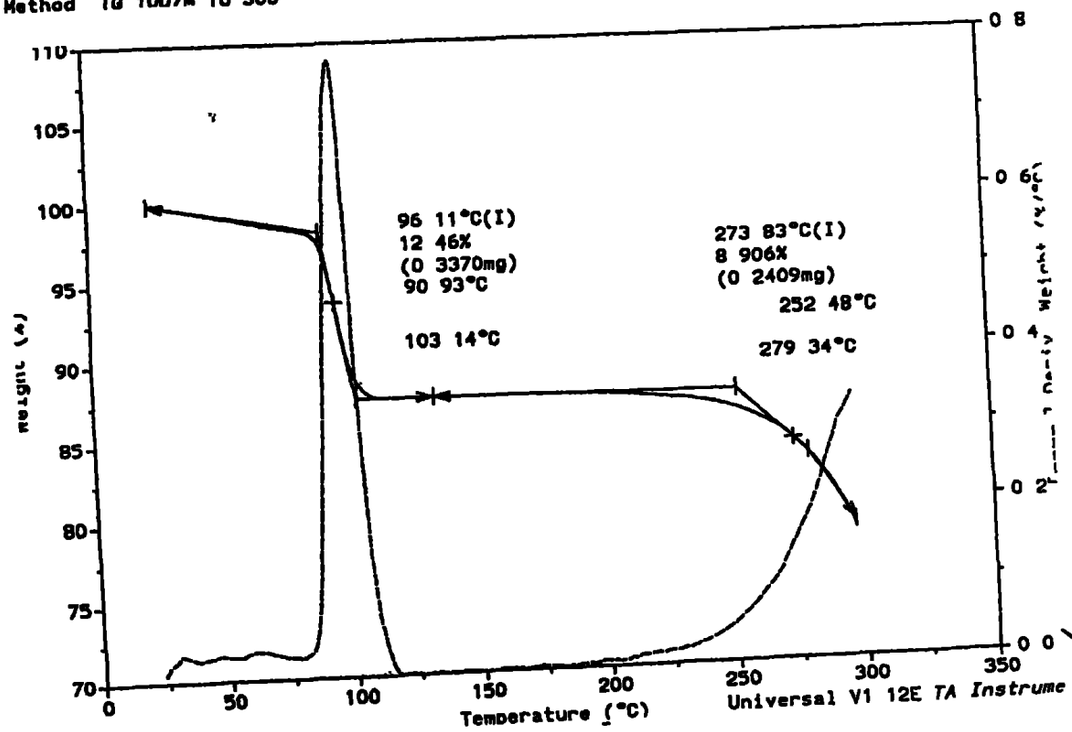


Figure 1Q

Size 2 2420 mg
Method TG 10D/M TO 300

n-octanol solvate

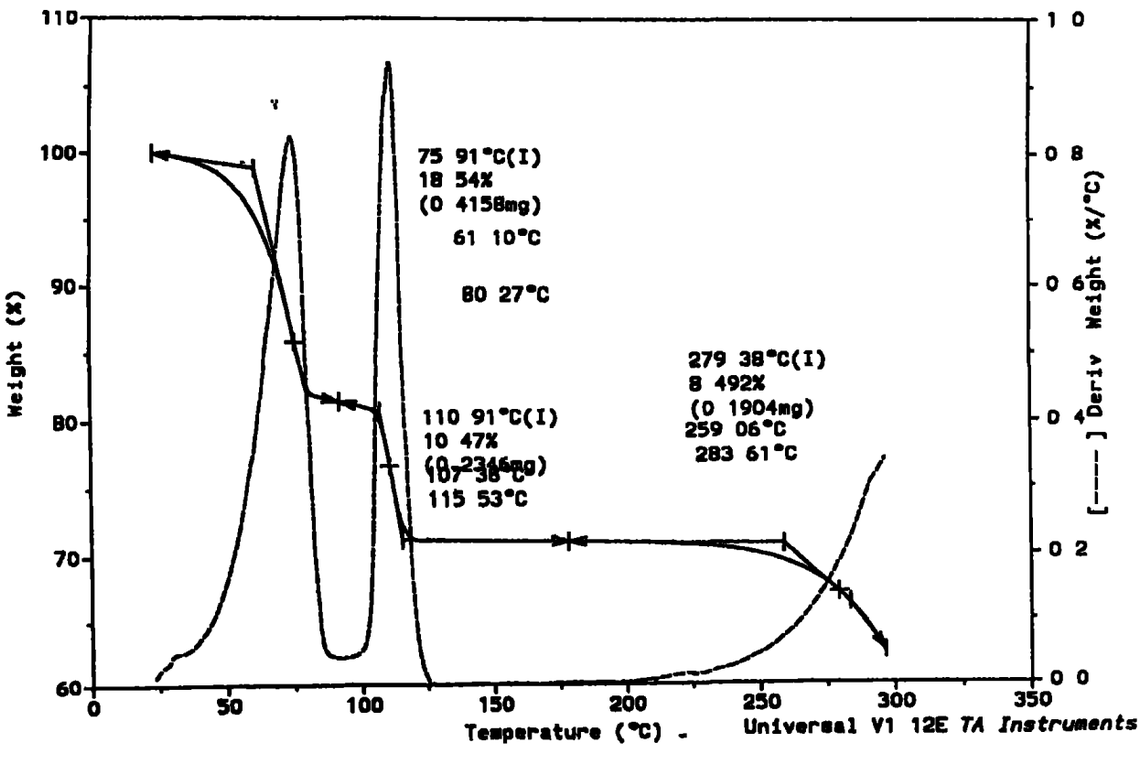
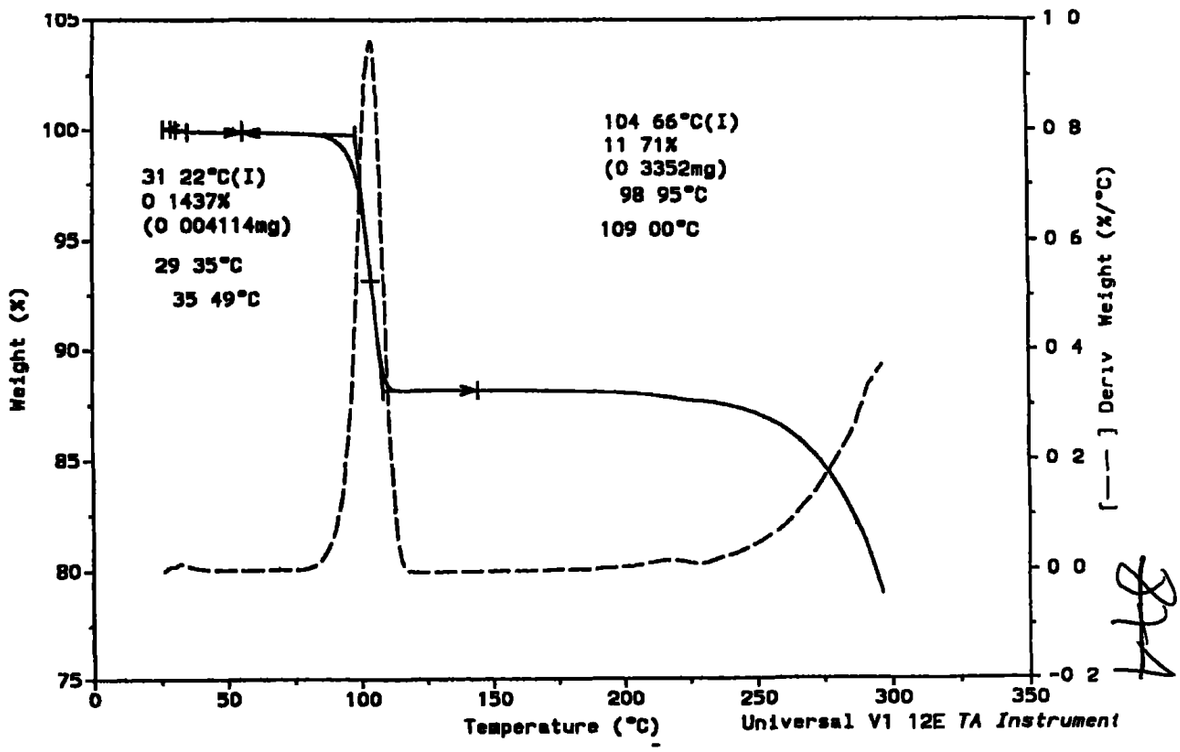


Figure [illegible]

Size 2 8630 mg
Method TG 10D/M TG 300

t-butyl acetate solvate



872
865

Figure 7

Figure 8

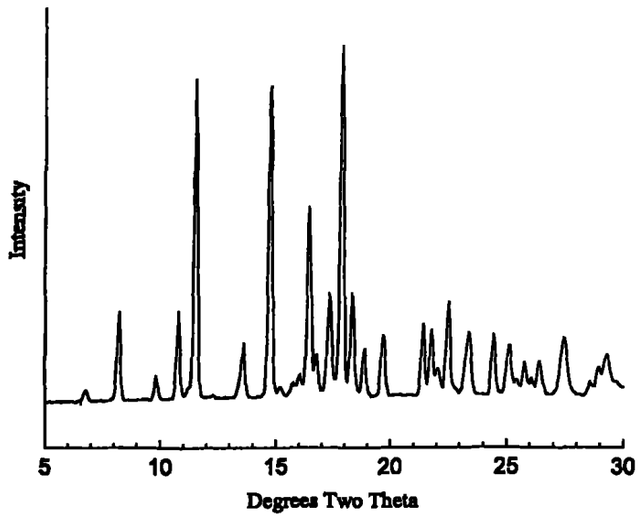


Figure 9

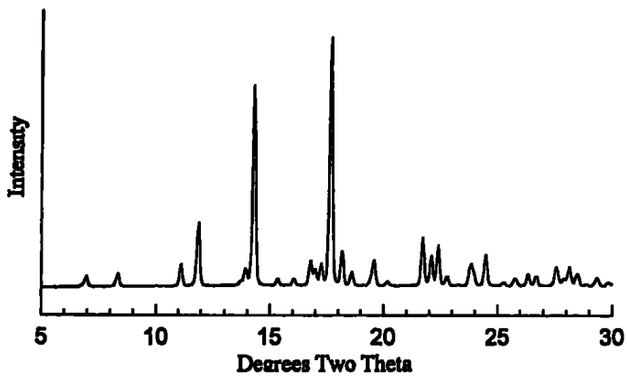


Figure 10

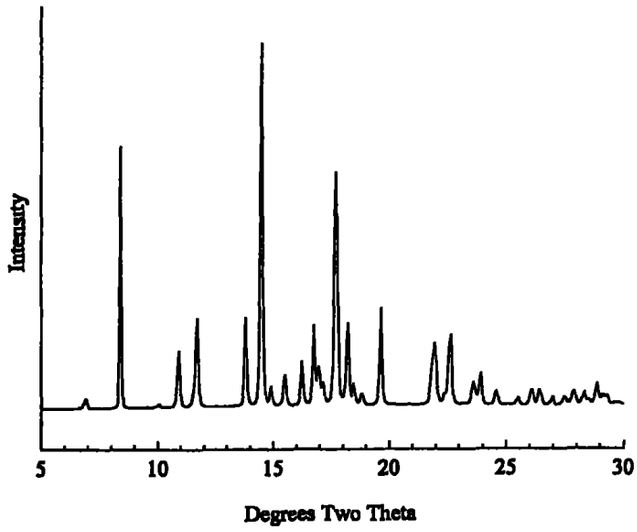


Figure 11

873

869

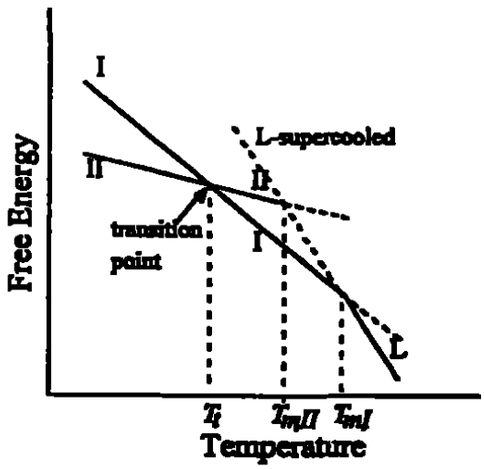


Figure 12

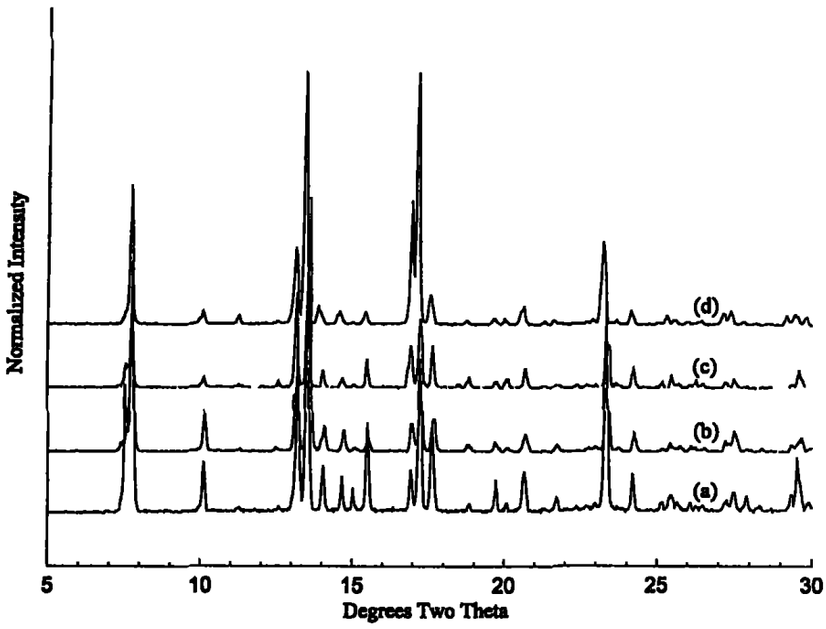


Figure 13

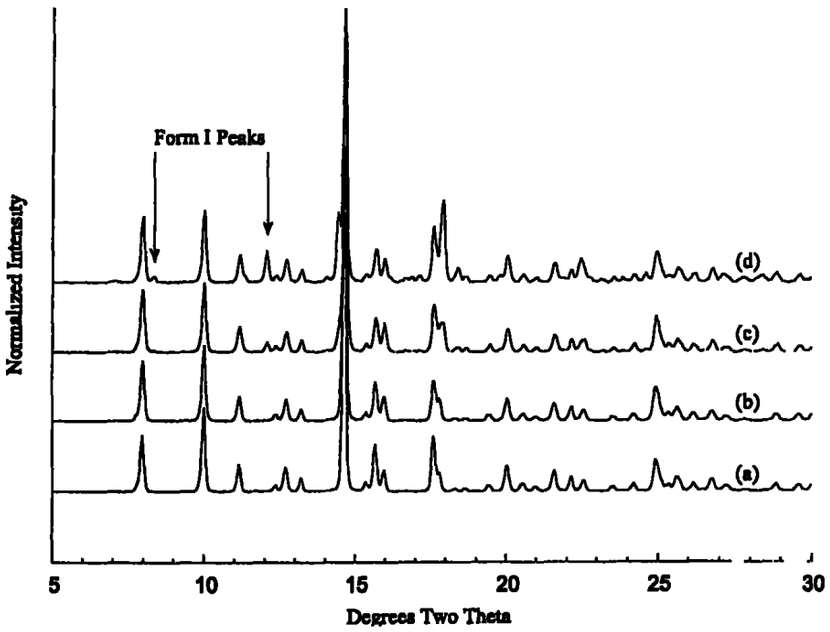

Form I Peaks
Normalized Intensity
(d)
(c)
(b)
(a)
5
10
15
20
25
30
Degrees Two Theta

Figure 14

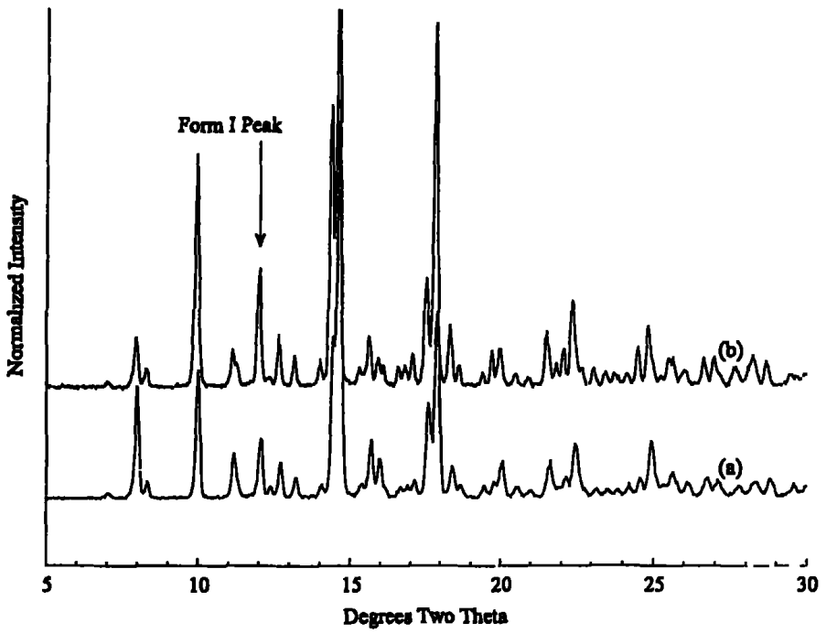

Form I Peak
Normalized Intensity
(b)
(a)
5
10
15
20
25
30
Degrees Two Theta

Figure 15

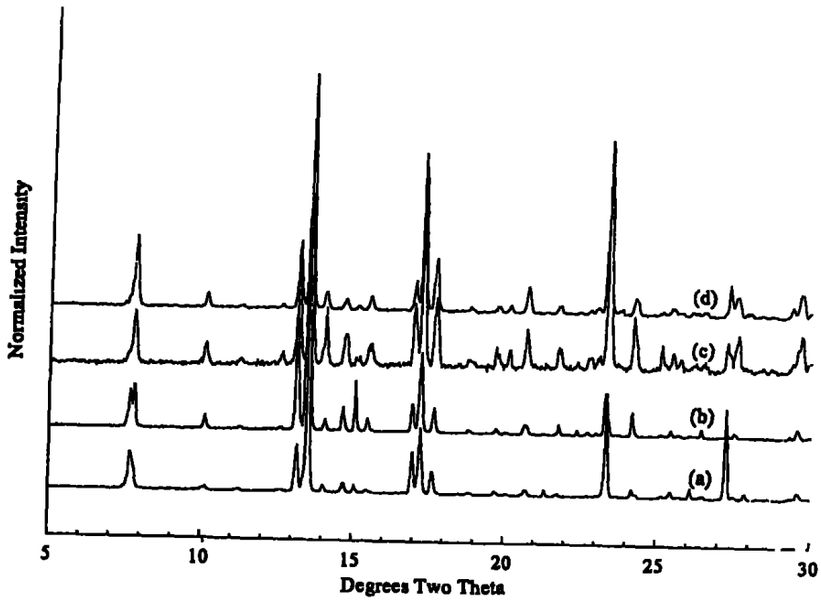


Figure 16

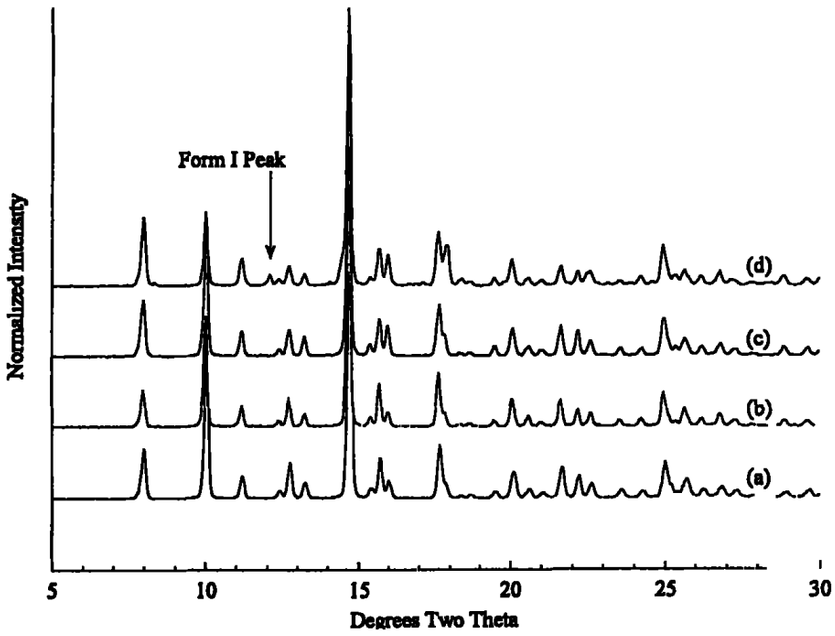

Form I Peak
Normalized Intensity
(d)
(c)
(b)
(a)
5
10
15
20
25
30
Degrees Two Theta

Figure 17

Figure 18

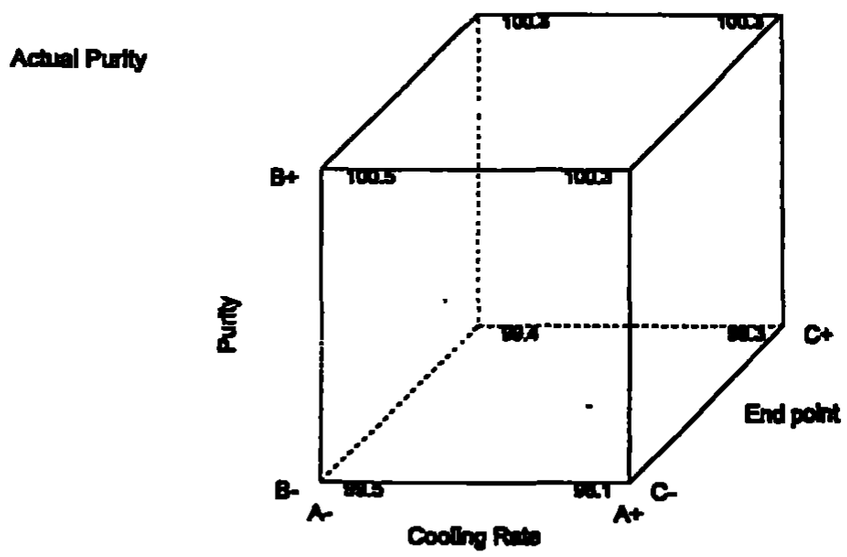

Actual Purity
100.3
100.3
B+
100.5
100.3
Purity
99.4
99.3
C+
End point
B-
98.5
98.1
C-
A-
A+
Cooling Rate

Figure 19

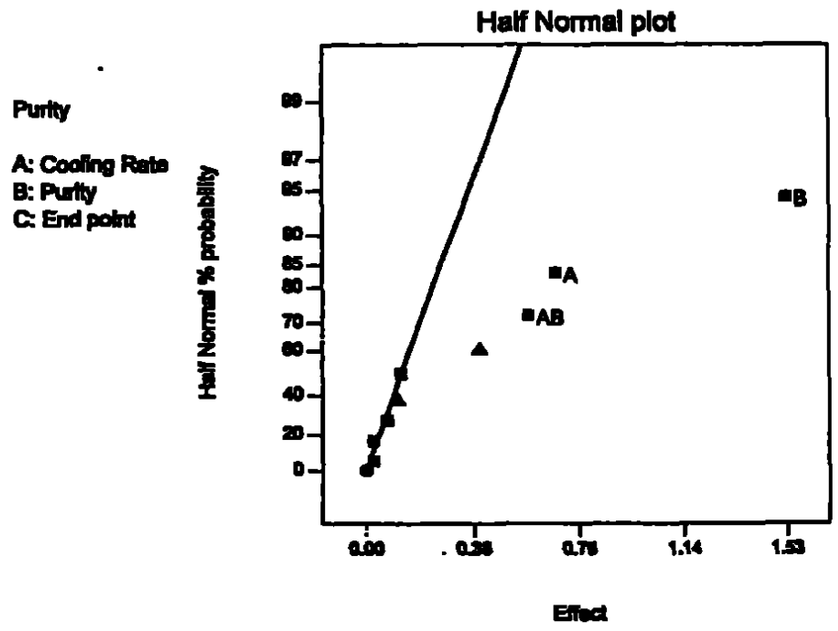

Half Normal plot
Purity
A: Cooling Rate
B: Purity
C: End point
Half Normal % probability
99
97
95
90
85
80
70
60
40
20
0
B
A
AB
0.00
0.38
0.76
1.14
1.53
Effect

Figure 20

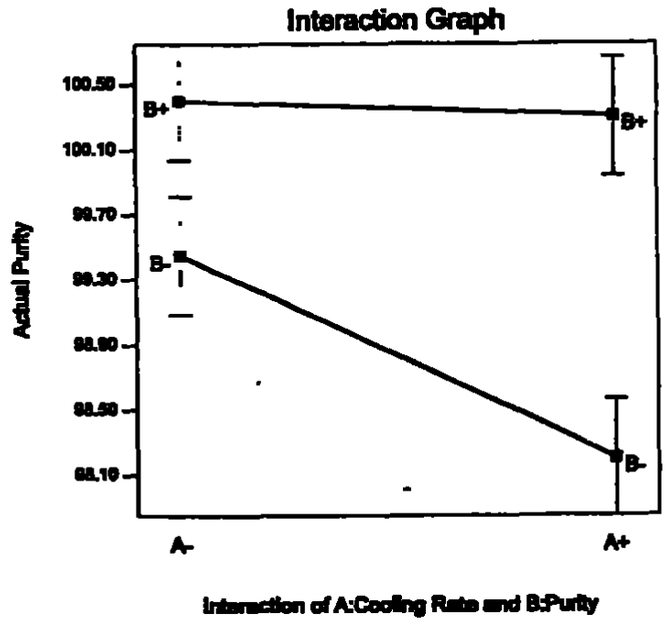

Interaction Graph
Actual Purity
100.50
100.10
99.70
99.30
98.90
98.50
98.10
B+
B-
B+
B-
A-
A+
Interaction of A:Cooling Rate and B:Purity

Figure 21

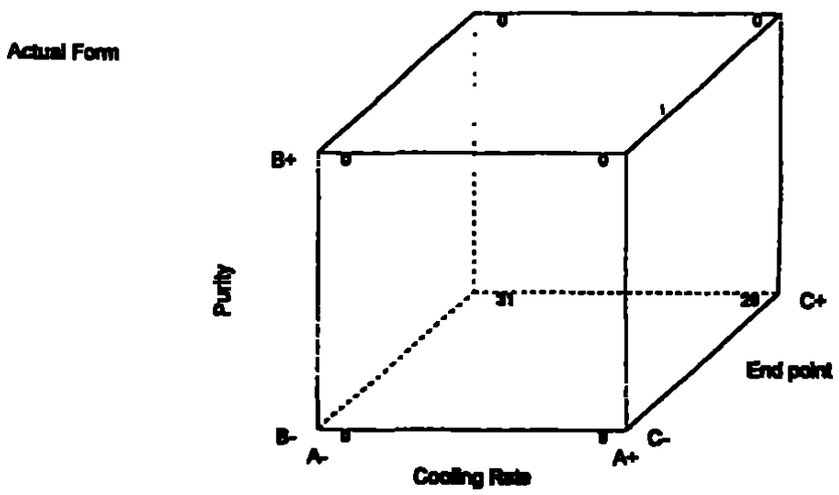

Actual Form
0
0
1
B+
0
0
Purity
31
28
C+
End point
B-
A-
0
0
C-
A+
Cooling Rate

Figure 22

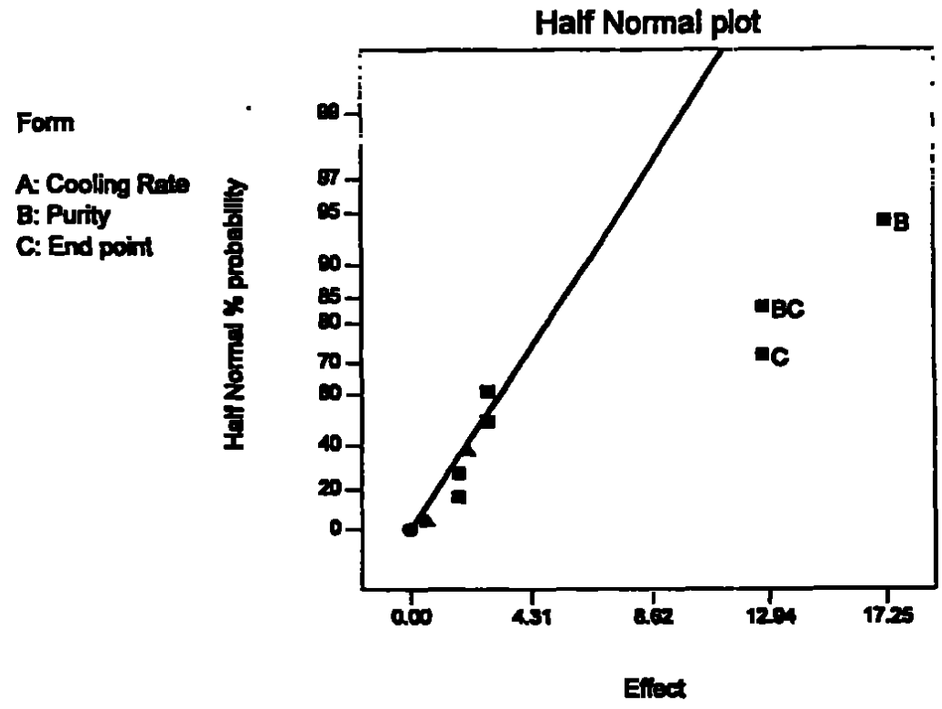

Half Normal plot
Form
A: Cooling Rate
B: Purity
C: End point
Half Normal % probability
99
97
95
90
85
80
70
60
40
20
0
B
BC
C
0.00
4.31
8.62
12.94
17.25
Effect

Figure 23

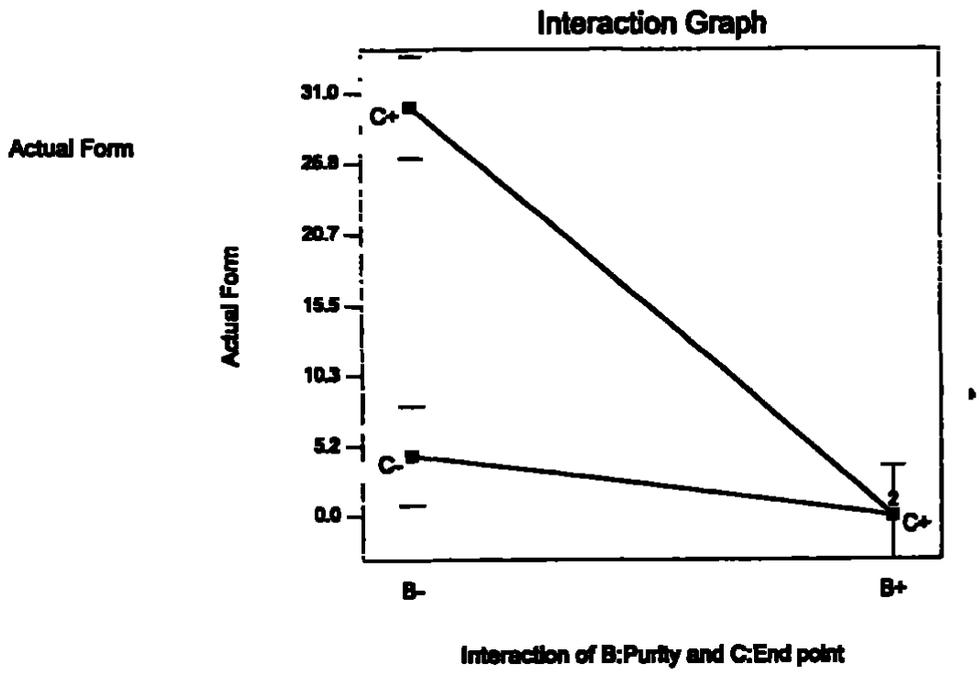
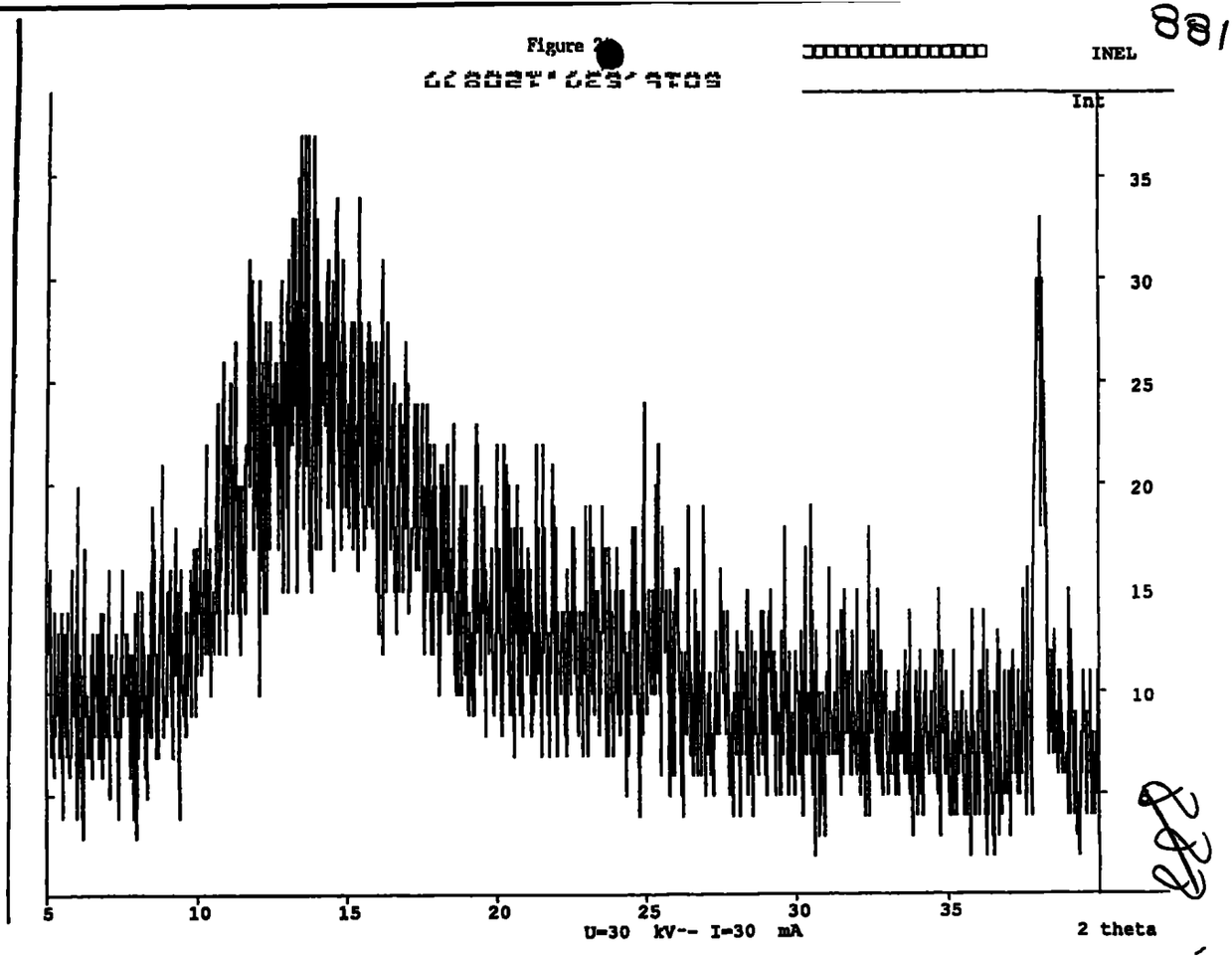
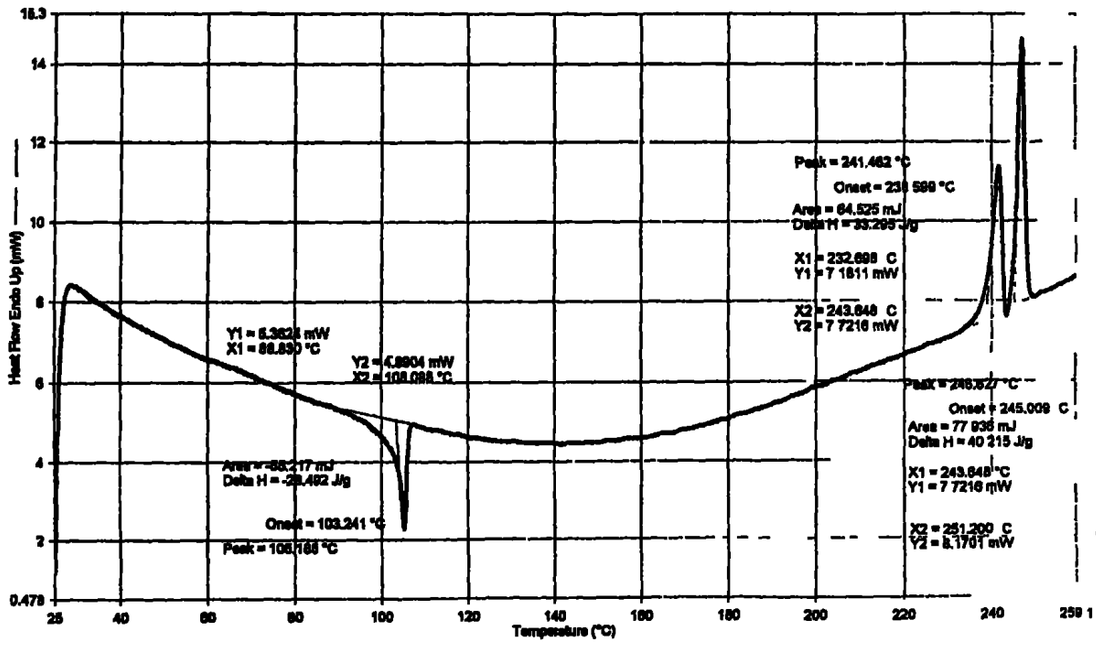

Interaction Graph
Actual Form
31.0
26.8
20.7
15.5
10.3
5.2
0.0
C+
C-
B-
B+
Interaction of B:Purity and C:End point

Figure 2



Figure

Sample Weight: 1.938 mg
Comment: Epierenone
Amorphous by freeze dry in water



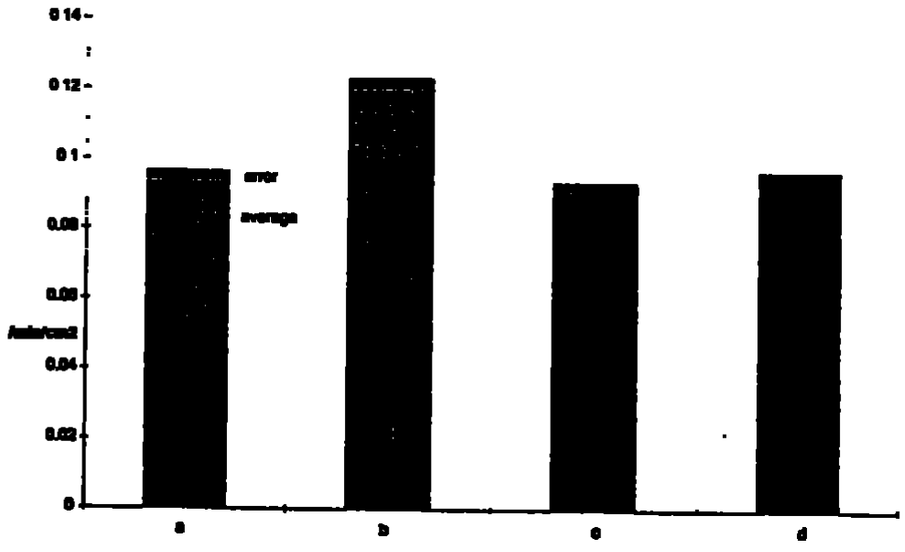


Figure 26:

Document Analysis

PR 125642

LOS ESTADOS UNIDOS DE AMERICA

A TODOS AQUELLOS A QUIENES LA PRESENTE CONCIERNE:
DEPARTAMENTO DE COMERCIO DE LOS ESTADOS UNIDOS

Oficina de Patentes y Marcas de los Estados Unidos

17 de enero de 2001

LA PRESENTE ES PARA CERTIFICAR QUE LA ANEXA A ESTA ES UNA COPIA FIEL DE LOS REGISTROS DE LA OFICINA DE PATENTES Y MARCAS DE LOS ESTADOS UNIDOS DE LOS DOCUMENTOS DE LA SOLICITUD DE PATENTE IDENTIFICADA MAS ADELANTE QUE SATISFACEN LOS REQUISITOS PARA QUE SE OTORGUE UNA FECHA DE PRIORIDAD BAJO 35 USC 111..

SOLICITUD NUMERO: 60/169,639

FECHA DE PRESENTACION: ***8 de diciembre de 1999***

Por la Autoridad del
COMISIONADO DE PATENTES Y MARCAS

Firmado por

L. EDELEN

Funcionario Certificador

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REIVINDICACIONES

1. Una combinación que comprende una primera forma de estado sólido de eplerenona y una segunda forma de estado sólido de eplerenona, donde dichas formas de estado sólido de eplerenona son seleccionadas de:

la forma cristalina de Forma I de eplerenona.

la forma cristalina de Forma II de eplerenona;

Una forma cristalina de eplerenona que comprende un solvente;

y

una eplerenona amorfa.

2. La combinación de la reivindicación 1 donde dichas formas de estado sólido de eplerenona se seleccionan de:

la forma cristalina de Forma I de eplerenona;

la forma cristalina de Forma II de eplerenona;

una forma cristalina de eplerenona que comprende un solvente, donde dicha forma no comprende metilen cloruro, etil éter o isopropanol; y

eplerenona amorfa.

3.La combinación de la reivindicación 1 donde dichas formas de estado sólido de eplerenona se seleccionan de:

la forma cristalina de Forma I de eplerenona;

la forma cristalina de Forma II de eplerenona;

una forma cristalina de eplerenona que comprende un solvente, donde dicha forma no comprende metilen cloruro, etil éter, isopropanol, metil etil cetona o etanol; y eplerenona amorfa.

4.La combinación de la reivindicación 1 donde la proporción en peso de dicha primera forma de estado sólido con relación a dicha segunda forma de estado sólido es al menos 1: 1.

5.La combinación de la reivindicación 4 donde la proporción en peso es al menos de 2:1.

6.La combinación de la reivindicación 4 donde la proporción en peso es al menos de 5:1.

7.La combinación de la reivindicación 4 donde la proporción en peso es al menos de 9:1.

8. La combinación de la reivindicación 1 donde la primera forma de estado sólido es la forma cristalina de Forma II de eplerenona, y la segunda forma de estado sólido es la forma cristalina de Forma I de eplerenona.

9.La combinación de la reivindicación 8 donde la relación en peso de dicha primera forma de estado sólido con relación a la segunda forma de estado sólido es al menos 1:9.

10.La combinación de la reivindicación 8 donde la proporción en peso es al menos de 1:1.

11.La combinación de la reivindicación 8 donde la proporción en peso es al menos de 2:1

12.La combinación de la reivindicación 8 donde la relación en peso es al menos de 5:1.

13.la combinación de la reivindicación 8 donde la proporción en peso es al menos de 9:1.

14.La combinación de la reivindicación 1 donde dicha primera forma de estado sólido y la segunda forma de estado sólido están en forma de partículas, donde al menos 90 por ciento de dichas partículas tienen un tamaño de partícula inferior a 400 micras.

15.La combinación de la reivindicación 14 donde al menos 90 por ciento de dichas partículas tienen un tamaño de partícula de 25 micras hasta 100 micras.

16.La combinación de la reivindicación 14 donde al menos 90 por ciento de dichas partículas tienen un tamaño de partículas de 50 micras hasta 150 micras.

17.La combinación de la reivindicación 14 donde al menos 90 por ciento de dichas partículas tiene un tamaño de partícula entre 75 micras hasta 125 micras.

18.La combinación de la reivindicación 14 donde al menos 90 por ciento de dichas partículas tiene un tamaño de partícula entre 40 micras hasta 100 micras.

19.La combinación de la reivindicación 14 donde al menos 90 por ciento de dichas partículas tienen un tamaño de partícula entre 30 micras hasta 50 micras.

20.Una composición farmacéutica que comprende una primera forma de estado sólido de eplerenona y una segunda forma de estado sólido de eplerenona en asociación con al menos un portador, adyuvante o diluyente farmacéuticamente aceptable;

donde dichas formas de estado sólido de eplerenona se seleccionan de:

la forma cristalina de Forma I de eplerenona;

la forma cristalina de Forma II de eplerenona;

una forma cristalina de eplerenona que comprende un solvente; y

eplerenona amorfa; y

donde dicha primera forma de estado sólido y una segunda forma de estado sólido en combinación comprenden una cantidad terapéuticamente efectiva de eplerenona.

21.La composición farmacéutica de la reivindicación 20 donde dichas formas de estado sólido de eplerenona se seleccionan de :

La forma cristalina de Forma I de eplerenona;

La forma cristalina de Forma II de eplerenona;

Una forma cristalina de eplerenona que comprende un solvente, donde dicha forma no comprende metilen cloruro etil éter o isopropanol; y eplerenona amorfa.

22.la composición farmacéutica de la reivindicación 20 donde dichas formas de estado sólido de eplerenona se seleccionan de .

La forma cristalina de Forma I de eplerenona;

La forma cristalina de Forma II de eplerenona;

Una forma cristalina de eplerenona que comprende un solvente, donde dicha forma no comprende metilen cloruro, etil éter, isopropanol; metil etil cetona o etanol; y eplerenona amorfa.

23.La composición farmacéutica de la reivindicación 20 donde la proporción en peso de dicha primera forma de estado sólido con relación a la segunda forma de estado sólido es al menos 1:9.

24. La composición farmacéutica de la reivindicación 23 donde la proporción en peso es al menos de 1:1.

25. La composición farmacéutica de la reivindicación 23 donde la proporción en peso es al menos de 9:1.

26.La composición farmacéutica de la reivindicación 20 donde la primera forma de estado sólido es la forma cristalina de Forma II de eplerenona, y la segunda forma de estado sólido es la forma cristalina de Forma I de eplerenona.

27. La composición farmacéutica de la reivindicación 26 donde la proporción en peso de dicha primera forma de estado sólido con relación a la segunda forma de estado sólido es al menos 1:9.

28. La composición farmacéutica de la reivindicación 27 donde la proporción en peso es al menos de 1:1.

29. La composición farmacéutica de la reivindicación 27 donde la proporción en peso es al menos 2:1.

30.La composición farmacéutica de la reivindicación 27 donde la proporción en peso es al menos 5:1.

31. La composición farmacéutica de la reivindicación 27 donde la proporción en peso es al menos 9:1.

32. la composición farmacéutica de la reivindicación 1 donde dicha primera forma de estado sólido y dicha segunda forma de estado sólido, están presentes cada una en forma de partículas, donde al

menos 90 por ciento de dichas partículas tienen un tamaño de partícula inferior a 400 micras.

33.La composición farmacéutica de la reivindicación 32 donde al menos 90 por ciento de dichas partículas tienen un tamaño de partícula entre 25 a 400 micras.

34. La composición farmacéutica de la reivindicación 32 donde al menos 90 por ciento de dichas partículas tienen un tamaño de partícula entre 50 a 150 micras.

35. La composición farmacéutica de la reivindicación 32 donde al menos 90 por ciento de dichas partículas tienen un tamaño de partícula entre 75 a 125 micras.

36. La composición farmacéutica de la reivindicación 32 donde al menos 90 por ciento de dichas partículas tienen un tamaño de partícula entre 40 a 100 micras.

37. La composición farmacéutica de la reivindicación 32 donde al menos 90 por ciento de dichas partículas tienen un tamaño de partícula entre30a 50 micras.

38.El método para tratar o prevenir una condición o desorden mediada por aldosterona en un sujeto, dicho método comprende administrar a un sujeto que tiene o es susceptible de dicha condición o desorden una primera forma de estado sólido de eplerenona y una segunda

forma de estado sólido de eplerenona donde dichas formas de estado sólido de eplerenona se seleccionan de:

la forma cristalina de Forma I de eplerenona;

la forma cristalina de Forma II de eplerenona;

la forma cristalina de eplerenona que comprende un solvente;

y

eplerenona amorfa; y

donde dicha primera forma de estado sólido y dicha segunda forma de estado sólido en combinación comprenden una cantidad terapéuticamente efectiva de eplerenona.

39. El método de la reivindicación 38 que comprende administrar a un sujeto que tiene o es susceptible a dicha condición o desorden una primera forma de estado sólido de eplerenona y una segunda forma de estado sólido de eplerenona en asociación con al menos un portador, adyuvante o diluyente farmacéuticamente aceptable;

donde dichas formas de estado sólido de eplerenona se seleccionan de:

La forma cristalina de Forma I de eplerenona;

La forma cristalina de Forma II de eplerenona; una forma cristalina de eplerenona que comprende un solvente;

y

Eplerenona amorfa; y

donde dicha primera forma de estado sólido y una segunda forma de estado sólido en combinación comprenden una cantidad terapéuticamente efectiva de eplerenona.

40. El método de la reivindicación 39 para ser usado en el tratamiento de hiperaldosteronismo.

41. El método de la reivindicación 39 para ser usado en tratamiento de hipertensión.

42. El método de la reivindicación 39 para ser usado en el tratamiento de fallas del corazón.

43.El método de la reivindicación 39 para ser usado en el tratamiento cirrosis de hígado.

44.El método de la reivindicación 39 para ser usado en el tratamiento de una condición seleccionada del grupo consistente de exceso de colágeno / fibrosis, hipertrofia benigna de próstata y depresión.

45.Una forma amorfa de eplerenona que tiene un patrón de difracción en polvo en rayos X sustancialmente carente de algunos picos característicos.

46.La forma de la reivindicación 45 que tiene un diferencial de escaneo calorímetrico en el termograma sustancialmente al observado en la figura 27.

47.Las partículas de la forma de la reivindicación 45 donde al menos 90 por ciento de dichas partículas tienen un tamaño de partícula inferior a 400 micras.

48. Las partículas de la forma de la reivindicación 45 donde al menos 90 por ciento de dichas partículas tienen un tamaño de partícula entre 25 a 400 micras.

49. Las partículas de la forma de la reivindicación 45 donde al menos 90 por ciento de dichas partículas tienen un tamaño de partícula entre 50 y 150 micras.

50. Las partículas de la forma de la reivindicación 45 donde al menos 90 por ciento de dichas partículas tienen un tamaño de partícula entre 75 a 125 micras.

51. Las partículas de la forma de la reivindicación 45 donde al menos 90 por ciento de dichas partículas tienen un tamaño de partícula entre 50 a 100 micras.

52. Las partículas de la forma de la reivindicación 45 donde al menos 90 por ciento de dichas partículas tienen un tamaño de partícula entre 30 a 50 micras.

53. El método para la preparación de eplerenona que comprende liofilizar una solución que comprende eplerenona.

54.El método de la reivindicación 53 donde la solución es una solución acuosa.

55.El método de preparación de eplerenona que comprende moler eplerenona sólida.

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THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

December 30, 2000

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE UNDER 35 USC 111.

APPLICATION NUMBER: ***60/169,683***
FILING DATE: ***December 08, 1999***

By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

T. Lawrence

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Certifying Officer

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

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[x] Additional inventors are being named on the 3rd separately numbered sheet(s) attached hereto.

Title of the Invention: FORM II CRYSTALLINE EPLERENONE

Correspondence Address: Customer Number 000321

The application includes:

[x] Specification - Number of Pages 102

[x] Drawings - Number of Sheets 92

[ ] Small Entity Statement

[x] Return Receipt Postcard

[ ] Other _____

Method of Payment of Filing Fee:

[X] A check in the amount of $ 150.00 is enclosed to cover the Provisional Filing Fee.

[ ] The Commissioner is hereby authorized to charge filing fees and credit Deposit Account No. 19-1345.

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

[X] No
[ ] Yes, the name of the U.S. Government agency and the Government contract number are: _____

Respectfully submitted,

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FORM II CRYSTALLINE EPLERENONE

Field of the Invention

This invention is in the field of aldosterone receptor antagonist pharmaceutical agents and specifically relates to the novel Form II crystalline form of eplerenone, methods of preparing this crystalline form of eplerenone, pharmaceutical compositions comprising this crystalline form of eplerenone, and methods for the treatment and/or prophylaxis of aldosterone-mediated conditions and/or disorders, including conditions and disorders associated with hyperaldosteronism such as hypertension.

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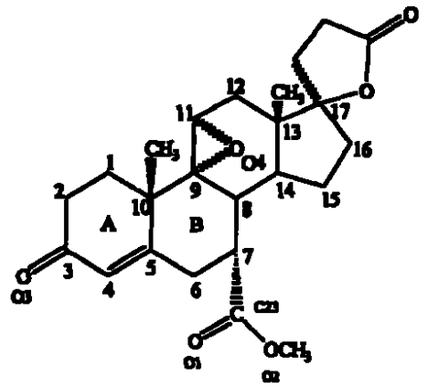
Background of the Invention

The compound methyl hydrogen 9,11-epoxy-17-hydroxy-3-oxopregn-4-ene-7,21-dicarboxylate, γ-lactone 1 (also referred to herein as "eplerenone") was first reported in Grob et al., U.S. Patent 4,559,332 which discloses a class of 9,11-epoxy steroid compounds and their salts. Eplerenone is an aldosterone receptor antagonist that can be administered in a therapeutically effective amount where use of an aldosterone receptor antagonist is indicated, such as in the treatment of pathological conditions associated with hyperaldosteronism including hypertension, heart failure including cardiac insufficiency, and cirrhosis of the liver.



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U.S. Patent 4,559,332 generally discloses the preparation of eplerenone and the preparation of pharmaceutical formulations comprising eplerenone.

Additional processes for the preparation of 9,11-epoxy steroid compounds and their salts, including eplerenone, are disclosed in Ng et al., WO97/21720 and Ng et al., WO98/25948.

Grob et al., "Steroidal Aldosterone Antagonists: Increased Selectivity of 9α,11-Epoxy Derivatives", Helvetica Chimica Acta, Vol. 80, pp. 566-585 (1997), discloses an X-ray crystal structure analysis of a solvate of eplerenone prepared by crystallizing eplerenone from a methylene chloride/diethyl ether solvent system.

De Gasparo et al., "Antialdosterones: Incidence and Prevention of Sexual Side Effects", J. Steroid Biochem., Vol. 32, No. 13, pp. 223-227 (1989), discloses the use of non-formulated eplerenone having a 20 micron particle size in a single dose study of eplerenone.

Spironolactone 2, a 20-spiroxane-steroid having activity as an aldosterone receptor antagonist, is commercially available for the treatment of hypertension. Spironolactone, however, has antiandrogenic activity that can result in gynecomastia and impotence in men. It also has weak progestational activity that can produce menstrual irregularities in women. Accordingly, there is interest in the development of additional active aldosterone receptor antagonists such as eplerenone that do not interact with other steroid receptor systems such as glucocorticoid, progestin and androgen steroid receptor systems and/or that provide for a broader range of treatment.

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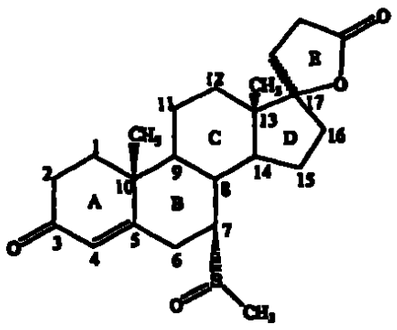

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Agafonov et al., "Polymorphism of Spironolactone", J. Pharm. Sciences, Vol. 80, No. 2, pp 181-185 (1991), discloses an acetonitrile solvate, an ethanol solvate, an ethyl acetate solvate, a methanol solvate and two non-solvated polymorphic crystalline forms of spironolactone. Brittan, Polymorphism in Pharmaceutical Solids, pp. 114-116, 207, 235, and 261 (Marcel Dekker, Inc. 1999), likewise discloses these solid state forms of spironolactone.

Summary of the Invention

A first aspect of the invention comprises the novel Form II crystalline form of eplerenone. This crystalline form possesses activity as an aldosterone receptor antagonist and has unique properties relative to other solid state forms of eplerenone. In particular, the Form II crystalline form has greater physical stability than the Form I crystalline form at typical storage temperatures.

A second aspect of the invention comprises particles of the novel Form II crystalline form of eplerenone wherein at least 90% of the particles have a particle size less than about 400 microns.

A third aspect of the invention comprises substantially phase pure Form II eplerenone.

A fourth aspect of the invention comprises pharmaceutical compositions comprising Form II eplerenone.

A fifth aspect of the invention comprises a method for the prophylaxis and/or treatment of an aldosterone-mediated condition and/or disorder comprising administering to a subject a therapeutically-effective amount of Form II eplerenone.

A sixth aspect of the invention comprises methods for the preparation of Form II eplerenone.

Additional aspects of the invention are discussed throughout the specification of this application.

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Brief Description of the Drawings

Fig. 1-A shows X-ray powder diffraction patterns of Form I eplerenone.

Fig. 1-B shows X-ray powder diffraction patterns of Form II eplerenone.

Fig. 1-C shows X-ray powder diffraction patterns of the methyl ethyl ketone solvate of eplerenone.

Figs. 1-D through 1-O show X-ray powder diffraction patterns for the following eplerenone solvates: n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, acetone solvate, toluene solvate, isopropanol solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, methyl acetate solvate, and propyl acetate solvate, respectively.

Fig. 2-A shows a differential scanning calorimetry (DSC) thermogram of non-milled Form II directly crystallized from methyl ethyl ketone.

Fig. 2-B shows a differential scanning calorimetry (DSC) thermogram of non-milled Form II prepared by desolvation of a solvate obtained by crystallization of a high purity eplerenone from methyl ethyl ketone.

Fig. 2-C shows a differential scanning calorimetry (DSC) thermogram of Form II prepared by crystallizing a solvate from a solution of high purity eplerenone in methyl ethyl ketone, desolvating the solvate to yield Form II, and milling the resulting Form II.

Fig. 2-D shows a differential scanning calorimetry (DSC) thermogram of non-milled Form I prepared by desolvation of a solvate obtained by digestion of low purity eplerenone from appropriate solvents.

Figs. 2-E through 2-T show DSC thermograms for the following eplerenone solvates: n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, chloroform solvate, acetone solvate, toluene solvate, isopropanol solvate, t-butyl acetate solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, methyl acetate solvate, propyl acetate solvate, n-butanol solvate, and n-octanol solvate, respectively.

Fig. 3-A shows the infrared spectra (diffuse reflectance, DRIFTS) of Form I eplerenone.

Fig. 3-B shows the infrared spectra (diffuse reflectance, DRIFTS) of Form II eplerenone.

Fig. 3-C shows the infrared spectra (diffuse reflectance, DRIFTS) of the methyl ethyl ketone solvate of eplerenone.

Fig. 3-D shows the infrared spectra (diffuse reflectance, DRIFTS) of eplerenone in chloroform solution.

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Figs. 3-E through 3-Q show infrared absorption spectra for the following eplerenone solvates: n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, acetone solvate, toluene solvate, isopropanol solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, propyl acetate solvate, methyl acetate solvate, propylene glycol solvate, and t-butyl acetate solvate respectively.

Fig. 4 shows ^{13}C NMR spectra for Form I of eplerenone.

Fig. 5 shows ^{13}C NMR spectra for Form II of eplerenone.

Figs. 6-A through 6-R show thermogravimetry analysis profiles for the following eplerenone solvates: methyl ethyl ketone solvate, n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, chloroform solvate, acetone solvate, toluene solvate, isopropanol solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, methyl acetate solvate, propyl acetate solvate, propylene glycol solvate, n-butanol solvate, n-octanol solvate, and t-butyl acetate solvate, respectively.

Fig. 7 is a scanning electron micrograph of Form II eplerenone prepared by desolvation of the methyl ethyl ketone solvate.

Fig. 8 is a scanning electron micrograph of Form II eplerenone prepared by direct crystallization from ethyl acetate.

Fig. 9 shows an X-ray powder diffraction pattern of a crystalline form of 7-methyl hydrogen 4α,5α:9α,11α-diepoxy-17-hydroxy-3-oxo-17α-pregnane-7α,21-dicarboxylate, γ-lactone isolated from methyl ethyl ketone.

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Fig. 10 shows an X-ray powder diffraction pattern of the crystalline form of 7-methyl hydrogen 11α,12α-epoxy-17-hydroxy-3-oxo-17α-pregn-4-ene-7α,21-dicarboxylate, γ-lactone isolated from isopropanol.

Fig. 11 shows an X-ray powder diffraction pattern of the crystalline form of 7-methyl hydrogen 17-hydroxy-3-oxo-17α-pregna-4,9(11)-diene-7α,21-dicarboxylate, γ-lactone isolated from n-butanol.

Fig. 12 illustrates the Gibbs free energy-temperature relationship for enantiotropically related polymorphs.

Fig. 13 shows the X-ray powder diffraction patterns for the wet cake (methyl ethyl ketone solvate) obtained from (a) 0%, (b) 1%, (c) 3%, and (d) 5% diepoxide-doped methyl ethyl ketone crystallizations.

Fig. 14 shows the X-ray powder diffraction patterns for the dried solids obtained from (a) 0%, (b) 1%, (c) 3%, and (d) 5% diepoxide-doped methyl ethyl ketone crystallizations.

Fig. 15 shows the X-ray powder diffraction patterns for the dried solids from the methyl ethyl ketone crystallization with 3% doping of diepoxide (a) without grinding of the solvate prior to drying, and (b) with grinding of the solvate prior to drying.

Fig. 16 shows the X-ray powder diffraction patterns for the wet cake (methyl ethyl ketone solvate) obtained from (a) 0%, (b) 1%, (c) 5%, and (d) 10% 11,12-epoxide-doped methyl ethyl ketone crystallizations.

Fig. 17 shows the X-ray powder diffraction patterns for the dried solids obtained from (a) 0%, (b) 1%, (c) 5%, and (d) 10% 11,12-epoxide-doped methyl ethyl ketone crystallizations.

Fig. 18 shows a cube plot of product purity, starting material purity, cooling rate and endpoint temperature based on the data reported in Table X-7A.

Fig. 19 shows a half normal plot prepared using the cube plot of Fig. 18 to determine those variables having a statistically significant effect on the purity of the final material.

Fig. 20 is an interaction graph based on the results reported in Table X-7A showing the interaction between starting material purity and cooling rate on final material purity.

Fig. 21 shows a cube plot of Form I weight fraction, starting material purity, cooling rate and endpoint temperature based on the data reported in Table X-7A.

Fig. 22 shows a half normal plot prepared using the cube plot of Fig. 21 to determine those variables having a statistically significant effect on the purity of the final material.

Fig. 23 is an interaction graph based on the results reported in Table X-7A showing the interaction between starting material purity and endpoint temperature on final material purity.

Fig. 24 shows an X-ray diffraction pattern of amorphous eplerenone.

Fig. 25 shows a DSC thermogram of amorphous eplerenone.

Fig. 26 shows the dissolution rates measured for four polymorph samples.

Detailed Description of the Preferred Embodiments

As with all pharmaceutical compounds and compositions, the chemical and physical properties of eplerenone are

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important in its commercial development. These properties include, but are not limited to: (1) packing properties such as molar volume, density and hygroscopicity, (2) thermodynamic properties such as melting temperature, vapor pressure and solubility, (3) kinetic properties such as dissolution rate and stability (including stability at ambient conditions, especially to moisture and under storage conditions), (4) surface properties such as surface area, wettability, interfacial tension and shape, (5) mechanical properties such as hardness, tensile strength, compactibility, handling, flow and blend; and (6) filtration properties. These properties can affect, for example, the processing and storage of pharmaceutical compositions comprising eplerenone. Solid state forms of eplerenone that provide an improvement in one or more of these properties relative to other solid state forms of eplerenone are desirable.

The present invention comprises such novel solid state forms of eplerenone. Specifically, these novel solid state forms include solvated crystalline forms, non-solvated and non-hydrated crystalline forms (designated "Form I" and "Form II"), and an amorphous form of eplerenone. Each solid state form of eplerenone described in the present application possesses one or more of the above-described advantageous chemical and/or physical properties relative to the other solid state forms described herein or otherwise disclosed in the literature.

In one embodiment of the invention, the solid state form comprises Form I eplerenone. Form I exhibits a faster dissolution rate (approximately 30% higher) at lower temperatures (i.e., temperatures below the enantiotropic

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transition temperature as later discussed) than, for example, Form II. Where dissolution of eplerenone in the gastrointestinal tract is the rate-controlling step for delivery of the eplerenone to the target cells, faster dissolution generally results in improved bioavailability. Form I, therefore, can provide an improved bioavailability profile relative to Form II. In addition, selection of a solid state form of eplerenone having a faster dissolution rate likewise provides greater flexibility in the selection of excipients for, and in the formulation of, immediate release pharmaceutical compositions relative to other solid state forms having a slower dissolution rate.

In another embodiment of the invention, the solid state form comprises Form II eplerenone. Form II possesses greater physical stability at lower temperatures (i.e., at temperatures below the enantiotropic transition temperature as later discussed) than, for example, Form I. Solid state forms of eplerenone such as Form II that do not require the use of special processing or storage conditions, and that avoid the need for frequent inventory replacement, are desirable. For example, selection of a solid state form of eplerenone that is physically stable during the manufacturing process (such as during milling of eplerenone to obtain a material with reduced particle size and increased surface area) can avoid the need for special processing conditions and the increased costs generally associated with such special processing conditions. Similarly, selection of a solid state form of eplerenone that is physically stable at different conditions of storage (especially considering the different possible storage conditions during the lifetime of the eplerenone product) can help avoid polymorphic or other

degradative changes in the eplerenone that can lead to product loss or deterioration of product efficacy. Therefore, the selection of a solid state form of eplerenone such as Form II having greater physical stability provides a meaningful benefit over less stable eplerenone forms.

In another embodiment of the invention, the solid state form of eplerenone comprises a solvated crystalline form of eplerenone. Such solvated crystalline forms are useful as intermediates in the preparation of Form I and Form II eplerenone. An additional benefit from the use of a solvated crystalline form as an intermediate is the "intrinsic micronizing" of the crystal that results upon desolvation as discussed later in this application. Such "intrinsic micronizing" can reduce or eliminate milling requirements. Further, where additional milling is still required, it is easier to mill certain solvates before the desolvation step than to mill Form I or Form II after desolvation of the solvate.

Pharmaceutically acceptable solvated crystalline forms also can be used directly in pharmaceutical compositions. These solvated crystalline forms preferably do not comprise methylene chloride, isopropanol or ethyl ether; and, in yet another embodiment, do not comprise methylene chloride, isopropanol, ethyl ether, methyl ethyl ketone or ethanol; and, in yet another embodiment, do not comprise methylene chloride, isopropanol, ethyl ether, methyl ethyl ketone, ethanol, ethyl acetate or acetone. More preferably, the solvated crystalline forms are substantially exclusive of solvents that are not pharmaceutically-acceptable solvents. Because Form I and Form II typically are more physically stable than the crystalline solvates at room temperature and under atmospheric pressure,

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the solvated crystalline forms used in such compositions generally comprise a pharmaceutically acceptable higher boiling and/or hydrogen bonding solvent such as, but not limited to, butanol. It is believed that the solvated crystalline forms collectively can offer a range of different dissolution rates and, where dissolution of eplerenone in the gastrointestinal tract is the rate-controlling step for delivery of the eplerenone to the target cells, bioavailabilities relative to Form I and Form II.

In another embodiment of the invention, the solid state form of eplerenone comprises an amorphous form of eplerenone. Amorphous eplerenone is useful as an intermediate in the preparation of Form I and Form II eplerenone. In addition, it is believed that amorphous eplerenone possesses a different dissolution rate and, where dissolution of eplerenone in the gastrointestinal tract is the rate-controlling step for delivery of the eplerenone to the target cells, bioavailability relative to Form I and Form II.

In another embodiment, the invention comprises combinations of solid state eplerenone forms selected from the group consisting of Form I eplerenone, Form II eplerenone, solvated crystalline eplerenone and amorphous eplerenone. Such combinations are useful, for example, in the preparation of pharmaceutical compositions having differing dissolution profiles, including controlled release compositions.

In addition, depending upon the intended use of the solid state form of eplerenone, processing considerations may favor the selection of a specific solid state form of eplerenone or a specific combination of such solid state forms. Pure phase Form II, for example, generally is more easily prepared than

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pure phase Form I. A mixture of Form I and Form II, however, generally is more easily prepared than phase pure Form II and allows for the use of a low purity eplerenone starting material. Use of a solvated crystalline form instead of Form I or Form II in a composition eliminates a processing step, desolvation, for those processes that otherwise would proceed by desolvation of the solvated crystalline form. Alternatively, the desolvation step can be eliminated, for example, if Form II is directly crystallized from an appropriate solvent without the intervening preparation and desolvation of the intermediate solvated crystalline form. These processes are described in greater detail later in the application.

Although each of the above solid state eplerenone embodiments can embrace a broad range of eplerenone particle sizes, it has been discovered that reduction of the particle size of the solid state form of eplerenone to a D_{90} particle size (later defined herein) between about 25 microns to about 400 microns can improve the bioavailability of unformulated eplerenone and pharmaceutical compositions comprising that solid state form of eplerenone. Accordingly, the D_{90} particle size of the unformulated eplerenone or the eplerenone used as a starting material in the pharmaceutical composition generally is less than about 400 microns, preferably less than about 200 microns, more preferably less than about 150 microns, still more preferably less than about 100 microns, and still more preferably less than about 90 microns. In one embodiment, the D_{90} particle size is between about 40 microns to about 100 microns. In another embodiment, the D_{90} particle size is between about 30 microns to about 50 microns. In another embodiment, the D_{90} particle size is between about 50

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microns to about 150 microns. In another embodiment, the D_{90} particle size is between about 75 microns to about 125 microns.

Definitions

The term "amorphous" as applied to eplerenone refers to a solid state wherein the eplerenone molecules are present in a disordered arrangement and do not form a distinguishable crystal lattice or unit cell. When subjected to X-ray powder diffraction, amorphous eplerenone does not produce any characteristic crystalline peaks.

Where reference is made in this application to the "boiling point" of a substance or solution, the term "boiling point" means the boiling point of the substance or solution under the applicable process conditions.

The term "crystalline form" as applied to eplerenone refers to a solid state form wherein the eplarenone molecules are arranged to form a distinguishable crystal lattice (i) comprising distinguishable unit cells, and (ii) yielding diffraction peaks when subjected to X-ray radiation.

The term "crystallization" as used throughout this application can refer to crystallization and/or recrystallization depending upon the applicable circumstances relating to the preparation of the eplerenone starting material.

The term "digestion" means a process in which a slurry of solid epleranone in a solvent or mixture of solvents is heated at the boiling point of the solvent or mixture of solvents under the applicable process conditions.

The term "direct crystallization" as used herein refers to the crystallization of eplerenone directly from a suitable

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solvent without the formation and desolvation of an intermediate solvated crystalline solid state form of eplerenone.

The term "particle size" as used herein refers to particle size as measured by conventional particle size measuring techniques well known in the art, such as laser light scattering, sedimentation field flow fractionation, photon correlation spectroscopy, or disk centrifugation. The term "D_{90} particle size" means the particle size of at least 90% of the particles as measured by such conventional particle size measuring techniques.

The term "DSC" means differential scanning calorimetry.

The term "HPLC" means high pressure liquid chromatography.

The term "IR" means infra red.

The term "min" means minute.

The term "psi" means pounds per square inch absolute.

The term "purity" means the chemical purity of eplerenone according to conventional HPLC assay. As used herein, "low purity eplerenone" generally means eplerenone that contains an effective amount of a Form I growth promoter and/or a Form II growth inhibitor. As used herein, "high purity eplerenone" generally means eplerenone that does not contain, or contains less than an effective amount of, a Form I growth promoter and/or a Form II growth inhibitor.

The term "phase purity" means the solid state purity of eplerenone with regard to a particular crystalline or amorphous form of the eplerenone as determined by the infrared spectroscopy analytical methods described herein.

The term "XPRD" means X-ray powder diffraction.

The term "rpm" means revolutions per minute.

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The term "TGA" means thermogravimetric analysis.

The term "T_m" means melting temperature.

Characterization of Crystalline Form

1. Molecular Conformation

Single crystal X-ray analysis indicates that the eplerenone molecular conformation differs between Form I and Form II, particularly with respect to the orientation of the ester group at the 7-position of the steroid ring. The orientation of the ester group can be defined by the C8-C7-C23-02 torsion angle.

In the Form I crystal lattice, the eplerenone molecule adopts a conformation in which the methoxy group of the ester is approximately aligned with the C-H bond at the 7-position and the carbonyl group is approximately positioned over the center of the B-steroid ring. The C8-C7-C23-02 torsion angle is approximately -73.0° in this conformation. In this orientation, the carbonyl oxygen atom of the ester group (01) is in close contact with the oxygen atom of the 9,11-epoxide ring (04). The 01-04 distance is about 2.97 Å, which is just below the van der Waal's contact distance of 3.0 Å (assuming van der Waal's radii of 1.5Å for the oxygen).

In the Form II crystal lattice, the eplerenone molecule adopts a conformation in which the ester group is rotated approximately 150° relative to that of Form I and has a C8-C7-C23-02 torsion angle of approximately +76.9°. In this orientation, the methoxy group of the ester is directed toward the 4,5-alkene segment of the A-steroid ring. In this orientation, the distance between either oxygen atom of the ester group (01,02) and the oxygen atom of the 9,11-epoxide

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ring is increased relative to the distance determined for Form I. The 02-04 distance is approximately 3.04Å, falling just above the van der Waal's contact distance. The 01-04 distance is about 3.45Å.

The eplerenone molecule appears to adopt a conformation characteristic of Form II in the solvated crystalline forms analyzed by single crystal X-ray diffraction to date.

2. X-Ray Powder Diffraction

The various crystalline forms of eplerenone were analyzed with either a Siemens D5000 powder diffractometer or an Inel Multipurpose Diffractometer. For the Siemens D500 powder diffractometer, the raw data was measured for 2q values from 2 to 50, with steps of 0.020 and step periods of two seconds. For the Inel Multipurpose Diffractometer, samples were placed in an aluminum sample holder and raw data was collected for 30 minutes at all two theta values simultaneously.

Tables 1A, 1B and 1C set out the significant parameters of the main peaks in terms of 2q values and intensities for the Form I (prepared by desolvation of the ethanol solvate obtained by digestion of low purity eplerenone), Form II (prepared by desolvation of the methyl ethyl ketone solvate obtained by recrystallization of high purity eplerenone), and methyl ethyl ketone solvate (prepared by room temperature slurry conversion of high purity eplerenone in methyl ethyl ketone) crystalline forms of eplerenone, respectively (X-ray radiation at a wavelength of 1.54056 Angstroms).

Minor shifts in peak positioning may be present in the diffraction patterns of Form I and Form II as a result of imperfections in the spacing of the crystal diffraction planes

due to the route of manufacture of Form I and Form II (i.e. desolvation of a solvate). In addition, Form I is isolated from a solvate prepared by digestion of crude eplerenone. This method results in a lower overall chemical purity (approximately 90%) of the Form I. Finally, the solvated forms of eplerenone are expected to show some shifting in the positioning of the diffraction peaks due to the increased mobility of the solvent molecules within the solvent channels in the crystal lattice.

TABLE 1A: FORM I DATA

Angle 2-theta	d-spacing Angstrom	Intensity	Intensity %
6.994	12.628	1166	7.2
8.291	10.655	2137	13
10.012	8.827	577	3.5
11.264	7.849	1854	11.3
12.04	7.344	7707	46.8
14.116	6.269	3121	19
14.438	6.13	16336	99.2
15.524	5.703	637	3.9
16.189	5.477	1349	8.2
16.699	5.305	1663	10.1
16.94	5.23	1692	10.3
17.147	5.167	2139	13
17.66	5.018	6883	41.8
17.91	4.949	16456	100
18.379	4.823	3108	18.9
18.658	4.752	1216	7.4
19.799	4.48	1496	9.1
20.235	4.385	383	2.3
21.707	4.091	1267	7.7
21.8	4.073	1260	7.7
21.959	4.044	1279	7.8
22.461	3.956	4264	25.9
23.191	3.832	1028	6.2
23.879	3.723	1000	6.1
24.599	3.616	1688	10.3
25.837	3.445	931	5.7
26.034	3.42	688	4.2
26.868	3.316	912	5.5
27.093	3.288	1322	8
27.782	3.209	1236	7.5
28.34	3.147	1845	11.2
28.861	3.091	957	5.8
29.866	2.9892	746	4.5
30.827	2.9166	992	6
31.108	2.8728	1205	7.3
33.215	2.8951	1287	7.8
33.718	2.656	802	4.9
34.434	2.6024	914	5.6

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TABLE 1B: FORM II DATA

Angle 2-Theta	d-spacing Angstrom	Intensity Cps	Intensity %
7.992	11.054	11596	26.6
10.044	8.799	12048	27.6
11.208	7.889	4929	11.3
12.441	7.109	1747	4
12.752	6.936	4340	9.9
13.257	6.673	2444	5.6
14.705	6.019	43646	100
15.48	5.727	2670	6.1
15.727	5.63	7982	18.3
16.016	5.529	3519	8.1
17.871	5.015	8897	20.4
17.9	4.951	2873	6.6
18.352	4.83	612	1.4
18.703	4.74	669	1.6
19.524	4.543	1128	2.6
20.103	4.413	3753	8.6
20.63	4.302	1451	3.3
21.067	4.214	876	2
21.675	4.097	2760	6.3
22.232	3.995	1951	4.5
22.652	3.922	1657	3.8
23.624	3.763	827	1.9
24.279	3.663	1242	2.8
25.021	3.556	5144	11.8
25.486	3.492	1702	3.9
25.707	3.463	2493	5.7
26.251	3.392	1371	3.1
26.85	3.318	1970	4.5
27.319	3.262	1029	2.4
27.931	3.192	440	1
27.969	3.187	440	1
28.937	3.083	1128	2.6
29.703	3.006	1211	2.8
30.173	2.9594	1606	3.6
30.664	2.9206	1602	3.7
30.885	2.8928	1550	3.6
31.217	2.8628	1068	2.4
31.605	2.8285	1038	2.4
32.059	2.7895	1211	2.8
32.84	2.7412	684	1.6
32.747	2.7324	758	1.7
33.46	2.6759	506	1.2
34.194	2.6201	1085	2.5
34.645	2.5943	915	2.1

TABLE 1C: METHYL ETHYL KETONE DATA

Angle 2-Theta	d-spacing Angstrom	Intensity Cps	Intensity %
7.584	11.648	5629	32.6
7.753	11.393	15929	92.3
10.151	8.707	2877	16.7
11.31	7.817	701	4.1
12.646	6.994	1027	5.9
13.193	6.705	15186	88
13.556	6.528	14225	82.4
14.074	6.287	1966	11.4
14.746	6.002	2759	16
15.185	5.837	801	4.6
15.548	5.694	1896	11
17.031	5.202	7980	46.2
17.28	5.127	17267	100
17.706	5.005	6873	39.8
18.565	4.778	545	3.2
18.871	4.699	1112	6.4
19.786	4.486	1704	9.9
20.158	4.401	1396	8.1
20.725	4.282	2644	15.3
21.787	4.076	1127	6.5
22.06	4.028	451	2.6
22.884	3.886	1542	8.9
23.412	3.798	14186	82.2
23.76	3.743	1154	6.7
24.288	3.662	3063	17.7
25.253	3.524	1318	7.6
25.503	3.49	1736	10.1
25.761	3.455	1225	7.1
26.176	3.402	1346	7.8
26.548	3.355	1096	6.4
27.367	3.257	1944	11.3
27.605	3.229	2116	12.3
27.9	3.195	858	5
28.378	3.142	583	3.4
28.749	3.103	763	4.4
29.3	3.046	1162	6.8
29.679	3.008	2606	15.1
30.402	2.9377	2184	12.6
30.738	2.9063	648	3.8

Graphical examples of the x-ray diffraction patterns for Form I, Form II, and the methyl ethyl ketone solvate

crystalline forms of eplerenone are shown in Figs. 1-A, 1-B, and 1-C, respectively. Form I shows distinguishing peaks at 7.0 ± 0.2, 8.3 ± 0.2, and 12.0 ± 0.2 degrees two theta. Form II shows distinguishing peaks at 8.0 ± 0.2, 12.4 ± 0.2, 12.8 ± 0.2, and 13.3 ± 0.2 degrees two theta. The methyl ethyl ketone solvated crystalline form shows distinguishing peaks at 7.6 ± 0.2, 7.8 ± 0.2, and 13.6 ± 0.2 degrees two theta.

Examples of the x-ray diffraction patterns are shown in Figs. 1-D through 1-O for the following solvate crystalline forms of eplerenone: n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, acetone solvate, toluene solvate, isopropanol solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, methyl acetate solvate, and propyl acetate solvate, respectively.

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3. Melting/Decomposition Temperature

The temperatures of melting and/or decomposition of non-solvated eplerenone crystalline forms were determined using a TA Instruments 2920 differential scanning calorimeter. Each sample (1-2 mg) was placed in either a sealed or unsealed aluminum pan and heated at 10°C/minute. Melting/decomposition ranges were defined from the extrapolated onset to the maximum of the melting/decomposition endotherm.

The melting of the non-solvated eplerenone crystals forms (Form I and Form II) was associated with chemical decomposition and loss of trapped solvent from the crystal lattice. The melting/decomposition temperature also was affected by the manipulation of the solid prior to analysis. For example, non-milled Form II (approximate D_{90} particle size of about 180-450 microns) prepared by direct crystallization

from an appropriate solvent or from desolvation of a solvate obtained from crystallization of high purity eplerenone in an appropriate solvent or mixture of solvents generally had a melting range of about 237-242°C. Milled Form II (approximate D_{90} particle size of about 80-100 microns) (Form II prepared by crystallizing a solvate from a solution of high purity eplerenone in an appropriate solvent or mixture of solvents, desolvating the solvate to yield Form II, and milling the resulting Form II) generally had a lower and broader melting/decomposition range of about 223-234°C. Non-milled Form I (approximate D_{90} particle size of about 180-450 microns) prepared by desolvation of a solvate obtained by digestion of low purity eplerenone generally had a higher melting/decomposition range of about 247-251°C. Examples of the DSC thermograms of (a) non-milled Form II directly crystallized from methyl ethyl ketone, (b) non-milled Form II prepared by desolvation of a solvate obtained by crystallization of a high purity eplerenone from methyl ethyl ketone, (c) Form II prepared by milling a desolvated solvate obtained by crystallization of high purity eplerenone from methyl ethyl ketone, and (d) non-milled Form I prepared by desolvation of a solvate obtained by digestion of low purity eplerenone from methyl ethyl ketone are given in Figures 2-A, 2-B, 2-C and 2-D, respectively.

DSC thermograms of solvated forms of eplerenone were determined using a Perkin Elmer Pyris 1 differential scanning calorimeter. Each sample (1-10 mg) was placed in an unsealed aluminum pan and heated at 10°C/minute. One or more endothermal events at lower temperatures were associated with enthalpy changes that occurred as solvent was lost from the solvate crystal lattice. The highest temperature endotherm

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or endotherms were associated with the melting/decomposition of Form II or Form I eplerenone. Examples of the DSC thermograms are shown in Figs. 2-E through 2-T for the following solvated crystalline forms of eplerenone: n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, chloroform solvate, acetone solvate, toluene solvate, isopropanol solvate, t-butyl acetate solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, methyl acetate solvate, propyl acetate solvate, n-butanol solvate, and n-octanol solvate, respectively.

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4. Infrared Absorption Spectroscopy

Infrared absorption spectra of the non-solvated forms of eplerenone (Form I and Form II) were obtained with a Nicolet DRIFT (diffuse reflectance infrared fourier transform) Magna System 550 spectrophotometer. A Spectra-Tech Collector system and a microsample cup were used. Samples (5%) were analyzed in potassium bromide and scanned from 400-4000 cm^{-1}. Infrared absorption spectra of eplerenone in dilute chloroform solution (3%) or in the solvated crystal forms were obtained with a Bio-rad FTS-45 spectrophotometer. Chloroform solution samples were analyzed using a solution cell of 0.2 mm path length with sodium chloride salt plates. Solvate FTIR spectra were collected using an IBM micro-MIR (multiple internal reflectance) accessory. Samples were scanned from 400-4000 cm^{-1}. Examples of the infrared absorption spectra of (a) Form I, (b) Form II, (c) the methyl ethyl ketone solvate, and (d) eplerenone in chloroform solution are shown in Figures 3-A, 3-B, 3-C and 3-D, respectively.

Table 2 discloses illustrative absorption bands for

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eplerenone in the Form I, Form II, and methyl ethyl ketone solvate crystal forms. Illustrative absorption bands for eplerenone in chloroform solution are also disclosed for comparison. Differences between Form I and either Form II or the methyl ethyl ketone solvate were observed, for example, in the carbonyl region of the spectrum. Form I has an ester carbonyl stretch of approximately 1739 cm^{-1} while both Form II and the methyl ethyl ketone solvate have the corresponding stretch at approximately 1724 and 1722 cm^{-1}, respectively. The ester carbonyl stretch occurs at approximately 1727 cm^{-1} in the eplerenone in chloroform solution. The change in stretching frequency of the ester carbonyl between Form I and Form II reflects the change in orientation of the ester group between the two crystal forms. In addition, the stretch of the ester of the conjugated ketone in the A-steroid ring shifts from approximately 1664-1667 cm^{-1} in either Form I or the methyl ethyl ketone solvate to approximately 1655 cm^{-1} in Form II. The corresponding carbonyl stretch occurs at approximately 1665 cm^{-1} in dilute solution.

Another difference between Form I and Form II was seen in the C-H bending region. Form I has an absorption at approximately 1399 cm^{-1} which is not observed in Form II, the methyl ethyl ketone solvate, or the eplerenone in chloroform solution. The 1399 cm^{-1} stretch occurs in the region of CH_2 scissoring for the C2 and C21 methylene groups adjacent to carbonyl groups.

Table 2

Absorption Region	Form I (cm^{-1})	Form II (cm^{-1})	Methyl Ethyl Ketone Solvate (cm^{-1})	Eplerenone in Chloroform (cm^{-1})
ν C=O(lactone)	1773	1775	1767	1768
ν C=O(ester)	1739	1724	1722	1727
ν C=O(3keto)	1664	1655	1667	1665
ν C=C (3,4-olefin)	1619	1619	1622	1623
δ_{as}CH3,δCH2, δCH2(α to carbonyl)	1460, 1444, 1426	1467, 1438, 1422, 1399	1467, 1438, 1422	1464, 1438, 1422
δ_{s}CH3	1380	1381	~1380	1378

Examples of infrared absorption spectra are shown in Figs. 3-E through 3-S for the following solvated crystalline forms of eplerenone: n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, acetone solvate, toluene solvate, isopropanol solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, propyl acetate solvate, methyl acetate solvate, propylene glycol solvate, and t-butyl acetate solvate respectively.

5. Nuclear Magnetic Resonance

^{13}C NMR spectra were obtained at a field of 31.94 MHz. Examples of the ^{13}C NMR spectra of Form I and Form II eplerenone are shown in Figs. 4 and 5, respectively. The Form I eplerenone analyzed to obtain the data reflected in Fig. 4 was not phase pure and included a small amount of Form II eplerenone. Form I is most clearly distinguished by the carbon resonances at around 64.8 ppm, 24.7 ppm and 19.2 ppm.

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Form II is most clearly distinguished by the carbon resonances at around 67.1 ppm and 16.0 ppm.

6. Thermogravimetry

Thermogravimetric analysis was performed using a TA Instruments TGA 2950 thermogravimetric analyzer. Samples were placed in an unsealed aluminum pan under nitrogen purge. Starting temperature was 25°C with the temperature increased at a rate of about 10°C/minute.

Examples of thermogravimetry analysis profiles are shown in Figs. 6-A through 6-R for the following solvated crystalline forms of eplerenone: methyl ethyl ketone solvate, n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, chloroform solvate, acetone solvate, toluene solvate, isopropanol solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, methyl acetate solvate, propyl acetate solvate, propylene glycol solvate, n-butanol solvate, n-octanol solvate, and t-butyl acetate solvate, respectively.

7. Microscopy

Hot-stage microscopy was performed on single crystals of the methyl ethyl ketone solvate using a Linkam THMS 600 Hot Stage with Zeiss Universal Polarized Light Microscope. Under polarized light at room temperature the solvate crystal was birefringent and translucent indicating that the crystal lattice was highly ordered. As the temperature increased to about 60°C, noticeable defects began to emerge along the long crystal dimension. A scanning electron micrograph of Form II eplerenone obtained by desolvation of the methyl ethyl ketone solvate is shown in Fig. 7 and reveals surface defects,

pores, cracks and fractures within the crystal lattice. A scanning electron micrograph of Form II eplerenone obtained by direct crystallization from ethyl acetate is shown in Fig. 8 and does not exhibit similar surface defects, pores, cracks and fractures within the crystal lattice.

8. Unit Cell Parameters

Tables 3A, 3B and 3C below summarize the unit cell parameters determined for Form I, Form II, and several solvated crystalline forms.

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TABLE 3A

Parameter	Form I	Form II	Methyl ethyl ketone Solvate
Crystal system	Ortho-rhombic	Monoclinic	Orthorhombic
Space group	$P2_12_12_1$	$P2_1$	$P2_12_12_1$
a	21.22 Å	8.78 Å	23.53 Å
b	15.40 Å	11.14 Å	8.16 Å
c	6.34 Å	11.06 Å	13.08 Å
α	90°	90°	90°
β	90°	93.52°	90°
γ	90°	90°	90°
z	4	2	4
Volume (Å)	2071.3	1081.8	2511.4
ρ (calculated)	1.329 g/cm³	1.275 g/cm³	1.287 g/cm³
R	0.0667	0.062	0.088

TABLE 3B

Parameter	Acetone Solvate	Toluene Solvate	Butyl Acetate Solvate[1]
Crystal system	Ortho-rhombic	Ortho-rhombic	Ortho-rhombic
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
a	23.31 Å	23.64 Å	23.07 Å
b	13.13 Å	13.46 Å	13.10 Å
c	8.28 Å	8.16 Å	8.24 Å
α	90°	90°	90°
β	90°	90°	90°
γ	90°	90°	90°
z	4	4	4
Volume (Å)	2533.7	2596.6	2490.0
ρ (calculated)	1.239 g/cm³	1.296 g/cm³	1.334 g/cm³
R	0.058	0.089	0.093

[1]The solvate molecules were not completely refined due to disorder of the solvent molecules in the channels.

TABLE 3C

Parameter	Isobutyl Acetate Solvate[1]	Isopropanol Solvate[1]	Ethanol Solvate[1]
Crystal system	Ortho-rhombic	Ortho-rhombic	Ortho-rhombic
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
a	23.19 Å	23.15 Å	23.51 Å
b	12.95 Å	12.73 Å	13.11 Å
c	8.25 Å	8.25 Å	8.27 Å
α	90°	90°	90°
β	90°	90°	90°
γ	90°	90°	90°
z	4	4	4
Volume (Å)	2476.4	2433.2	2548.6
ρ (calculated)	1.337 g/cm³	1.296 g/cm³	1.234 g/cm³
R	0.098	0.152	0.067

[1]The solvate molecules were not refined completely due to disorder of the solvent molecules in the channels.

Additional information on selected solvated crystalline forms of eplerenone is reported in Table 4 below. The unit cell data reported in Table 3A above for the methyl ethyl ketone solvate also are representative of the unit cell parameters for many of these additional eplerenone crystalline solvates. Most of the eplerenone crystalline solvates tested are substantially isostructural to each other. While there may be some minor shifting in the X-ray powder diffraction peaks from one solvated crystalline form to the next due to the size of the incorporated solvent molecule, the overall diffraction patterns are substantially the same and the unit cell parameters and molecular positions are substantially identical for most of the solvates tested.

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TABLE 4

Solvent	Stoichiometry (Solvent: Eplerenone)	Isostructural to Methyl Ethyl Ketone Solvate?	Desolvation Temperature[1] (°C)
Methyl Ethyl Ketone	1:1	N/A	89
2-Pentanone	---	---	---
Acetic Acid	1:2	Yes	203
Acetone	1:1	Yes	117
Butyl Acetate	1:2	Yes	108
Chloroform	---	Yes	125
Ethanol	1:1	Yes	166
Isobutanol	---	---	---
Isobutyl Acetate	1:2	Yes	112
Isopropanol	1:1	Yes	121
Methyl Acetate	1:1	Yes	103
Ethyl Propionate	1:1	Yes	122
n-Butanol	1:1	Yes	103
n-Octanol	---	Yes	116
n-Propanol	1:1	Yes	129
Propyl Acetate	1:1	Yes	130
Propylene Glycol	---	Yes	188
t-Butanol	---	---	---
Tetrahydrofuran	1:1	Yes	136
Toluene	1:1	Yes	83
t-Butyl Acetate	---	Yes	109

[1]Defined as the extrapolated desolvation temperature from the final solvent weight loss step as determined by thermogravimetric analysis at a heating rate of 10°C/minute under nitrogen purge. Desolvation temperatures, however, can be affected by the method of manufacture of the solvate. Different methods can produce different numbers of nucleation sites capable of initiating desolvation in the solvate at lower temperatures.

The unit cell of the solvate is composed of four eplerenone molecules. The stoichiometry of the eplerenone molecules and solvent molecules in the unit cell is also reported in Table 4 above for a number of solvates. The unit cell of Form I is composed of four eplerenone molecules. The unit cell of Form II is composed of two eplerenone molecules. The solvate unit cells are converted during desolvation into Form I and/or Form II unit cells when the eplerenone

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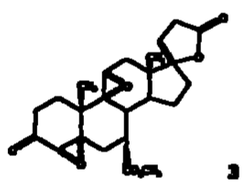
molecules undergo translation and rotation to fill the spaces left by the solvent molecules. Table 4 also reports the desolvation temperatures for a number of different solvates.

9. Crystal Properties of Impurity Molecules

Selected impurities in eplerenone can induce the formation of Form I during the desolvation of the solvate. In particular, the effect of the following two impurity molecules was evaluated: 7-methyl hydrogen 4α,5α:9α,11α-diepoxy-17-hydroxy-3-oxo-17α-pregnane-7α,21-dicarboxylate, γ-lactone 3 (the "diepoxide"); and 7-methyl hydrogen 11α,12α-epoxy-17-hydroxy-3-oxo-17α-pregn-4-ene-7α,21-dicarboxylate, γ-lactone 4 (the "11,12-epoxide").



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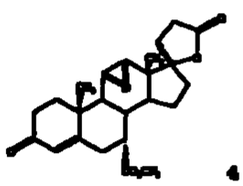

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The effect of these impurity molecules on the eplerenone crystalline form resulting from desolvation is described in greater detail in the examples of this application.

Given the similarity in single crystal structure of 7-methyl hydrogen 17-hydroxy-3-oxo-17α-pregna-4,9(11)-diene-7α

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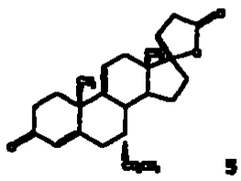
,21-dicarboxylate, γ-lactone 5 (the "9,11-olefin") and Form I, it is hypothesized that the 9,11-olefin also can induce the formation of Form I during the desolvation of the solvate.



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A single crystal form was isolated for each impurity compound. Representative X-ray powder diffraction patterns for the crystal forms isolated for the diepoxide, 11,12-epoxide and 9,11-olefin are given in Figs. 9, 10 and 11, respectively. The X-ray powder diffraction pattern of each impurity molecule is similar to the X-ray powder diffraction pattern of Form I, suggesting that Form I and the three impurity compounds have similar single crystal structures.

Single crystals of each impurity compound also were isolated and subjected to X-ray structure determination to verify that these three compounds adopt single crystal structures similar to that of Form I. Single crystals of the diepoxide were isolated from methyl ethyl ketone. Single crystals of the 11,12-epoxide were isolated from isopropanol. Single crystals of the 9,11-olefin were isolated from n-butanol. Crystal structure data determined for the crystalline form of each impurity compound are given in Table 5. The resulting crystal system and cell parameters were substantially the same for the Form I, diepoxide, 11,12-epoxide, and 9,11-olefin crystalline forms.

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TABLE 5

Parameter	Form I	Diepoxide	11,12 Epoxide	9,11 olefin
Crystal system	Ortho-rhombic	Ortho-rhombic	Ortho-rhombic	Ortho-rhombic
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
a	21.22 Å	21.328 Å	20.90 Å	20.90 Å
b	15.40 Å	16.16 Å	15.55 Å	15.74 Å
c	6.34 Å	6.15 Å	6.38 Å	6.29 Å
α	90°	90°	90°	90°
β	90°	90°	90°	90°
γ	90°	90°	90°	90°
Z	4	4	4	4
Volume (Å)	2071.3	2119.0	2073.2	2069.3
ρ (calculated)	1.329 g/cm³	1.349 g/cm³	1.328 g/cm³	1.279 g/cm³
R	0.0667	0.0762	0.0865	0.0764

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The four compounds reported in Table 5 crystallize into the same space group and have similar cell parameters (i.e., they are isostructural). It is hypothesized that the diepoxide, 11,12-epoxide and 9,11-olefin adopt a Form I conformation. The relative ease of isolation of a Form I packing (directly from solution) for each impurity compound, indicates that the Form I lattice is a stable packing mode for this series of structurally similar compounds.

Preparation of Eplerenone

The eplerenone starting material used to prepare the novel crystalline forms of the present invention can be prepared using the methods set forth in Ng et al., WO97/21720; and Ng et al., WO98/25948, particularly scheme 1 set forth in WO97/21720 and WO98/25948.

Preparation of Crystalline Forms

1. Preparation of Solvated Crystalline Form

The solvated crystalline forms of eplerenone can be prepared by crystallization of eplerenone from a suitable solvent or a mixture of suitable solvents. A suitable solvent or mixture of suitable solvents generally comprises an organic solvent or a mixture of organic solvents that solubilizes the eplerenone together with any impurities at an elevated temperature, but upon cooling, preferentially crystallizes the solvate. The solubility of eplerenone in such solvents or mixtures of solvents generally is about 5 to about 200 mg/mL at room temperature. The solvent or mixtures of solvents preferably are selected from those solvents previously used in the process to prepare the eplerenone starting material, particularly those solvents that would be pharmaceutically acceptable if contained in the final pharmaceutical composition comprising the eplerenone crystalline form. For example, a solvent system comprising methylene chloride that yields a solvate comprising methylene chloride generally is not desirable.

Each solvent used preferably is a pharmaceutically acceptable solvent, particularly a Class 2 or Class 3 solvent as defined in "Impurities: Guideline For Residual Solvents", International Conference On Harmonisation Of Technical Requirements For Registration Of Pharmaceuticals For Human Use (Recommended for Adoption at Step 4 of the ICH Process on July 17, 1997 by the ICH Steering Committee). Still more preferably, the solvent or mixture of solvents is selected from the group consisting of methyl ethyl ketone, 1-propanol, 2-pentanone, acetic acid, acetone, butyl acetate, chloroform,

ethanol, isobutanol, isobutyl acetate, methyl acetate, ethyl propionate, n-butanol, n-octanol, isopropanol, propyl acetate, propylene glycol, t-butanol, tetrahydrofuran, toluene, methanol and t-butyl acetate. Still more preferably, the solvent is selected from the group consisting of methyl ethyl ketone and ethanol.

In another embodiment of this process, the solvent or mixture of solvents is selected from the group consisting of 1-propanol, 2-pentanone, acetic acid, acetone, butyl acetate, chloroform, isobutanol, isobutyl acetate, methyl acetate, ethyl propionate, n-butanol, n-octanol, propyl acetate, propylene glycol, t-butanol, tetrahydrofuran, toluene, methanol and t-butyl acetate.

In another embodiment of this process, the solvent or mixture of solvents is selected from the group consisting of 1-propanol, 2-pentanone, acetic acid, acetone, butyl acetate, chloroform, isobutanol, isobutyl acetate, methyl acetate, ethyl propionate, n-butanol, n-octanol, n-propanol, propyl acetate, propylene glycol, t-butanol, tetrahydrofuran, toluene, methanol and t-butyl acetate.

To prepare the solvated crystalline form of eplerenone, an amount of the eplerenone starting material is solubilized in a volume of the solvent and cooled until crystals form. The solvent temperature at which the eplerenone is added to the solvent generally will be selected based upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, this solvent temperature typically is at least about 25°C, preferably from about 30°C to the boiling point of the solvent, and more preferably from about 25°C below the boiling point of the solvent to the boiling point of the

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solvent.

Alternatively, hot solvent may be added to the eplerenone and the mixture can be cooled until crystals form. The solvent temperature at the time it is added to the eplerenone generally will be selected based upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, the solvent temperature typically is at least 25°C, preferably from about 50°C to the boiling point of the solvent, and more preferably from about 15°C below the boiling point of the solvent to the boiling point of the solvent.

The amount of the eplerenone starting material mixed with a given volume of solvent likewise will depend upon the solubility curve of the solvent or mixture of solvents. Typically, the amount of eplerenone added to the solvent will not completely solubilize in that volume of solvent at room temperature. For most of the solvents described herein, for example, the amount of eplerenone starting material mixed with a given volume of solvent usually is at least about 1.5 to about 4.0 times, preferably about 2.0 to about 3.5 times, and more preferably about 2.5 times, the amount of eplerenone that will solubilize in that volume of solvent at room temperature.

After the eplerenone starting material has completely solubilized in the solvent, the solution typically is cooled slowly to crystallize the solvated crystalline form of eplerenone. For most of the solvents described herein, for example, the solution is cooled at a rate slower than about 20°C/minute, preferably at a rate of about 10°C/minute or slower, more preferably at a rate of about 5°C/minute or

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slower, and still more preferably at a rate of about 1°C/minute or slower.

The endpoint temperature at which the solvated crystalline form is harvested will depend upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, the endpoint temperature typically is less than about 25°C, preferably less than about 5°C, and more preferably less than about -5°C. Decreasing the endpoint temperature generally favors the formation of the solvated crystalline form.

Alternatively, other techniques may be used to prepare the solvate. Examples of such techniques include, but are not limited to, (i) dissolving the eplerenone starting material in one solvent and adding a co-solvent to aid in the crystallization of the solvate crystalline form, (ii) vapor diffusion growth of the solvate, (iii) isolation of the solvate by evaporation, such as rotary evaporation, and (iv) slurry conversion.

The crystals of the solvated crystalline form prepared as described above can be separated from the solvent by any suitable conventional means such as by filtration or centrifugation. Increased agitation of the solvent system during crystallization generally results in smaller crystal particle sizes.

2. Preparation of Form II From Solvate

Form II eplerenone can be prepared directly from the solvated crystalline form by desolvation. Desolvation can be accomplished by any suitable desolvation means such as, but not limited to, heating the solvate, reducing the ambient pressure surrounding the solvate, or combinations thereof.

If the solvate is heated to remove the solvent, such as in an oven, the temperature of the solvate during this process typically does not exceed the enantiotropic transition temperature for Form I and Form II. This temperature preferably does not exceed about 150°C.

The desolvation pressure and time of desolvation are not narrowly critical. The desolvation pressure preferably is about one atmosphere or less. As the desolvation pressure is reduced, however, the temperature at which the desolvation can be carried out and/or the time of desolvation likewise is reduced. Particularly for solvates having higher desolvation temperatures, drying under vacuum will permit the use of lower drying temperatures. The time of desolvation need only be sufficient to allow for the desolvation, and thus the formation of Form II, to reach completion.

To ensure the preparation of a product that comprises substantially all Form II, the eplerenone starting material typically is a high purity eplerenone, preferably substantially pure eplerenone. The eplerenone starting material used to prepare Form II eplerenone generally is at least 90% pure, preferably at least 95% pure, and more preferably at least 99% pure. As discussed in greater detail elsewhere in this application, certain impurities in the eplerenone starting material can adversely affect the yield and Form II content of the product obtained from the process.

The crystallized eplerenone product prepared in this manner from a high purity eplerenone starting material generally comprises at least 10% Form II, preferably at least 50% Form II, more preferably at least 75% Form II, still more preferably at least 90% Form II, still more preferably at

least about 95% Form II, and still more preferably substantially phase pure Form II.

3. Preparation of Form I From Solvate

A product comprising Form I can be prepared in substantially the same manner as set forth above for the preparation of Form II by (i) using a low purity eplerenone starting material instead of a high purity eplerenone starting material, (ii) seeding the solvent system with phase pure Form I crystals, or (iii) a combination of (i) and (ii).

A. Use Of Impurities As Growth Promoters and Inhibitors

The presence and amount of selected impurities in the eplerenone starting material, rather than the total amount of all impurities in the eplerenone starting material, affect the potential for Form I crystal formation during the desolvation of the solvate. The selected impurity generally is a Form I growth promoter or Form II growth inhibitor. It may be contained in the eplerenone starting material, contained in the solvent or mixture of solvents before the eplerenone starting material is added, and/or added to the solvent or mixture of solvents after the eplerenone starting material is added. Bonafede et al., "Selective Nucleation and Growth of an Organic Polymorph by Ledge-Directed Epitaxy on a Molecular Crystal Substate", J. Amer. Chem. Soc., Vol. 117, No. 30 (August 2, 1995) discusses the use of growth promoters and growth inhibitors in polymorph systems and is incorporated by reference herein. For the present invention, the impurity generally comprises a compound having a single crystal structure substantially identical to the single crystal structure of Form I. The impurity preferably is a compound

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having an X-ray powder diffraction pattern substantially identical to the X-ray powder diffraction pattern of Form I, and more preferably is selected from the group consisting of the diepoxide, the 11,12-epoxide, the 9,11-olefin and combinations thereof.

The amount of impurity needed to prepare Form I crystals typically can depend, in part, upon the solvent or mixture of solvents and the solubility of the impurity relative to eplerenone. In the crystallization of Form I from a methyl ethyl ketone solvent, for example, the weight ratio of diepoxide to low purity eplerenone starting material typically is at least about 1:100, preferably at least about 3:100, more preferably between about 3:100 and about 1:5, and still more preferably between about 3:100 and about 1:10. The 11,12-epoxide has a higher solubility in methyl ethyl ketone than the diepoxide and generally requires a larger amount of the 11,12-epoxide generally is necessary to prepare Form I crystals. Where the impurity comprises the 11,12-epoxide, the weight ratio of the diepoxide to the low purity eplerenone starting material typically is at least about 1:5, more preferably at least about 3:25, and still more preferably between about 3:25 and about 1:5. Where both the diexpoxide and the 11,12-epoxide impurities are used in the preparation of the Form I crystals, the weight ratio of each impurity to the eplerenone starting material may be lower than the corresponding ratio when only that impurity is used in the preparation of the Form I crystals.

A mixture of Form I and Form II is generally obtained when a solvate comprising the selected impurity is desolvated. The weight fraction of Form I in the product resulting from the initial desolvation of the solvate

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typically is less than about 50%. Further treatment of this product by crystallization or digestion, as discussed below, generally will increase the weight fraction of Form II in the product.

B. Seeding

Form I crystals also can be prepared by seeding the solvent system with phase pure Form I crystals (or a Form I growth promoter and/or Form II growth inhibitor as previously discussed above) prior to crystallization of the eplerenone. The eplerenone starting material can be either a low purity eplerenone or a high purity eplerenone. When the resulting solvate prepared from either starting material is desolvated, the weight fraction of Form I in the product typically is at least about 70% and may be as great as about 100%.

The weight ratio of Form I seed crystals added to the solvent system to the eplerenone starting material added to the solvent system generally is at least about 0.75:100, preferably between about 0.75:100 to about 1:20, and more preferably between about 1:100 to about 1:50. The Form I seed crystals can be prepared by any of the methods discussed in this application for the preparation of Form I crystals, particularly the preparation of Form I crystals by digestion as discussed below.

The Form I seed crystals may be added at one time, in multiple additions or substantially continually over a period of time. The addition of the Form I seed crystals, however, generally is completed before the eplerenone begins to crystallize from solution, i.e., the seeding is completed before the cloud point (the lower end of the metastable zone) is reached. Seeding typically is performed when the solution

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temperature ranges from about 0.5°C above the cloud point to about 10°C above the cloud point, preferably within about 2°C to about 3°C above the cloud point. As the temperature above the cloud point at which the seeds are added increases, the amount of seeding needed for crystallization of Form I crystals generally increases.

The seeding preferably occurs not only above the cloud point, but within the metastable zone. Both the cloud point and the metastable zone are dependant on the eplerenone solubility and concentration in the solvent or mixture of solvents. For a 12 volume dilution of methyl ethyl ketone, for example, the high end of the metastable zone generally is between about 70°C to about 73°C and the lower end of the metastable zone (i.e., the cloud point) is between about 57°C and 63°C. For a concentration of 8 volumes of methyl ethyl ketone, the metastable zone is even narrower because the solution is supersaturated. At this concentration, the cloud point of the solution occurs at about 75°C to about 76°C. Because the boiling point of methyl ethyl ketone is about 80° C under ambient conditions, seeding for this solution typically occurs between about 76.5°C and the boiling point.

An illustrative non-limiting example of seeding with Form I is set forth below in Example 7.

The crystallized eplerenone product obtained using a Form I growth promoter or Form II growth inhibitor, and/or Form I seeding generally comprises at least 2% Form I, preferably at least 5% Form I, more preferably at least 7% Form I, and still more preferably at least about 10% Form I.

The remaining crystallized eplerenone product generally is Form II.

C. Form I Prepared By Grinding Eplerenone

In yet another alternative, it has been discovered that a small amount of Form I can be prepared by suitable grinding eplerenone. Concentrations of Form I in ground eplerenone as high as about 3% have been observed.

4. Preparation of Form II From Solvate Prepared From Low Purity Eplerenone

As discussed above, crystallization of low purity eplerenone to form a solvate followed by desolvation of the solvate generally yields a product comprising both Form I and Form II. A product having a greater Form II content can be prepared from low purity eplerenone in substantially the same manner as set forth above for the preparation of Form I by seeding the solvent system with phase pure Form II crystals, or by using a Form II growth promoter and/or Form I growth inhibitor. The seeding protocol and the weight ratio of the amount of Form II seed crystals added to the solvent system to the amount of the eplerenone starting material added to the solvent system generally are similar to those ratios previously discussed above for the preparation of Form I eplerenone by seeding with phase pure Form I crystals.

The crystallized eplerenone product prepared in this manner generally comprises at least 10% Form II, preferably at least 50% Form II, more preferably at least 75% Form II, more preferably at least 90% Form II, still more preferably at least about 95% Form II, and still more preferably substantially phase pure Form II.

The seeding protocols described in this section and in the prior section relating to the preparation of Form I eplerenone also may allow for improved control of the particle size of the crystallized eplerenone.

5. Crystallization of Form II Directly From Solution

Form II eplerenone also can be prepared by the direct crystallization of eplerenone from a suitable solvent or mixture of solvents without the formation of an intermediate solvate and the accompanying need for desolvation. Typically, (i) the solvent has a molecular size that is incompatible with the available channel space in the solvate crystal lattice, (ii) the eplerenone and any impurities are soluble in the solvent at elevated temperatures, and (iii) upon cooling, results in the crystallization of the non-solvated Form II eplerenone. The solubility of eplerenone in the solvent or mixture of solvents generally is about 5 to about 200 mg/mL at room temperature. The solvent or mixture of solvents preferably comprises one or more solvents selected from the group consisting of methanol, ethyl acetate, isopropyl acetate, acetonitrile, nitrobenzene, water and ethyl benzene.

To crystallize Form II eplerenone directly from solution, an amount of the eplerenone starting material is solubilized in a volume of the solvent and cooled until crystals form. The solvent temperature at which the eplerenone is added to the solvent generally will be selected based upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, this solvent temperature typically is at least about 25°C, preferably from about 30°C to the boiling point of the

solvent, and more preferably from about 25°C below the boiling point of the solvent to the boiling point of the solvent.

Alternatively, hot solvent may be added to the eplerenone and the mixture can be cooled until crystals form. The solvent temperature at the time it is added to the eplerenone generally will be selected based upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, the solvent temperature typically is at least 25°C, preferably from about 50°C to the boiling point of the solvent, and more preferably from about 15°C below the boiling point of the solvent to the boiling point of the solvent.

The amount of the eplerenone starting material mixed with a given volume of solvent likewise will depend upon the solubility curve of the solvent or mixture of solvents. Typically, the amount of eplerenone added to the solvent will not completely solubilize in that volume of solvent at room temperature. For most of the solvents described herein, for example, the amount of eplerenone starting material mixed with a given volume of solvent usually is at least about 1.5 to about 4.0 times, preferably about 2.0 to about 3.5 times, and more preferably about 2.5 times, the amount of eplerenone that will solubilize in that volume of solvent at room temperature.

To ensure the preparation of a product that comprises substantially phase pure Form II, the eplerenone starting material generally is a high purity eplerenone. The eplerenone starting material preferably is at least 65% pure, more preferably at least 90% pure, still more preferably at least 98% pure, and still more preferably at least 99% pure.

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After the eplerenone starting material has completely solubilized in the solvent, the solution typically is cooled slowly to crystallize the solvated crystalline form of eplerenone. For most of the solvents described herein, for example, the solution is cooled at a rate slower than about 1.0°C/minute, preferably at a rate of about 0.2°C/minute or slower, and more preferably at a rate between about 5°C/minute and about 0.1°C/minute.

The endpoint temperature at which the Form II crystals are harvested will depend upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, the endpoint temperature typically is less than about 25°C, preferably less than about 5°C, and more preferably less than about -5°C.

Alternatively, other techniques may be used to prepare the Form II crystals. Examples of such techniques include, but are not limited to, (i) dissolving the eplerenone starting material in one solvent and adding a co-solvent to aid in the crystallization of Form II eplerenone, (ii) vapor diffusion growth of Form II eplerenone, (iii) isolation of Form II eplerenone by evaporation, such as rotary evaporation, and (iv) slurry conversion.

The crystals of the solvated crystalline form prepared as described above can be separated from the solvent by any suitable conventional means such as by filtration or centrifugation.

In addition, Form II eplerenone also can be prepared by digesting (as described below) a slurry of high purity eplerenone in methyl ethyl ketone and filtering the digested eplerenone at the boiling point of the slurry.

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6. Preparation of Form I Directly From Solution

It is hypothesized that if the crystallization is performed above the enantiotropic transition temperature (T_t) for Form I and Form II, particularly if Form I growth promoters or Form II growth inhibitors are present or the solvent is seeded with phase pure Form I crystals, Form I should crystallize directly from solution since Form I is more stable at these higher temperatures. The solvent system used preferably comprises a high boiling solvent such as nitrobenzene. Suitable Form I growth promoters would include, but would not be limited to, the diepoxide and the 11,12-olefin.

7. Digestion of Eplerenone With A Solvent

The solvated crystalline forms, Form I and Form II of eplerenone also can be prepared by digestion of an eplerenone starting material in a suitable solvent or mixture of solvents. In the digestion process, a slurry of eplerenone is heated at the boiling point of the solvent or mixture of solvents. For example, an amount of eplerenone starting material is combined with a volume of solvent or mixture of solvents, heated to reflux, and the distillate is removed while an additional amount of the solvent is added simultaneously with the removal of the distillate. Alternatively, the distillate can be condensed and recycled without the addition of more solvent during the digestion process. Typically, once the original volume of solvent has been removed or condensed and recycled, the slurry is cooled and solvated crystals form. The solvated crystals can be separated from the solvent by any suitable conventional means such as by filtration or centrifugation. Desolvation of the

solvate as previously described yields either Form I or Form II eplerenone depending upon the presence or absence of the selected impurities in the solvated crystals.

A suitable solvent or mixture of solvents generally comprises one or more of the solvents previously disclosed herein. The solvent may be selected, for example, from the group consisting of methyl ethyl ketone and ethanol.

The amount of eplerenone starting material added to the solvent used in the digestion process generally is sufficient to maintain a slurry (i.e., the eplerenone in the solvent or mixture of solvents is not completely solubilized) at the boiling point of the solvent or mixture of solvents. Illustrative values include, but are not limited to, about one gram of eplerenone per four mL methyl ethyl ketone and about one gram of eplerenone per eight mL ethanol.

The solution generally is cooled slowly once solvent turnover is complete to crystallize the solvated crystalline form of eplerenone. For the solvents tested, for example, the solution is cooled at a rate slower than about 20°C/minute, preferably about 10°C/minute or slower, more preferably about 5°C/minute or slower, and still more preferably about 1°C/minute or slower.

The endpoint temperature at which the solvated crystalline form is harvested will depend upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, the endpoint temperature typically is less than about 25°C, preferably less than about 5°C, and more preferably less than about -5°C.

If a product comprising primarily or exclusively Form II is desired, a high purity eplerenone starting material

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typically is digested. The high purity eplerenone starting material preferably is at least 98% pure, more preferably at least 99% pure, and still more preferably at least 99.5% pure. The digested eplerenone product prepared in this manner generally comprises at least 10% Form II, preferably at least 50% Form II, more preferably at least 75% Form II, more preferably at least 90% Form II, still more preferably at least about 95% Form II, and still more preferably substantially phase pure Form II.

If a product comprising primarily or exclusively Form I is desired, a low purity eplerenone starting material typically is digested. The low purity eplerenone starting material generally contains only as much Form I growth promoter and/or Form II growth inhibitor as is needed to yield Form I. Preferably, the low purity eplerenone starting material is at least 65% pure, more preferably at least 75% pure, and still more preferably at least 80% pure. The digested eplerenone product prepared in this manner generally comprises at least 10% Form I, preferably at least 50% Form I, more preferably at least 75% Form I, more preferably at least 90% Form I, still more preferably at least about 95% Form I, and still more preferably substantially phase pure Form I.

8. Preparation of Amorphous Eplerenone

Amorphous eplerenone can be prepared in small quantities by suitable comminution of solid eplerenone, such as by crushing, grinding and/or micronizing. Phase pure amorphous eplerenone can be prepared, for example, by lyophilizing a solution of eplerenone, particularly an aqueous solution of

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eplerenone. These processes are illustrated in Examples 13 and 14 below.

Additional Processing Considerations

1. Thermodynamic Stability Considerations

Form II is more thermodynamically stable than Form I at ambient temperatures. As described in Example 5 below, when an organic slurry containing equivalent amounts of Form I and Form II was allowed to stand overnight at room temperature and the residual solids were then collected and analyzed by X-ray powder diffraction, the analytical results indicated that the eplerenone had completely converted to Form II. The differential scanning calorimetry ("DSC") data (discussed previously) indicate that Form I is more thermodynamically stable than Form II at higher temperatures since it has the higher melting/decomposition temperature. Taken together, the slurry conversion and DSC data indicate that Form I and Form II are related enantiotropically, i.e., a change in the stability relationship between the two polymorphs occurs around a transition temperature (T_t), with Form II being the more stable at lower temperatures. Fig. 12 shows the Gibbs free energy-temperature relationship generally observed for enantiotropically related polymorphs such as Form I and Form II, wherein I and II refer to the crystal forms, T_t refers to the transition temperature, T_m refers to the melting points of Form I and Form II, and L represents the liquid or melt state.

Accordingly, processing temperatures preferably are maintained below the transition temperature during the preparation of compositions comprising Form II. For example, drying temperatures employed for desolvation typically are

less than about 150°C, preferably less than about 125°C, more preferably less than about 115°C, more preferably less than about 110°C, and still more preferably between about 80°C to about 110°C. In addition, cooling (such as by use of liquid nitrogen) may be necessary during particle size reduction process steps to maintain the temperature of the Form II crystals below the transition temperature.

2. Intrinsic Micronizing Considerations

The method used to prepare crystalline eplerenone may affect the properties of the resulting crystal. For example, Form II prepared by desolvation of a solvate exhibits a higher incidence of surface defects, pores, cracks and fractures within the crystal lattice than Form II prepared by direct crystallization from solution. This "intrinsic micronizing" of the desolvated crystal results in an increase in both the available surface area of the crystal and the dissolution rate of the crystal. Dissolution times, therefore, can be shortened by selection of Form II crystals prepared by desolvation, lengthened by selection of Form II crystals prepared by direct crystallization, or otherwise adjusted by selection of an appropriate combination of Form II crystals prepared by desolvation and Form II crystals prepared by direct crystallization.

This "intrinsic micronizing" also may effectively reduce or eliminate the need for crystal particle size reduction during processing steps where Form II crystals prepared by desolvation are used in the preparation of the pharmaceutical composition. One disadvantage to using such Form II crystals, however, is the need for a desolvation step that is

not required for Form II crystals prepared by direct crystallization.

Product-By-Process Solid State Forms

The present invention also comprises each of the solid state eplerenone forms prepared in accordance with the processes disclosed in this application. In particular, the invention comprises solvated eplerenone, Form I eplerenone, Form II eplerenone, and combinations thereof, prepared as set forth in this application.

Combinations of Solid State Forms

Also embraced within this invention are combinations comprising a first solid state form of eplerenone and a second solid state form of eplerenone, wherein the first and second solid state forms of eplerenone are selected from Form I, Form II, solvated eplerenone and amorphous eplerenone. In general, the weight ratio of said first solid state form to said second solid state form preferably is at least about 1:9, more preferably is at least about 1:1, more preferably at least about 2:1, more preferably at least about 5:1, and still more preferably at least about 9:1.

In one embodiment, for example, the first solid state form is Form I and the second solid state form is Form II.

In another embodiment, for example, the first solid state form is Form II and the second solid state form is Form I.

Pharmaceutical Compositions

Also embraced within this invention is a class of pharmaceutical compositions comprising a first solid state

form of eplerenone and a second solid state form of eplerenone in association with one or more pharmaceutically-acceptable carriers and/or diluents and/or adjuvants (collectively referred to herein as "carrier" materials) and, if desired, other active ingredients. The first solid state form of eplerenone and the second solid state form of eplerenone are selected from Form I, Form II, solvated eplerenone and amorphous eplerenone. In general, the weight ratio of said first solid state form to said second solid state form preferably is at least about 1:9, more preferably is at least about 1:1, more preferably at least about 2:1, more preferably at least about 5:1, and still more preferably at least about 9:1.

In one embodiment of the invention, the entire amount of eplerenone contained in the composition is present as phase pure Form II.

In another embodiment of the invention, the entire amount of eplerenone contained in the composition is present as phase pure Form I.

In still another embodiment of the invention, the entire amount of eplerenone contained in the composition is present as phase pure solvated crystalline eplerenone.

In still another embodiment of the invention, the entire amount of eplerenone contained in the composition is present as amorphous eplerenone.

In still another embodiment of the invention, the composition comprises both Form I and Form II. The ratio of the amount of Form II to Form I in the composition generally is between about 1:20 to about 20:1. In other embodiments, for example, this ratio is between about 10:1 to about 1:10;

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about 5:1 to about 1:5; about 2:1 to about 1:2; or is about 1:1.

In still another embodiment of the invention, the composition comprises a first solid state form of eplerenone and a second solid state form of eplerenone, wherein the first and second solid state forms of eplerenone are selected from Form I, Form II, solvated eplerenone and amorphous eplerenone. In general, the weight ratio of said first solid state form to said second solid state form preferably is at least about 1:9, preferably about 1:1, more preferably at least about 2:1, more preferably at least about 5:1, and still more preferably at least about 9:1.

In each of the above combination and pharmaceutical composition embodiments, the D_{90} particle size of the eplerenone used as a starting material in the pharmaceutical composition generally is between about 25 microns to about 400 microns, preferably less than about 200 microns, more preferably less than about 150 microns, still more preferably less than about 100 microns, and still more preferably less than about 90 microns. In one embodiment, the D_{90} particle size is between about 40 microns to about 100 microns. In another embodiment, the D_{90} particle size is between about 30 microns to about 50 microns. In another embodiment, the D_{90} particle size is between about 50 microns to about 150 microns. In another embodiment, the D_{90} particle size is between about 75 microns to about 125 microns.

These solid state forms of eplerenone of the present invention may be administered by any suitable route, preferably in the form of a pharmaceutical composition adapted to such a route, and in a dose effective for the treatment intended. The solid state forms of eplerenone and

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compositions thereof may, for example, be administered orally, intravascularly, intraperitoneally, subcutaneously, intramuscularly or topically.

The amount of eplerenone that is administered and the dosage regimen for treating a condition or disorder with the solid state forms of eplerenone and/or compositions of this invention depends on a variety of factors, including the age, weight, sex and medical condition of the subject, the severity of the disease, the route and frequency of administration, and the particular compound employed, and thus may vary widely. The pharmaceutical compositions may contain eplerenone in the range of about 0.1 to 2000 mg, preferably in the range of about 0.5 to 500 mg and most preferably between about 1 and 200 mg. A daily dose of about 0.01 to 100 mg/kg body weight, preferably between about 0.5 and about 20 mg/kg body weight and most preferably between about 0.1 to 10 mg/kg body weight, may be appropriate. The daily dose can be administered in one to four doses per day.

For oral administration, the pharmaceutical composition may be in the form of, for example, a tablet, capsule, or suspension. The pharmaceutical composition is preferably made in the form of a dosage unit containing a particular amount of eplerenone. Examples of such dosage units are tablets or capsules. The eplerenone may also be administered by injection of a suspension wherein, for example, saline, dextrose or water may be used as a suitable carrier.

Other formulations may be in the form of a topical ointment or cream, or a suppository, containing eplerenone in a total amount of, for example, 0.075 to 30% w/w, preferably 0.2 to 20% w/w and most preferably 0.4 to 15% w/w. When formulated in an ointment, the eplerenone may be employed

with either paraffinic or a water-miscible ointment base. Alternatively, the eplerenone may be formulated in a cream with an oil-in-water cream base. If desired, the aqueous phase of the cream base may include, for example at least 30% w/w of a polyhydric alcohol such as propylene glycol, butane-1,3-diol, mannitol, sorbitol, glycerol, polyethylene glycol and mixtures thereof. The topical formulation may desirably include a compound which enhances absorption or penetration of the eplerenone through the skin or other affected areas. Examples of such dermal penetration enhancers include dimethylsulfoxide and related analogs.

The novel solid state forms of eplerenone also can be administered by a transdermal device. Preferably, topical administration will be accomplished using a patch either of the reservoir and porous membrane type or of a solid matrix variety. In either case, the eplerenone is delivered continuously from the reservoir or microcapsules through a membrane into the eplerenone permeable adhesive, which is in contact with the skin or mucosa of the recipient. If the eplerenone is absorbed through the skin, a controlled and predetermined flow of the eplerenone is administered to the recipient. In the case of microcapsules, the encapsulating agent may also function as the membrane.

The oily phase of the emulsions of this invention may be constituted from known ingredients in a known manner. While the phase may comprise merely an emulsifier, it may comprise a mixture of at least one emulsifier with a fat or an oil or with both a fat and an oil. Preferably, a hydrophilic emulsifier is included together with a lipophilic emulsifier which acts as a stabilizer. It is also preferred to include both an oil and a fat. Together, the emulsifier(s) with or

without stabilizer(s) make-up the so-called emulsifying wax, and the wax together with the oil and fat make up the so-called emulsifying ointment base which forms the oily dispersed phase of the cream formulations. Emulsifiers and emulsion stabilizers suitable for use in the formulation of the present invention include Tween 60, Span 80, cetostearyl alcohol, myristyl alcohol, glyceryl monostearate, and sodium lauryl sulfate, among others.

The choice of suitable oils or fats for the formulation is based on achieving the desired cosmetic properties, since the solubility of the eplerenone in most oils likely to be used in pharmaceutical emulsion formulations is very low. Thus, the cream should preferably be a non-greasy, non-staining and washable product with suitable consistency to avoid leakage from tubes or other containers. Straight or branched chain, mono- or dibasic alkyl esters such as di-isoadipate, isocetyl stearate, propylene glycol diester of coconut fatty acids, isopropyl myristate, decyl oleate, isopropyl palmitate, butyl stearate, 2-ethylhexyl palmitate or a blend of branched chain esters may be used. These may be used alone or in combination depending on the properties required. Alternatively, high melting point lipids such as white soft paraffin and/or liquid paraffin or other mineral oils can be used.

Formulations suitable for topical administration to the eye also include eye drops wherein the eplerenone is suspended in suitable carrier, especially an aqueous carrier for the eplerenone. The eplerenone is preferably present in such formulations in a concentration of 0.5 to 20%, advantageously 0.5 to 10% and particularly about 1.5% w/w.

For therapeutic purposes, the novel solid state forms of eplerenone ordinarily are combined with one or more adjuvants appropriate to the indicated route of administration. If administered per os, the compound may be admixed with lactose, sucrose, starch powder, cellulose esters of alkanoic acids, cellulose alkyl esters, talc, stearic acid, magnesium stearate, magnesium oxide, sodium and calcium salts of phosphoric and sulfuric acids, gelatin, acacia gum, sodium alginate, polyvinylpyrrolidone, and/or polyvinyl alcohol, and then tableted or encapsulated for convenient administration. Such capsules or tablets may contain an immediate release formulation, or may contain a controlled-release formulation such as a dispersion of the eplerenone in hydroxypropylmethyl cellulose. Formulations for parenteral administration may be in the form of aqueous or non-aqueous isotonic sterile injection suspensions. These suspensions may be prepared from sterile powders or granules having one or more of the carriers or diluents mentioned for use in the formulations for oral administration. Other adjuvants and modes of administration are well and widely known in the pharmaceutical art.

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Methods of Treatment or Prophylaxis

The present invention also comprises methods for the treatment and/or prophylaxis of an aldosterone-mediated condition and/or disorder such as hypertension in a subject; the methods comprising treating the subject having or susceptible to such condition or disorder with a therapeutically-effective amount of Form I, Form II, solvated crystalline form, or an amorphous form of eplerenone, or combinations thereof. These solid state forms of eplerenone

would be useful for the treatment and/or prophylaxis of a condition and/or disorder in a subject where administration of an aldosterone-antagonist is indicated including, but not limited to, the treatment of hyperaldosteronism such as hypertension, heart failure including cardiac insufficiency, cirrhosis of the liver, excess collagen/fibrosis, benign prostate hypertrophy and depression.

Besides being useful for human treatment, these solid state forms of eplerenone are also useful for veterinary treatment of companion animals, exotic animals and farm animals, including mammals, rodents, and the like. More preferred animals include horses, dogs, and cats.

These solid state forms of eplerenone also may be used (i) in combination therapies partially or completely in place of other conventional aldosterone receptor antagonists, or (ii) in combination therapies with other active drugs. The phrase "combination therapy" is intended to embrace the administration of each agent in a sequential manner in a regimen that will provide beneficial effects of the drug combination, and is intended as well to embrace co-administration of these agents in a substantially simultaneous manner, such as in a single capsule or injection having a fixed ratio of these active agents or in multiple, separate capsules or injections for each agent. Non-limiting examples of combination therapy include the treatment of cardiovascular diseases using a combination of an aldosterone receptor antagonist and an angiotensin II receptor antagonist as described in WO96/24373; the treatment of congestive heart failure using a combination of an aldosterone receptor antagonist and an angiotensin II antagonist as described in WO96/40257; and the treatment of heart failure using a

combination of an aldosterone receptor antagonist, an ACE inhibitor and a diuretic as described in WO96/24372.

Working Examples

The following examples contain detailed descriptions of the methods of preparation of the various solid state forms of eplerenone described in this application. These detailed descriptions fall within the scope, and serve to exemplify the invention. These detailed descriptions are presented for illustrative purposes only and are not intended as a restriction on the scope of the invention. All parts are by weight and temperatures are in degrees Centigrade unless otherwise indicated. The eplerenone starting material used in each of the following examples was prepared in accordance with scheme 1 set forth in Ng et al., WO98/25948.

Example 1: Preparation of (a) methyl ethyl ketone solvate from high purity eplerenone starting material and (b) Form II crystalline eplerenone from resulting solvate

A. Preparation of Methyl Ethyl Ketone Solvate:

High purity eplerenone (437 mg; greater than 99% purity with less than 0.2% diepoxide and 11,12 epoxide present) was dissolved in 10 mL of methyl ethyl ketone by heating to boiling on a hot plate with magnetic stirring at 900 rpm. The resulting solution was allowed to cool to room temperature with continuous magnetic stirring. Once at room temperature, the solution was transferred to a 1°C bath with maintenance of the stirring for one hour. After one hour, the solid methyl ethyl ketone solvate was collected by vacuum filtration.

B. Preparation of Form II crystalline eplerenone:

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The solid methyl ethyl ketone solvate prepared in Step A above was dried in an oven at 100°C for four hours at ambient pressure. The dried solid was determined to be pure Form II by DSC and XPRD analysis.

Example 2: Preparation of additional solvates from high purity eplerenone starting material

Additional solvated crystalline forms were prepared by replacing methyl ethyl ketone with one of the following solvents: n-propanol, 2-pentanone, acetic acid, acetone, butyl acetate, chloroform, ethanol, isobutanol, isobutyl acetate, isopropanol, methyl acetate, ethyl propionate, n-butanol, n-octanol, propyl acetate, propylene glycol, t-butanol, tetrahydrofuran, and toluene and carrying out the crystallization substantially as described above in Step A of Example 1.

Example 3: Preparation of Methyl Ethyl Ketone Solvate by Vapor Diffusion Growth

Eplerenone (400 mg; greater than 99.9% purity) was dissolved in 20 mL of methyl ethyl ketone by warming on a hot plate to form a stock solution. An 8 mL amount of the stock solution was transferred to a first 20 mL scintillation vial and diluted to 10 mL with methyl ethyl ketone (80%). A 10 mL amount of the stock solution was transferred to a second 20 mL scintillation vial and diluted to 10 mL with methyl ethyl ketone (40%). The final 2 mL of the stock solution was diluted to 10 mL with methyl ethyl ketone (20%). The four vials containing the dilutions were transferred to a dessicator jar containing a small amount of hexane as an anti-solvent. The dessicator jar was sealed and the hexane

vapor allowed to diffuse into the methyl ethyl ketone solutions. Methyl ethyl ketone solvate crystals grew in the 80% dilution sample by the next day.

Example 4: Preparation of Methyl Ethyl Ketone Solvate by Rotary Evaporation

About 400 mg of eplerenone (greater than 99.9% purity) is weighed into a 250 mL round bottom flask. Solvent (150 mL) is added to the flask and, if necessary, the solution is heated gently until the solid is dissolved. The resulting clear solution is placed on a Buchi rotary evaporator with a bath temperature of about 85°C and the solvent is removed under vacuum. Solvent removal is stopped when approximately 10 mL of solvent remain in the round bottom flask. The resulting solids are analyzed by appropriate method (XPRD, DSC, TGA, microscopy, etc.) for determination of form.

Example 5: Slurry Conversion

Approximately 150 mg of Form II eplerenone and 150 mg of Form I eplerenone were added to 5 mL of ethyl acetate. The resulting slurry was allowed to stir at 300 rpm (magnetic stirring) overnight. The next day a sample of the solid was collected by filtration. Analysis of the sample by XPRD indicated that the sample was entirely composed of Form II eplerenone.

Example 6: Preparation of (a) solvate from low purity eplerenone starting material and (b) Form I crystalline eplerenone from resulting solvate

Samples containing varying amounts of the impurity 7-methyl hydrogen 4α,5α:9α,11α-diepoxy-17-hydroxy-3-oxo-17α-

pregnane-7α,21-dicarboxylate, γ-lactone (the "diepoxide") or the impurity 7-methyl hydrogen 11α,12α-epoxy-17-hydroxy-3-oxo-17α-pregn-4-ene-7α,21-dicarboxylate, γ-lactone (the "11,12-epoxide") were prepared by adding the desired amount of the impurity to a 7 mL scintillation vial together with an amount of eplerenone sufficient to provide a total sample mass of 100 mg. The weight percent of the diepoxide or 11,12-epoxide in each sample is given in Tables X-6A and X-6B, respectively. A micro-flea magnetic stirrer was added to each scintillation vial along with 1 mL of methyl ethyl ketone. The vials were loosely capped and the solid dissolved by heating to reflux on a hot plate with magnetic stirring. Once the solids were dissolved, the solutions were allowed to cool to room temperature on the hot plate. Magnetic stirring was maintained during the cooling period. After the solutions reached room temperature, the solids were collected by vacuum filtration and immediately analyzed by X-ray powder diffraction (XPRD). The solids were then placed in a 100°C oven and dried for one hour at ambient pressure. The dried solids were analyzed by XPRD for Form I content by monitoring the area of the Form I diffraction peak at about 12.1 degrees two theta. All XPRD diffraction patterns were recorded using an Inel Multipurpose Diffractometer.

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TABLE X-6A

Weight Percent Diepoxide	Weight Eplerenone (mg)	Weight Diepoxide (mg)
0%	100.44	--
1%	99.08	1.24
2%	98.09	2.24
3%	97.08	3.04
5%	95.09	5.04

TABLE X-6B

Weight Percent 11,12-Epoxide	Weight Eplerenone (mg)	Weight 11,12-Epoxide (mg)
0%	101.38	0
1%	99.23	1.10
5%	94.97	5.36
10%	90.13	10.86

A. Diepoxide Results

Fig. 13 shows the X-ray powder diffraction patterns for the wet cake (methyl ethyl ketone solvate) obtained from the (a) 0%, (b) 1%, (c) 3%, and (d) 5% diepoxide-doped methyl ethyl ketone crystallizations. The peak intensities have been

normalized for ease of comparison. No peaks characteristic of Form I or the diepoxide are present in the diffraction patterns. The patterns are characteristic of the methyl ethyl ketone solvate of eplerenone.

Fig. 14 shows the X-ray powder diffraction patterns for the dried solids obtained from the (a) 0%, (b) 1%, (c) 3%, and (d) 5% diepoxide-doped methyl ethyl ketone crystallizations. The peak intensities have been normalized for ease of comparison. No Form I was detected for the dried samples corresponding to the methyl ethyl ketone crystallizations performed at doping levels of 0 and 1%. Form I was detected in the dried samples corresponding to the methyl ethyl ketone crystallizations performed at doping levels of 3 and 5%. The area for the Form I diffraction peak at about 12.1 degrees two theta and the estimated Form I content for each sample are given in Table X-6C below.

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TABLE X-6C

Weight Percent of Diepoxide in Starting Eplerenone Mixture	Weight Percent of Diepoxide in Resulting Crystals (HPLC)	Form I Peak Area 12° Two Theta Peak	Estimated Weight Percent of Form I
0%	---	None Detected	None Detected
1%	0.29%	None Detected	None Detected
3%	0.58%	1168	10%
5%	1.05%	4175	30%

The results reported in Table X-6C confirm that the presence of the diepoxide affects the formation of Form I during the desolvation. These results indicate that the diepoxide is effective in inducing the formation of Form I

eplerenone when it is incorporated into and/or adsorbed onto the methyl ethyl ketone solvate crystals.

The 3% diepoxide doping experiment was repeated to analyze the impact of the route of preparation on the amount of Form I formed during the desolvation. In this experiment, the methyl ethyl ketone solvate obtained from the doped crystallization was divided into two portions. The first portion was left untreated while the second portion was lightly ground in a mortar and pestle to induce a higher level of crystal defects. The two portions were both dried at 100 °C for one hour at ambient pressure. The dried solids were analyzed by XPRD. The XPRD patterns are given in Fig. 15 for the dried solids from the methyl ethyl ketone crystallization with 3% doping of diepoxide (a) without grinding of the solvate prior to drying, and (b) with grinding of the solvate prior to drying. The XPRD patterns indicated a greater amount of Form I in the ground sample relative to the unground sample. These results suggest that the conditions under which the methyl ethyl ketone solvate is isolated and handled can affect the crystal form that results from the desolvation.

B. 11,12-Epoxide Results

Fig. 16 shows the X-ray powder diffraction patterns for the wet cake (methyl ethyl ketone solvate) obtained from the (a) 0%, (b) 1%, (c) 5%, and (d) 10% 11,12-epoxide-doped methyl ethyl ketone crystallizations. The peak intensities have been normalized for ease of comparison. No peaks characteristic of Form I or the 11,12-epoxide are present in the diffraction patterns. The patterns are characteristic of the methyl ethyl ketone solvate of eplerenone.

Fig. 17 shows the X-ray powder diffraction patterns for the dried solids obtained from the (a) 0%, (b) 1%, (c) 5%, and (d) 10% 11,12-epoxide-doped methyl ethyl ketone crystallizations. The peak intensities have been normalized for ease of comparison. No Form I was detected for the dried samples corresponding to the methyl ethyl ketone crystallizations performed at doping levels of 0, 1% and 5%. Form I was detected in the dried samples corresponding to the methyl ethyl ketone crystallization performed at a doping level of 10%. The area for the Form I diffraction peak at 12.1 degrees two theta and estimated Form I content for each sample are given in Table X-6D.

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TABLE X-6D

Weight Percent 11,12-Epoxide in Starting Eplerenone Mixture	Weight Percent 11,12-Epoxide in Resulting Crystals (HPLC)	Form I Peak Area 12° Two Theta Peak	Estimated Weight Percent of Form I
0%	Not Available	None Detected	None Detected
1%	Not Available	None Detected	None Detected
5%	Not Available	None Detected	None Detected
10%	Not Available	1541	10-15%

The results reported in Table X-6D confirm that the presence of the 11,12-epoxide impacts the formation of Form I during the desolvation. The percentage of impurity in the methyl ethyl ketone crystallization required to induce the formation of Form I eplerenone appears to be greater for the 11,12-epoxide than for the diepoxide.

Example 7: Effect of Crystallization and Drying on Final Crystal Form

The following four experiments analyzing the effect of crystallization and drying on the final crystal form were conducted: (i) methyl ethyl ketone crystallization of eplerenone (2^3+3 statistical design of experiment), (ii) crystallization of poor quality mother liquor residue, (iii) crystallization of high purity eplerenone with form I seeding, and (iv) crystallization of low purity eplerenone with form II seeding. Variables in the design of the experiments included cooling rate, starting material purity level, and end point temperature of crystallization. For purposes of this Example, high purity eplerenone was defined as ultra-pure milled eplerenone (HPLC analysis showed this material to be 100.8% pure) and low purity eplerenone was defined as 89% pure eplerenone. To prepare the low purity eplerenone, stripped-down mother liquors from the process for the preparation of eplerenone were analyzed and blended to yield a material that was 61.1% eplerenone, 12.8% diepoxide and 7.6% 11,12-epoxide. This material was then blended with a sufficient amount of high purity eplerenone to yield the 89% eplerenone.

A. Methyl Ethyl Ketone Crystallization

In the methyl ethyl ketone crystallization experiment, all runs were performed using 60 g of high purity eplerenone. High endpoint was defined as 45°C and low endpoint was defined as 5°C. High cooling rate was defined as 3°C/minute cooling and low cooling rate was defined as 0.1°C/minute cooling. Center points were 1.5°C/minute cooling, 94.5% pure eplerenone, and a 25°C endpoint.

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After a background reading was taken with the FTIR, 250 mL of methyl ethyl ketone was charged to a 1L Mettler RC-1, MP10 reactor and stirred at 100 rpm. After several scans, eplerenone was charged to the reactor followed by an additional 470 mL of methyl ethyl ketone. Agitation was increased to 500 rpm to suspend solids and the batch temperature was increased to 80°C. The batch temperature was held at 80°C to ensure dissolution of the eplerenone. Black or white specks generally were visible in the resulting transparent solution. The batch temperature was then ramp cooled at the desired rate to the desired endpoint, where it was maintained for one hour before being pulled into a transfer flask and filtered. The vacuum was reactor, transfer flask and cake were then washed with 120 mL methyl ethyl ketone. Once the wash was pulled through the cake, the stopped. About 10 grams of each wet cake were dried in a vacuum oven under nominal conditions of 75°C with a light nitrogen bleed. For the "high, high, high" and "low, low, low" experiments described below, fluid bed drying was operated under high and low conditions. High fluid bed drying was defined as 100°C with a blower setting of "4" while low fluid bed drying was defined as 40°C with a blower setting of "1".

B. Crystallization of Poor Quality Mother Liquor Residue

In the crystallization of poor quality mother liquor residue experiment, 60 g of the 61.1% pure material and 720 mL methyl ethyl ketone were charged directly to a 1L Mettler RC-1, MP10 reactor. The 61.1% pure material was not blended with high purity eplerenone prior to being charged to the reactor. The resulting mixture was heated to 80°C and was an opaque

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slurry at that temperature. The crystallization continued and the mixture was filtered at 45°C under fast cooling conditions.

C. Form I Seeding

In the Form I seeding experiment, 60 g of pure (100.8%) eplerenone and 720 mL of methyl ethyl ketone were charged to a 1L Mettler RC-1, MP10 reactor. The mixture was heated to 80°C and then cooled to 25°C at a rate of 1.5° C/minute. When the solution had cooled to 62°C, it was seeded with 3 g of phase pure Form I crystals to initiate crystallization. The Form I seed crystals were prepared by the digestion process described in Example 9 below.

D. Form II Seeding

In the Form II seeding experiment, 66.6 g of 89.3% eplerenone (prepared by mixing 48.3 g of 100% eplerenone with 18.3 g of 61.1% eplerenone) and 720 mL of methyl ethyl ketone were charged to a 1L Mettler RC-1, MP10 reactor. The mixture was heated to 80°C and then cooled to 25°C at a rate of 1.5° C/minute. When the solution had cooled to 63°C, it was seeded with 3 g of phase pure Form II crystals to initiate crystallization. The Form II seed crystals were prepared by the crystallization and desolvation process described in Example 1 above.

Results from the experiments are reported in Table X-7A. In the n+1 crystallization experiment, Form I was detected only in the experiments employing low purity eplerenone where the product contained the diepoxide. Elevated levels of the diepoxide in the final product were also observed with higher cooling rates.

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The crystallization of poor quality mother liquor residue experiment yielded poor quality material that appeared to be a mixture of the diepoxide and Form I when analyzed by X-ray powder diffraction.

The Form I seeding experiment (where high purity eplerenone was seeded with Form I) yielded a product that was 77% Form I based on X-ray powder diffraction analysis, but entirely Form I based on DSC. The X-ray powder diffraction model, however, had not been tested for linearity beyond about 15% Form I. This experiment was the only one of the four experiments of this Example where Form I was created in the absence of the diepoxide.

The Form II seeding experiment (where low purity eplerenone was seeded with Form II) yielded a product that was entirely Form II.

The data obtained for the high fluid bed drying of eplerenone appeared to correspond to the data obtained for the vacuum oven drying. The low fluid bed dryings yielded results that differed from those of the vacuum oven dryings.

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TABLE X 7A

Cooling Rate	Cooling Endpoint	Impurity Level	Nucleation Temperature (°C)	Weight Percent 11 12 Epoxide	Weight Percent Dieposide	Assay For Desolvated Crystal	Percent Yield	Weight Percent Form I (XPRD)
+	+		57 0	ND	ND	100 3	66 1	ND
+			54 9	ND	ND	100 3	98 1	ND
	+		60 9	ND	ND	100 3		ND
			63 4	ND	ND	100 5	79 3	ND
+	+	++	N/A	4 8	36 6	43 3	27	100[5]
+	+	+	52 2	0 49	0 88	98 3	62	29
+		+	53 3	0 56	1 0	98 1	87	9
0	0	0	59 0	0 18	0 36	99 4	75	5
	+	+	63 3	0 20	0 44	99 4	36	31
		+	61 4	0 18	0 40	99 5	87	ND
0	0	0	60 6	0 18	0 36	99 5	79 2	ND
0	0	0	55 9	0 38	0 80	98 6	80 5	<3%
0	0	100 8% eplerenone seeded with Form I		0 03	ND	100 4	82 2	77/100[6]
0	0	89 3% eplerenone seeded with Form II		0 33	0 50	97 5	80 2	ND

[1]Cooling Rate: (+) = 3°C/min (0) = 1 5 °C/min ; and ( ) = 0 1 °C/min
[2]Cooling Endpoint (+) = 45°C (0) = 25 °C and ( ) = 5°C
[3]Impurity Level: (+) = 89 3% purity eplerenone starting material; (++) = 61 1% purity eplerenone starting material (0) = 100 8% purity eplerenone starting material and ( ) = 94 5% purity eplerenone starting material
[4]Weight percent after drying solvate in a vacuum oven at 75 C
[5]Appears to be mixture of Form I and dieposide when analysed by XPRD
[6]Appears to be 77% Form I when analysed by XPRD and 100% Form I when analysed by DSC

A. Material Purity

A cube plot of product purity, starting material purity, cooling rate and endpoint temperature based on the data reported in Table X-7A is shown in Fig. 18. The cube plot suggests that the use of a higher purity material at the start of crystallization will yield a higher purity product. The endpoint temperature of crystallization does not appear to greatly affect the product purity. The cooling rate, however, appears to have an effect with slightly less pure product resulting from a faster cooling rate. In fact, the level of diepoxide generally was higher with faster cooling rates.

Fig. 19 shows a half normal plot that was prepared using the results of cube plot to determine which variables, if any, had a statistically significant effect on the product purity. Starting material purity had the greatest statistically significant effect on product purity, although cooling rate and the interaction between cooling rate and starting material purity were also seen as statistically significant effects.

Fig. 20 is an interaction graph based on these results and showing the interaction between starting material purity and cooling rate on product purity. With the high purity eplerenone (100.8% eplerenone starting material) the cooling rate appears to have little or no effect on final purity. With the low purity eplerenone (89.3% eplerenone starting material), however, the product purity decreases as cooling rate increases. This result suggests that more impurities crystallize out in eplerenone crystallizations conducted at higher cooling rates.

B. Form I Content

A cube plot of Form I weight fraction, starting material product purity, cooling rate and endpoint temperature based on the data reported in Table X-7A is shown in Fig. 21. The cube plot suggests that the use of a higher purity eplerenone at the start of crystallization will yield a lower amount of Form I. The endpoint temperature of crystallization also appears to have an effect on the form of the final product. The cooling rate does not appear to greatly affect the formation of Form I although some Form I may result from faster cooling at the low endpoint temperature in the presence of impurities.

Fig. 22 shows a half normal plot that was prepared using the results of the cube plot to determine which variables, if any, had a statistically significant effect on the amount of Form I in the final material. Starting material purity, endpoint temperature of the crystallization and the interaction between the two variables were seen as statistically significant effects.

Fig. 23 is an interaction graph based on these results and showing the interaction between starting material purity and endpoint temperature on final Form I content. With the high purity eplerenone (100.8% eplerenone starting material), endpoint temperature appears to have little effect on Form I content. No Form I resulted in either case with pure eplerenone. With low purity eplerenone (89.3% eplerenone starting material), however, Form I was present in both cases, with significantly more Form I at higher endpoint temperatures.

Table X-7B reports the weight fraction of Form I measured in materials dried using either a fluid bed (LAB-LINE/P.R.L.

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Hi-Speed Fluid Bed Dryer, Lab-Line Instruments, Inc.) or a vacuum oven (Baxter Scientific Products Vacuum Drying Oven, Model DP-32). Similar Form I content was observed for comparable materials dried in either the high fluid bed or the vacuum oven. A difference was observed, however, for comparable materials dried in the low fluid bed relative to the vacuum oven.

TABLE X-7B

Cooling Rate	End Point	Impurity Level	Drying Type	Weight Percent Form I
High	High	High	Vacuum Oven	29%
High	High	High	High Fluid Bed	25%
High	High	High	Low Fluid Bed	4.7%
Low	Low	Low	Vacuum Oven	ND
Low	Low	Low	High Fluid Bed	ND
Low	Low	Low	Low Fluid Bed	5.5%

Example 8: Crystallization of a Mixture of Form I and Form II From Methyl Ethyl Ketone To Prepare a Solvate, and (b) Desolvation of the Solvate to Prepare Form II

Form I eplerenone (10 g) was combined with 80 mL of methyl ethyl ketone. The mixture was heated to reflux (79°C) and stirred at this temperature for about 30 minutes. The resulting slurry was then cooled with a stepwise, holdpoint protocol by maintaining the slurry at 65°C, 50°C, 35°C and 25°C for about 90 minutes at each temperature. The slurry was filtered and rinsed with about 20 mL methyl ethyl ketone. The isolated solid was initially dried on the filter and then in a vacuum oven at 40-50°C. The drying was completed in the vacuum oven

at 90-100°C. The desolvated solid was obtained with an 82% recovery. XPRD, MIR and DSC confirmed that the solid had a Form II crystalline structure.

Example 9: Digestion of Low Purity Eplerenone Starting Material With a Solvent to Prepare Form I

A. Digestion With Ethanol Solvent:

Low purity eplerenone (24.6 g; 64% by weight assay via HPLC) was combined with 126 mL of ethanol 3A. The slurry was heated to reflux and the distillate removed. An additional 126 mL of ethanol 3A was simultaneously added as 126 ml of solvent was removed via atmospheric distillation. Upon completion of the solvent turnover, the mixture was cooled to 25 °C and stirred for one hour. The solid was filtered and rinsed with ethanol 3A. The solid was air-dried to give the ethanol solvate. The solvate was further dried in a vacuum oven at 90-100°C for six hours to obtain 14.9 g of Form I eplerenone.

B. Digestion With Methyl Ethyl Ketone Solvent

In an alternative digestion process, 1 gram of low purity eplerenone (about 65% pure) was digested in 4 mL of methyl ethyl ketone for two hours. After the two hours, the mixture was allowed to cool to room temperature. Once cooled, the solid was collected by vacuum filtration and determined to be the methyl ethyl ketone solvate by XPRD analysis. The solid was dried at 100°C for 30 to 60 minutes. The dried solids were determined to be pure Form I by XPRD.

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Example 10: Digestion of High Purity Eplerenone Starting Material With a Solvent to Prepare Form II

A. Digestion With Ethanol Solvent:

High purity eplerenone (1 gram) was digested in 8 mL of ethanol for approximately two hours. The solution was then allowed to cool to room temperature and the solids were collected by vacuum filtration. Analysis of the solids by XPRD immediately after filtration indicated that the solids were a solvate (presumably the ethanol solvate). The solids were subsequently dried at 100°C at atmospheric pressure for 30 minutes. The dried solid was analyzed by XPRD and determined to be predominately Form II (no Form I detected).

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B. Digestion with Methyl Ethyl Ketone Solvent:

High purity eplerenone (1 gram) was digested in 4 mL of methyl ethyl ketone for two hours. After the two hours, the solution was allowed to cool to room temperature and the solids collected by vacuum filtration. The solid was immediately analyzed by XPRD and determined to be a solvate of eplerenone (presumably the methyl ethyl ketone solvate). The solvate was subsequently dried at 100°C at ambient pressure for 30 to 60 minutes. The dried solids were analyzed by XPRD and determined to be primarily Form II with no diffraction peaks for Form I present.

Example 11: Crystallization of Form II Directly From Solution

Procedure A: Eplerenone (2.5 g) was dissolved in ethyl acetate by heating to 75°C. Once the eplerenone dissolved,

the solution was held at 75°C for 30 minutes to ensure complete dissolution. The solution was then cooled at 1 °C/min to 13°C. Once at 13°C, the slurry was allowed to stir for two hours at 750 rpm with an overhead stirrer. The crystals were collected by vacuum filtration and dried in a vacuum oven at 40°C for one hour. The XPRD pattern and DSC thermogram of the solid were characteristic of Form II eplerenone. Thermal gravimetric analysis (TGA) of the solid indicated no weight loss from the solid up to 200°C.

Procedure B: In an alternative procedure, 2 g of eplerenone was dissolved in 350 mL of 15/85% acetonitrile/water by heating on a hot plate with magnetic stirring. Once the eplerenone was dissolved, the solution was allowed to cool to room temperature overnight with magnetic stirring. The resulting solid was collected by vacuum filtration. The crystals were birefringent and had a triangular, plate-like crystal habit. The solid had an XPRD and DSC characteristic of Form II eplerenone. TGA indicated no weight loss up to 200°C.

Procedure C: In an alternative procedure, 640 mg of eplerenone was placed in a 50 mL flask with 20 mL of ethyl benzene. The resulting slurry was heated to 116°C and became a clear solution. The clear solution was cooled to 25°C over 30 minutes. Nucleation began at 84°C during the cooling period. The resulting solids were filtered from the solution and air-dried to give 530 mg of solids (83% recovery). Hot-stage microscopy and XPRD confirmed that the solids were Form II crystals.

Procedure D: In an alternative procedure, 1.55 g of eplerenone was added to 2.0 mL of nitrobenzene and heated to 200°C. The resulting slurry was stirred overnight at 200°C. The solution was allowed to cool to room temperature (natural air convection) the following day and the solid was isolated. The solid was determined to be Form II eplerenone by XPRD and polarized light microscopy.

Procedure E: In an alternative procedure, 5.0 g of eplerenone (purity greater than 99%) was added to 82 g of methanol (104 mL). Under stirring action (210 rpm), the solution was heated to 60°C and held at that temperature for 20 minutes to ensure complete dissolution. The solution was then cooled to -5°C at a rate of 0.16°C/minute under stirring. The crystals were collected by filtration and dried in a vacuum oven at 40°C for 20 hours. The dried solids were determined to be pure Form II eplerenone by DSC and XPRD analysis.

Procedure F: In an alternative procedure, 6.0 g of eplerenone (ethanol solvate containing 9% ethanol and having a corrected purity of 95.2%) was added to 82 g of methanol (104 mL). Under stirring action (210 rpm), the solution was heated to 60°C and held at that temperature for 20 minutes to ensure complete dissolution. The solution was then cooled to 50°C at a rate of 0.14°C/minute and then held at that temperature for about 2.5 hours. The solution was then cooled to -5°C at a rate of 0.13°C/minute under stirring. The crystals were collected by filtration and dried in a vacuum

oven at 40°C for 16 hours. The dried solids were determined to be pure Form II eplerenone by DSC and XPRD analysis.

Example 12: Crystallisation of Form I Directly From Solution

150.5 mg of the diepoxide and 2.85 g of eplerenone were added to 1.5 mL of nitrobenzene. The mixture was magnetically stirred at 200°C for several hours. The slurry was then allowed to cool to room temperature by natural air convection. The sample was dried and analyzed by polarized light microscopy and XPRD. The XPRD indicated that the sample was a mixture of Form I and Form II. The crystals were translucent by microscopy, indicating that desolvation (and conversion to either Form I or Form II) did not occur.

Example 13: Preparation of Amorphous Eplerenone By Comminution

Approximately one-half of a steel Wig-L-Bug container was filled with about 60 g of eplerenone (greater than 99.9% purity). A steel ball and cap were placed on the sample container and agitated for 30 seconds by the Wig-L-Bug apparatus. The eplerenone was scraped off the surface of the Wig-L-Bug container and the container agitated for an additional 30 seconds. The resulting solid was analyzed by XPRD and DSC and determined to be a mixture of amorphous eplerenone and Form II crystalline eplerenone.

Example 14: Preparation of Amorphous By Lyophilisation

Approximately 100 mg of crude eplerenone was weighed into a beaker containing 400 mL of water. The solution was heated slightly for five minutes, and then sonicated and heated with stirring for an additional five minutes.

Approximately 350 mL of the eplerenone solution was filtered into a 1000 mL round bottom flask containing 50 mL of HPLC water. The solution was flashed frozen in a dry ice/acetone bath over a time period of one to two minutes. The flask was attached to a Labconco Freezone 4.5 freeze dryer and dried overnight. The solids in the flask were transferred to a small brown bottle. A small aliquot was observed under polarized light microscopy at 10X, 1.25X optivar in cargille oil (1.404) and observed to be at least 95% amorphous eplerenone. Figures 24 and 25 show the XPRD pattern and DSC thermogram obtained for the amorphous eplerenone. The peak observed at 39 degrees two theta in Figure 24 is attributable to the aluminum sample container.

Example 15: Measurement of Form II Solubility

The aqueous solubility of Form II eplerenone was measured at pH 7 (100 mM phosphate buffer) at 5, 25, and 40°C. Approximately 30 mg of Form II eplerenone was mixed with approximately 10 mL of buffer to form a slurry of eplerenone at both 5 and 25 °C. Approximately 40 mg of Form II eplerenone was mixed with approximately 10 mL of buffer to form a slurry of eplerenone at 40 °C. Samples were prepared in duplicate for each condition. The slurries were allowed to equilibrate in appropriate temperature water shaker baths and the solutions were analyzed for eplerenone content by ultraviolet visible analysis (245 nm) at the 1, 5, 12, 19, 27, and 36 day time intervals. Data from each condition was appropriately averaged to determine the solubility of eplerenone at each condition and is reported in Table X-15. The residual solids from each time point were analyzed by DSC and TGA at the end of the 36 day equilibration and were

determined to be Form II.

TABLE X-15

Temperature (°C)	Form II Solubility
5	0.24 mg/mL
25	0.29 mg/mL
40	0.39 mg/mL

Example 16: Measurement of Intrinsic Dissolution Rates

Intrinsic dissolution rates were measured for the following four eplerenone polymorph samples: (i) Form II eplerenone prepared by direct crystallization from acetonitrile using water as an anti-solvent in the same manner as Example 12, Procedure B, (ii) Form I eplerenone prepared by digestion in ethanol in the same manner as Example 10, Procedure A, (iii) a mixture of Form I (5 weight percent) and Form II (95 weight percent), and (iv) Form II eplerenone that was micronized to provide the following particle size distribution: 10% of the particles under 9 microns, 50% of the particles under 22 microns, and 90% of the particles under 41 microns.

Eplerenone (150 mg) was weighed and placed into a VanKel intrinsic dissolution cavity. The powder was compressed at 1200 psi using a Carver press. The sample was then mounted onto the intrinsic dissolution apparatus. The dissolution media used was 1% SDS in HPLC grade water. All experiments were conducted at 37°C for two hours. Before the start of the experiment, 500 mL of the dissolution medium was equilibrated at 37°C for 30 minutes in the dissolution bathing chamber. An initial sample was taken from each dissolution vessel which served as time zero for the experiment. The eplerenone

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tablets were then lowered into the dissolution medium. Samples were drawn at determined intervals for the determination of rate of dissolution. Care was taken to avoid air bubbles forming at the surface of the tablet. The samples were analyzed by UV absorbance detection at 243 nm. Intrinsic dissolution rates were calculated from the slope of the linear portion of the concentration versus time profiles corrected for volume and normalized for the surface area of the dissolution tablet (0.5 cm^2).

Fig. 26 reports the intrinsic dissolution rates measured for the four samples. These studies appear to indicate that Form I eplerenone has faster intrinsic dissolution rate than Form II eplerenone. X-Ray powder diffraction measurements comparing compressed against uncompressed eplerenone confirmed that the polymorphs did not interconvert upon compression or during the course of the dissolution studies.

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Example 17: Eplerenone Polymorph Composition

Tablets containing 25 mg, 50 mg, 100 mg and 200 mg doses of Form II eplerenone are prepared and have the following composition:

Ingredient	Weight % of Tablet
Form II Eplerenone	29.41
Form I Eplerenone	Not Detected
Lactose Monohydrate (#310, NF)	42.00
Microcrystalline Cellulose (NF, Avicel PH101)	18.09
Croscarmellose Sodium (NF, Ac-Di-Sol)	5.00
Hydroxypropyl Methylcellulose (#2910, USP, Pharmacoat 603)	3.00
Sodium Lauryl Sulfate (NF)	1.00
Talc (USP)	1.00
Magnesium Stearate (NF)	0.5
Total	100.00

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Example 18: Eplerenone Polymorph Composition

Capsules (hard gelatin capsule, #0) are prepared containing a 100 mg dose of eplerenone and have the following composition:

Ingredient	Amount (mg)
Form II Eplerenone	90.0
Form I Eplerenone	10.0
Lactose, Hydrous, NF	231.4
Microcrystalline Cellulose, NF	45.4
Talc, USP	10.0
Croscarmellose Sodium, NF	8.0
Sodium Lauryl Sulfate, NF	2.0
Colloidal Silicon Dioxide, NF	2.0
Magnesium Stearate, NF	1.2
Total Capsule Fill Weight	400.0

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Example 19: Eplerenone Polymorph Composition

Capsules (hard gelatin capsule, size #0) are prepared containing a 200 mg dose of eplerenone and have the following composition:

Ingredient	Amount (mg)
Form II Eplerenone	190.0
Form I Eplerenone	10.0
Lactose, Hydrous, NF	147.8
Microcrystalline Cellulose, NF	29.0
Talc, USP	10.0
Croscarmellose Sodium, NF	8.0
Sodium Lauryl Sulfate, NF	2.0
Colloidal Silicon Dioxide, NF	2.0
Magnesium Stearate, NF	1.2
Total Capsule Fill Weight	400.0

Example 20: Preparation of Milled Eplerenone

Dried methyl ethyl ketone solvate is first delumped by passing the solvate through a 20 mesh screen on a Fitzmill. The delumped solid is then pin milled using an Alpine Hosakawa stud disk pin mill operating under liquid nitrogen cooling at a feed rate of approximately 250 kilograms/hour. Pin milling produces milled eplerenone with a D_{90} size of approximately 65-100 microns.

Example 21: Effect of Eplerenone Particle Size (Dog Study)

The effect of the particle size of Form II eplerenone on eplerenone plasma concentrations and relative bioavailability was studied in a dog model. Four heathy female beagle dogs

weighing between 8 to 12 kg were intragastrically administered one immediate release capsule containing the formulation described in Table X-21A below followed by about 10 mL of water.

Table X-21A

INGREDIENT	WEIGHT % OF TABLET	Amount (mg)
Eplerenone	50.00	200.00
Lactose, Fast-Flo, Hydrous	36.95	147.80
Microcrystalline Cellulose (Avicel PH102)	7.25	29.00
Sodium Lauryl Sulfate	0.50	2.00
Croscarmellose Sodium	2.00	8.00
Talc	2.50	10.00
Colloidal Silicon Dioxide	0.50	2.00
Magnesium Stearate	0.30	1.20
Total	100.00	400.00
Capsules, Size #0, White Opaque	1 Capsule	

The dogs were fasted for 15 to 20 hours prior to administration of the capsule and were not fed again until at least 4 hours after dose administration. Blood samples (approximately 3 mL) were collected by venipuncture in chilled tubes containing heparin at 0, 0.5, 1, 2, 3, 4, 6, 8 and 24 hours after dose administration. The blood samples were immediately placed on ice. Separation of plasma from the blood samples was complete after about 15 minutes of centrifugation. The resulting plasma samples were frozen at about -20°C and stored until analyzed. Analysis was performed using an LC/MS/MS procedure.

This experiment was conducted using the same four dogs for three formulations that were identical except for the

particle size of the eplerenone starting material. The three formulations tested used eplerenone starting materials having a D_{90} particle size (that is, at least 90% of the particles were) less than about 212 microns, less than about 86 microns, and less than about 36 microns, respectively. A minimum of a five day wash-out period was allowed between administration of each of the formulations. Reducing the D_{90} particle size of the eplerenone starting material from about 212 microns to above 86 microns increased relative bioavailability by almost 100%. The mean results are reported in Tables X-21B and X-21C below.

Table X-21B

Time (hours)	Blood Serum Eplerenone Concentration (μg/mL)		
	D_{90} = 212 microns	D_{90} = 86 microns	D_{90} = 36 microns
0	0	0	0
0.5	1.83	3.65	1.99
1.0	2.40	6.18	5.86
2.0	3.77	6.89	6.77
3.0	2.85	5.70	6.60
4.0	2.61	4.39	5.56
6.0	1.63	3.11	3.31
8.0	1.10	1.90	2.09
24.0	0.0252	0.032	0.0706

Table X-21C

Pharmacokinetic Parameter	Pharmacokinetic Parameter Value		
	D_{90} = 212 microns	D_{90} = 86 microns	D_{90} = 36 microns
C_{max} (μg/mL)	3.98	7.02	7.39
T_{max} (hours)	1.50	1.75	2.25
AUC ((μg/mL)hr)	26.6	49.2	53.1
Relative Bioavailability (%)	53.25	100	107.9

Example 22: Effect of Eplerenone Particle Size (Human Study)

The effect of the particle size of a Form II eplerenone starting material used in a pharmaceutical composition on eplerenone plasma concentrations and relative bioavailability was studied in a human model. Subjects received single doses of Form II eplerenone medication on Days 1, 8, 15, 22 and 29 according to a randomization schedule. All medication was administered with 180 mL of water at 0800 hours. Blood samples for eplerenone pharmacokinetic analyses were collected at -0.5 (predose), 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 16, 24, 36 and 48 hours postdose. Urine samples were collected and pooled between the hours of 0-24 and 24-48 hour postdose.

Plasma and urinary concentrations of eplerenone were determined using a validated high performance liquid chromatography method with MS/MS Detection. Pharmacokinetic data obtained is reported in Table X-23A. The particle size distributions of the eplerenone used in the preparation of the medications was determined in the dry powder state using laser light scattering.

Table X-23A

Pharmacokinetic Parameter	Pharmacokinetic Parameter Value		
	100 mg Capsule A (D_{90}=40 microns)	100 mg Tablet (D_{90}=82 microns)	100 mg Capsule B (D_{90}=96 microns)
C_{max} (ng/mL)	1747	1704	1669
T_{max} (hours)	1.8	1.8	1.3
AUC ((ng/mL)hr)	11349	11945	11981

The specific composition of each medication is reported in Table X-23B below.

Table X-23B

INGREDIENT	WEIGHT % OF TABLET/CAPSULE		
	Capsule A	Tablet A	Capsule B
Form II Eplerenone	25	30	25
Lactose Monohydrate	–	42	57.86 (fast flo lactose)
Lactose, Hydrous NF	57.8	–	–
Microcrystalline Cellulose (Avicel PH101)	11.4	17.5 (7.5% intragranular plus 10% extragranular)	11.34 (Avicel PH 102)
Croscarmellose Sodium (Ac-Di-Sol)	2	5	2
Hydroxypropyl Methylcellulose (Pharmacoat 603)	–	3	–
Sodium Lauryl Sulfate	0.5	1	0.5
Talc	2.5	1	2.5
Magnesium Stearate	0.3	0.5	0.3
Colloidal Silicon Dioxide	0.5	–	0.5
Total	100	100	100

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All mentioned references are expressly incorporated by reference herein as if fully set forth at length. In addition, U.S. Provisional Application Serial No. 60/111,646 filed December 9, 1998, and the corresponding nonprovisional application, U.S. Application Serial No. 09/_____ filed December 8, 1999, are expressly incorporated by reference herein as if fully set forth at length.

When introducing elements of the present invention or the preferred embodiment(s) thereof, the articles "a", "an", "the" and "said" are intended to mean that there are one or more of the elements. The terms "comprising", "including" and "having" are intended to be inclusive and mean that there may be additional elements other than the listed elements.

Although this invention has been described with respect to specific embodiments, the details of these embodiments are not to be construed as limitations.

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WHAT IS CLAIMED IS:

1. The Form II crystalline form of eplerenone.

2. A form of Claim 1 having an X-ray powder diffraction pattern with a peak at 8.0 ± 0.2 degrees two theta.

3. A form of Claim 1 having an X-ray powder diffraction pattern with peaks at 8.0 ± 0.2, 12.4 ± 0.2, 12.8 ± 0.2, and 13.3 ± 0.2 degrees two theta.

4. A form of Claim 1 having an X-ray powder diffraction pattern without a significant peak at 12.0 ± 0.2.

5. A form of Claim 1 having an X-ray powder diffraction pattern substantially as shown in Figure 1-B.

6. A form of Claim 1 having a melting range from about 223°C to about 234°C.

7. A form of Claim 1 having a melting range from about 237°C to about 242°C.

8. A form of Claim 1 having an IR spectrum with a peak at about 1724 cm^{-1}.

9. A form of Claim 1 having an IR spectrum without a significant peak at about 1739 cm^{-1}.

10. A form of Claim 1 having an IR spectrum without a significant peak at about 1399 cm^{-1}.

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11. A form of Claim 1 having an IR spectrum substantially as shown in Figure 3-B.

12. A form of Claim 1 having a ^{13}C NMR spectrum with a carbon resonance at about 67.1 ppm.

13. A form of Claim 1 having a ^{13}C NMR spectrum with carbon resonances at about 16.0 ppm and about 67.1 ppm.

14. A form of Claim 1 having a ^{13}C NMR spectrum without a carbon resonance at about 24.7 ppm.

15. A form of Claim 1 having a ^{13}C NMR spectrum substantially as shown in Figure 5.

16. A form of Claim 1 having a melting range from about 223°C to about 234°C, and an IR spectrum with a peak at about 1724 cm^{-1}.

17. A form of Claim 1 having a melting range from about 223°C to about 234°C, and an IR spectrum with a peak at about 1724 cm^{-1} and without significant peaks at about 1399 cm^{-1} and about 1739 cm^{-1}.

18. A form of Claim 2 having a melting range from about 223°C to about 234°C; an IR spectrum with a peak at about 1724 cm^{-1} and without significant peaks at about 1399 cm^{-1} and about 1739 cm^{-1}; and a ^{13}C NMR spectrum with a carbon resonance at about 67.1 ppm.

19. A form of Claim 1 having a melting range from about 237°C to about 242°C, and an IR spectrum with a peak at about 1724 cm^{-1}.

20. A form of Claim 1 having a melting range from about 237°C to about 242°C, and an IR spectrum with a peak at 1722 cm^{-1} and without a significant peak at 1739 cm^{-1}.

21. A form of Claim 2 having a melting range from about 237°C to about 242°C; an IR spectrum with a peak at about 1724 cm^{-1} and without significant peaks at about 1399 cm^{-1} and about 1739 cm^{-1}; and a ^{13}C NMR spectrum with a carbon resonance at about 67.1 ppm.

22. A form of Claim 1 having a monoclinic crystal system.

23. A form of Claim 1 having a $P2_1$ space group.

23. A form of Claim 1 having unit cell a, b and c values of about 8.78 Å, about 11.14 Å, and about 11.06 Å, respectively.

25. A form of Claim 1 having unit cell α, β and γ values of about 90°, about 90.52°, and about 90°, respectively.

26. Particles of the form of Claim 1 wherein at least 90% of said particles have a particle size less than about

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400 microns.

27. The particles of Claim 26 wherein at least 90% of said particles have a particle size between about 25 microns to about 400 microns.

28. The particles of Claim 26 wherein at least 90% of said particles have a particle size between about 50 microns to about 150 microns.

29. The particles of Claim 26 wherein at least 90% of said particles have a particle size between about 75 microns to about 125 microns.

30. The particles of Claim 26 wherein at least 90% of said particles have a particle size between about 40 microns to about 100 microns.

31. The particles of Claim 26 wherein at least 90% of said particles have a particle size between about 30 microns to about 50 microns.

32. Eplerenone comprising the crystalline form of Claim 1, wherein said eplerenone has a phase purity of at least about 50%.

33. The eplerenone of Claim 32 wherein said eplerenone has a phase purity of at least about 90%.

34. The eplerenone of Claim 32 wherein said eplerenone is a substantially phase pure form of Claim 1.

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35. A pharmaceutical composition comprising a therapeutically-effective amount of the form of any of Claims 1 to 35 in association with at least one pharmaceutically-acceptable carrier, adjuvant or diluent.

36. A composition of Claim 35 wherein the only eplerenone crystalline form present in the composition is a form of Claim 1.

37. A method of treating or preventing an aldosterone-mediated condition or disorder in a subject, said method comprising administering to a subject having or susceptible to said condition or disorder a therapeutically-effective amount of the form of Claim 1.

38. The method of Claim 37 for use in treatment of hyperaldosteronism.

39. The method of Claim 37 for use in treatment of hypertension.

40. The method of Claim 37 for use in treatment of heart failure.

41. The method of Claim 37 for use in treatment of cirrhosis of the liver.

42. The method of Claim 37 for use in treatment of a condition selected from the group consisting of excess collagen/fibrosis, benign prostate hypertrophy and

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depression.

43. A method of preparing eplerenone comprising the form of Claim 1, the method comprising crystallizing eplerenone from a pharmaceutically acceptable solvent or mixture of solvents to directly yield that form.

44. The method of Claim 43 wherein the solvent or mixture of solvents comprises a solvent selected from the group consisting of methanol, ethyl acetate, isopropyl acetate, acetonitrile, nitrobenzene, water and ethyl benzene.

45. The method of Claim 43 wherein the eplerenone used in the crystallization has a chemical purity of at least about 99%.

46. The method of Claim 43 wherein the crystallization is carried out in the presence of a Form II growth promoter or Form I growth inhibitor.

47. The method of Claim 43 wherein the solvent or mixture of solvents is seeded with the form of Claim 1 prior to crystallizing the eplerenone.

48. The method of Claim 43 wherein at least about 50 weight percent of the crystallized eplerenone is in the form of Claim 1.

49. The method of Claim 43 wherein at least about 90 weight percent of the crystallized eplerenone is in the form of Claim 1.

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50. The method of Claim 43 wherein at least about 99 weight percent of the crystallized eplerenone is in the form of Claim 1.

51. A method of preparing eplerenone comprising the crystalline form of Claim 1, the method comprising:

crystallizing eplerenone from a pharmaceutically acceptable solvent or mixture of solvents to form a solvate; and

desolvating the solvate to yield the form of Claim 1.

52. The method of Claim 51 wherein the solvent or mixture of solvents comprises a solvent selected from the group consisting of methylethyl ketone, 2-pentanone, acetic acid, acetone, butyl acetate, chloroform, ethanol, isobutanol, isobutyl acetate, methyl acetate, ethyl propionate, n-butanol, n-octanol, n-propanol, isopropanol, propyl acetate, propylene glycol, t-butanol, tetrahydrofuran, toluene and t-butyl acetate.

53. The method of Claim 51 wherein the solvent or mixture of solvents comprises a solvent selected from the group consisting of 2-pentanone, acetic acid, acetone, butyl acetate, chloroform, isobutanol, isobutyl acetate, methyl acetate, ethyl propionate, n-butanol, n-octanol, n-propanol, propyl acetate, propylene glycol, t-butanol, tetrahydrofuran, toluene and t-butyl acetate.

54. The method of Claim 51 wherein the solvent or mixture of solvents comprises a solvent selected from the

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group consisting of methylethyl ketone and ethanol.

55. The method of Claim 51 wherein the eplerenone used in the crystallization has a chemical purity of at least about 99%.

56. The method of Claim 51 wherein the crystallization is carried out in the presence of a Form II growth promoter or Form I growth inhibitor.

57. The method of Claim 51 wherein the solvent or mixture of solvents is seeded with the form of Claim 1 prior to crystallizing the eplerenone.

58. The method of Claim 51 wherein at least about 50 weight percent of the crystallized eplerenone is in the form of Claim 1.

59. The method of Claim 51 wherein at least about 90 weight percent of the crystallized eplerenone is in the form of Claim 1.

60. The method of Claim 51 wherein at least about 99 weight percent of the crystallized eplerenone is in the form of Claim 1.

61. A method of preparing eplerenone comprising a crystalline form of Claim 1, the method comprising digesting eplerenone in a pharmaceutically acceptable solvent or mixture of solvents.

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62. The method of Claim 61 wherein the solvent comprises ethanol or methylethyl ketone.

63. The method of Claim 61 wherein at least about 50 weight percent of the eplerenone prepared is in the form of Claim 1.

64. The method of Claim 61 wherein at least about 90 weight percent of the eplerenone prepared is in the form of Claim 1.

65. The method of Claim 61 wherein at least about 99 weight percent of the eplerenone prepared is in the form of Claim 1.

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ABSTRACT OF THE DISCLOSURE

The novel Form II crystalline form of eplerenone is described. This crystalline form has unique chemical and physical properties relative to other solid state forms of eplerenone and is characterized by its x-ray powder diffraction pattern, infrared spectrum, nuclear magnetic resonance spectrum, differential scanning calorimetric thermogram, and other physical characterizations.

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Figure 1-A

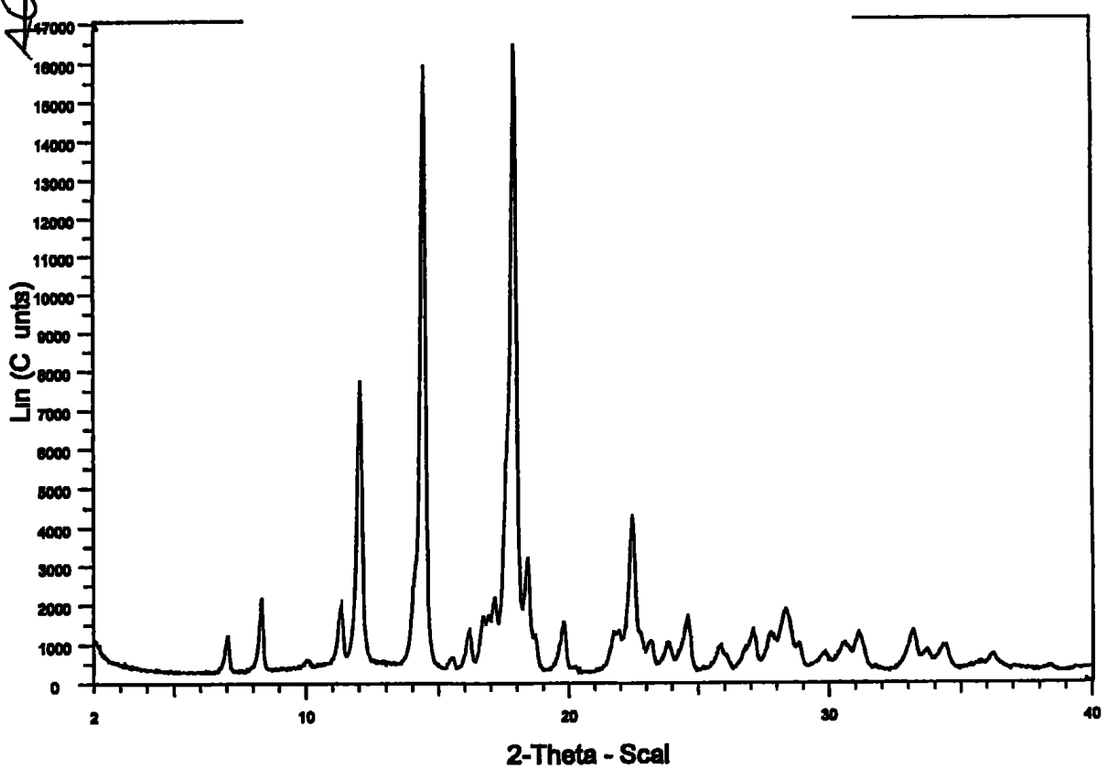

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Figure 1-B

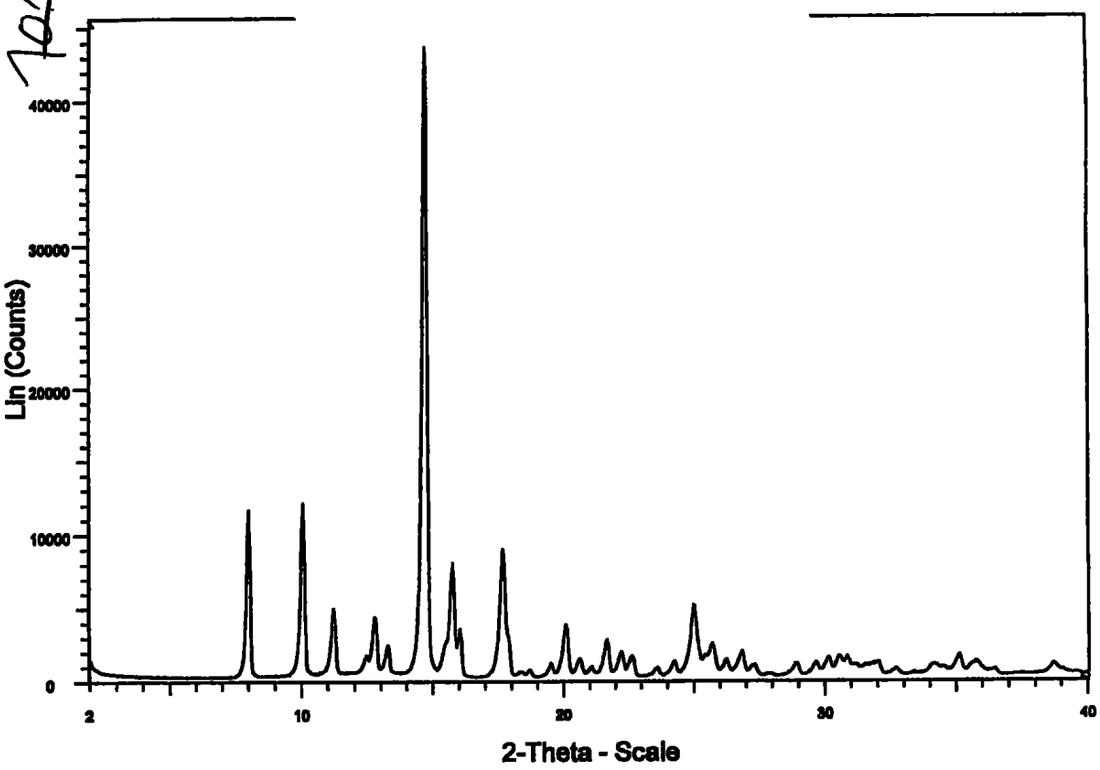
Lin (Counts)
40000
30000
20000
10000
0
2
10
20
30
40
2-Theta - Scale

Figure 1-C

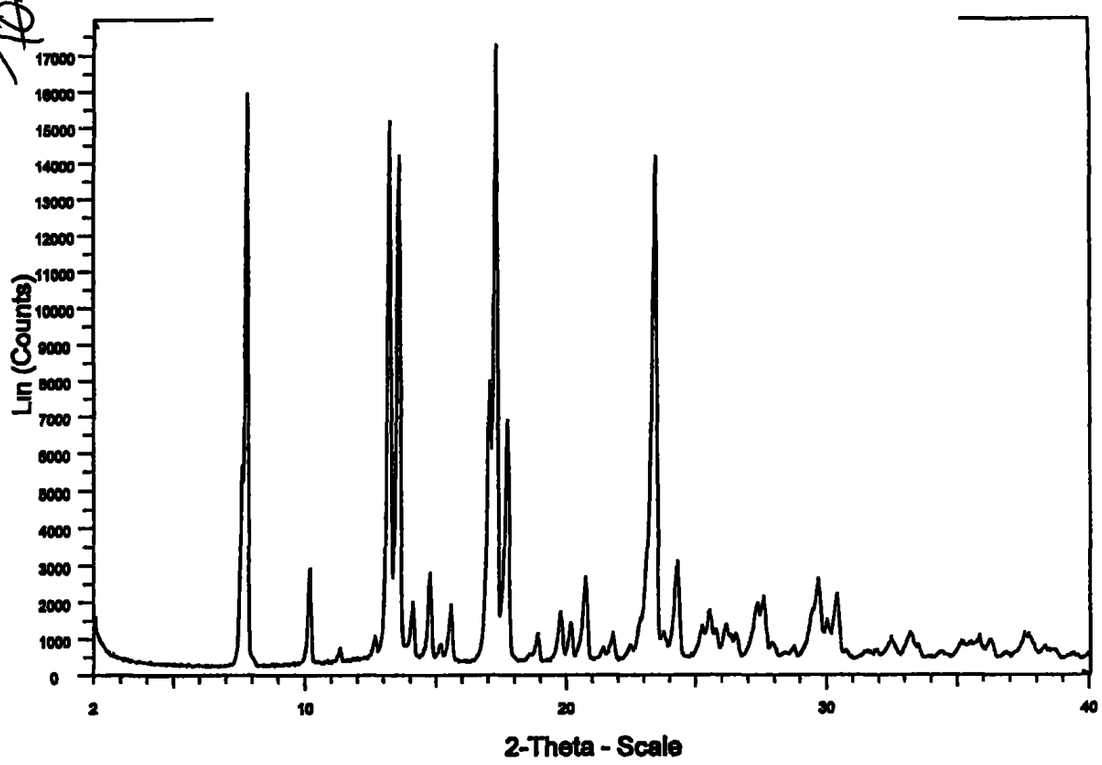

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Figure 1-D

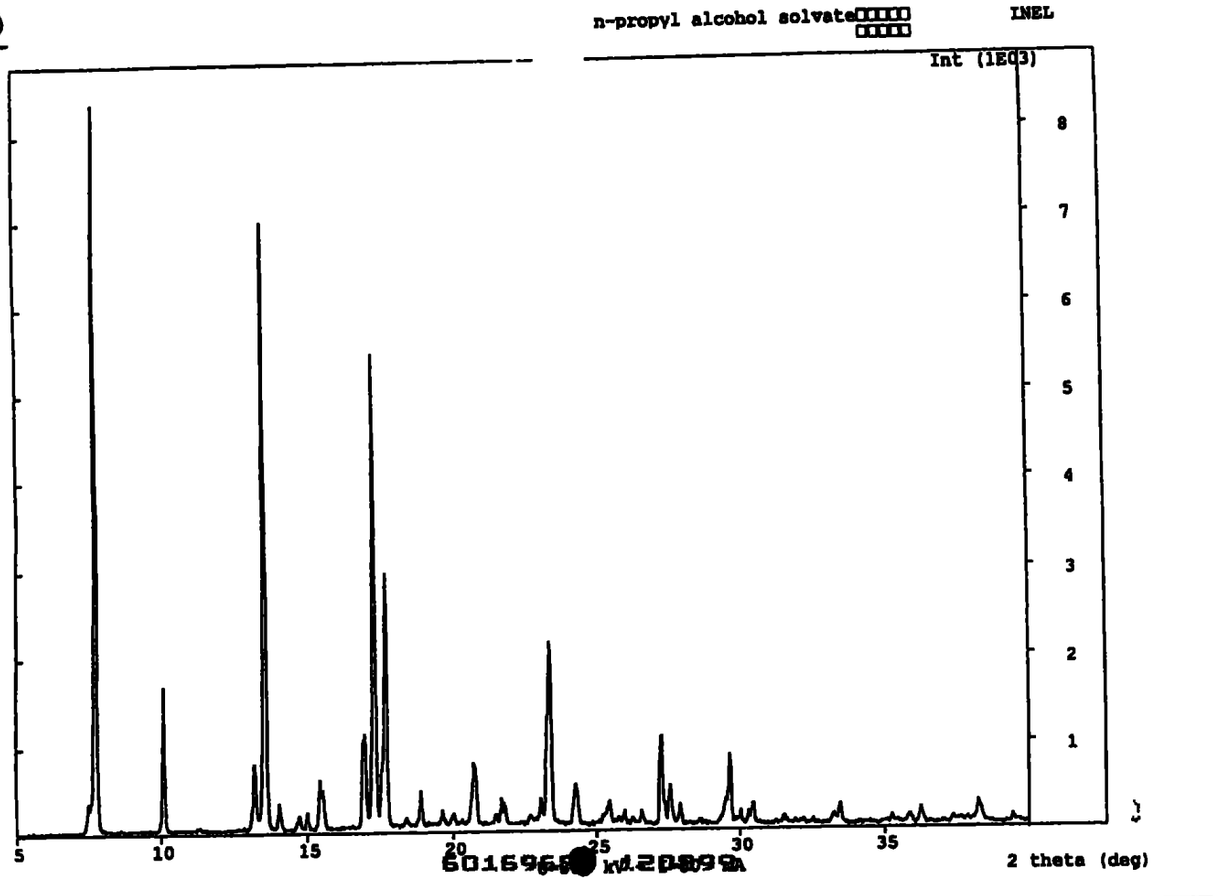

Figure 1-E

THF solvate[redacted] INEL

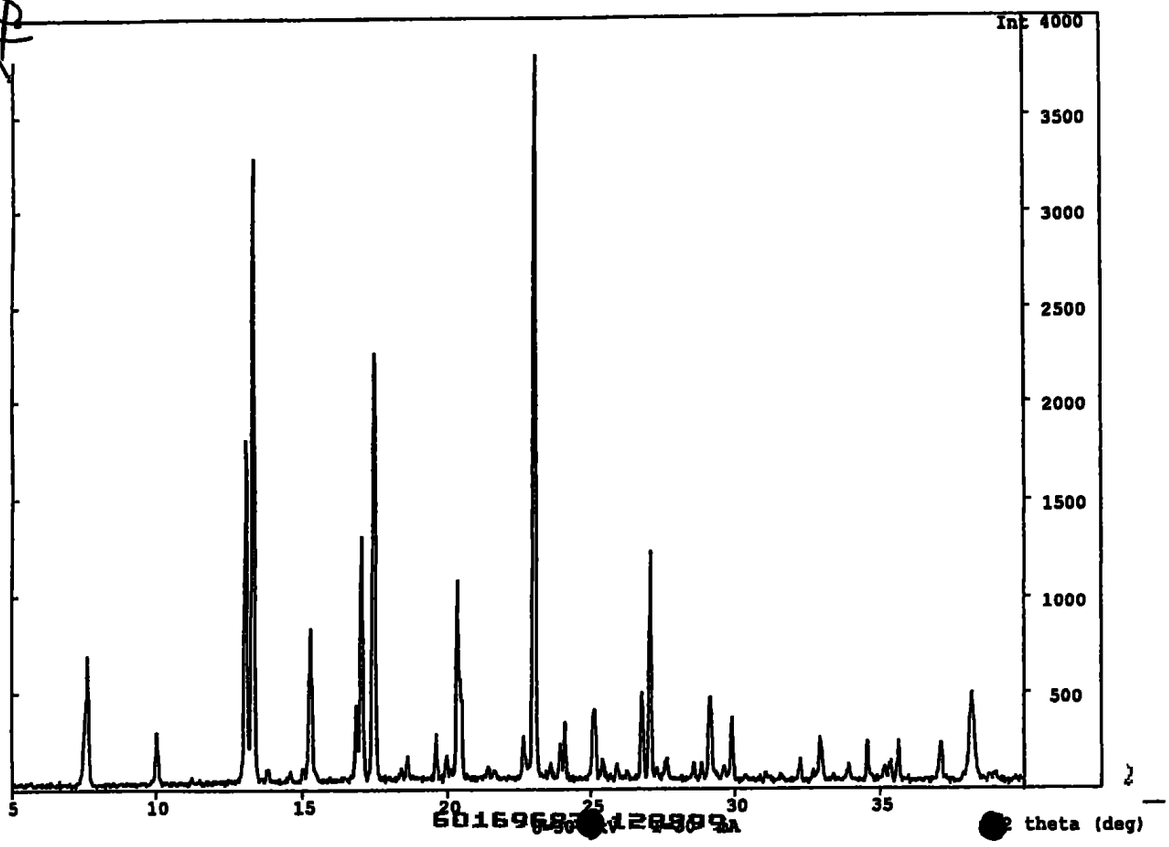

Figure 1-F

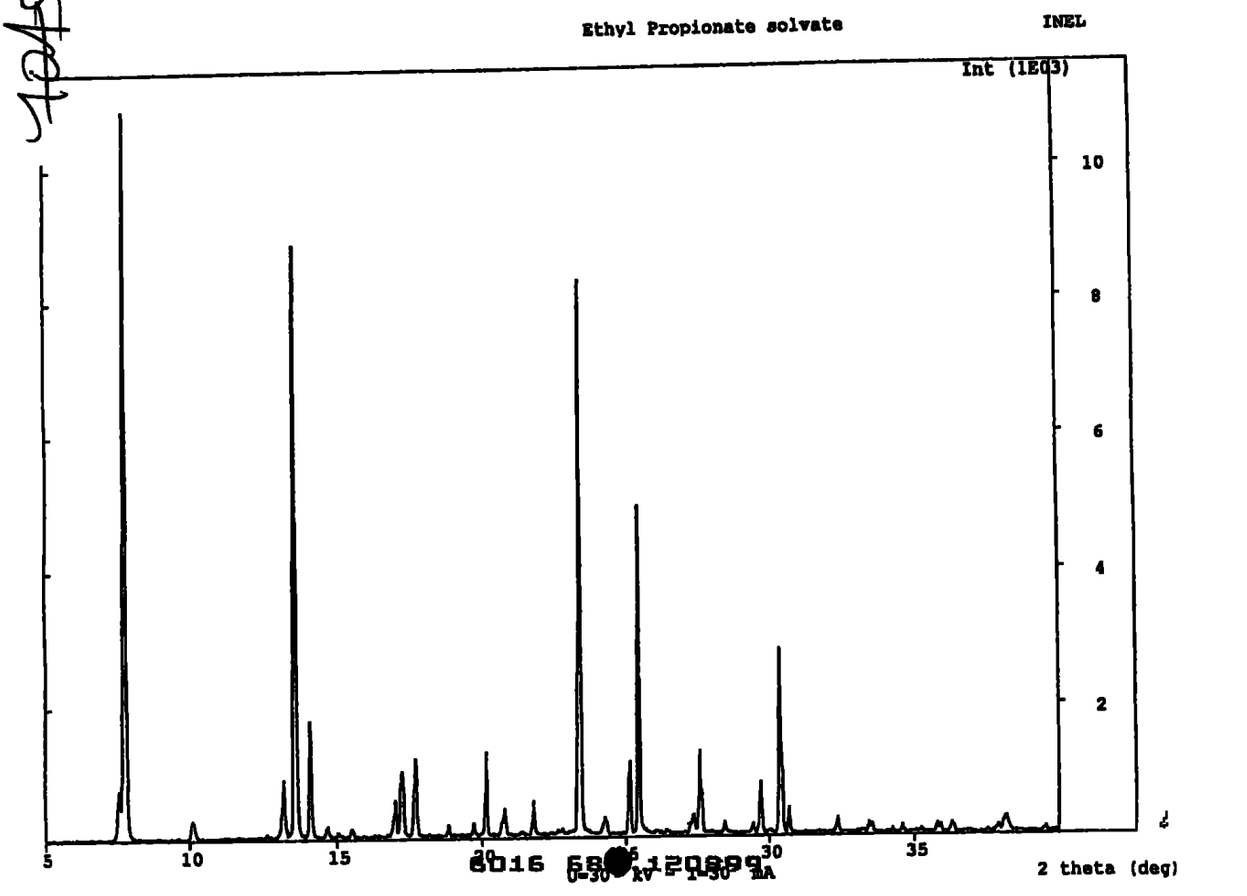
Ethyl Propionate solvate
INEL
Int (1E03)
10
8
6
4
2
5
10
15
20
30
35
2 theta (deg)

Figure 1-G

Acetic Acid solvate [redacted] INEL

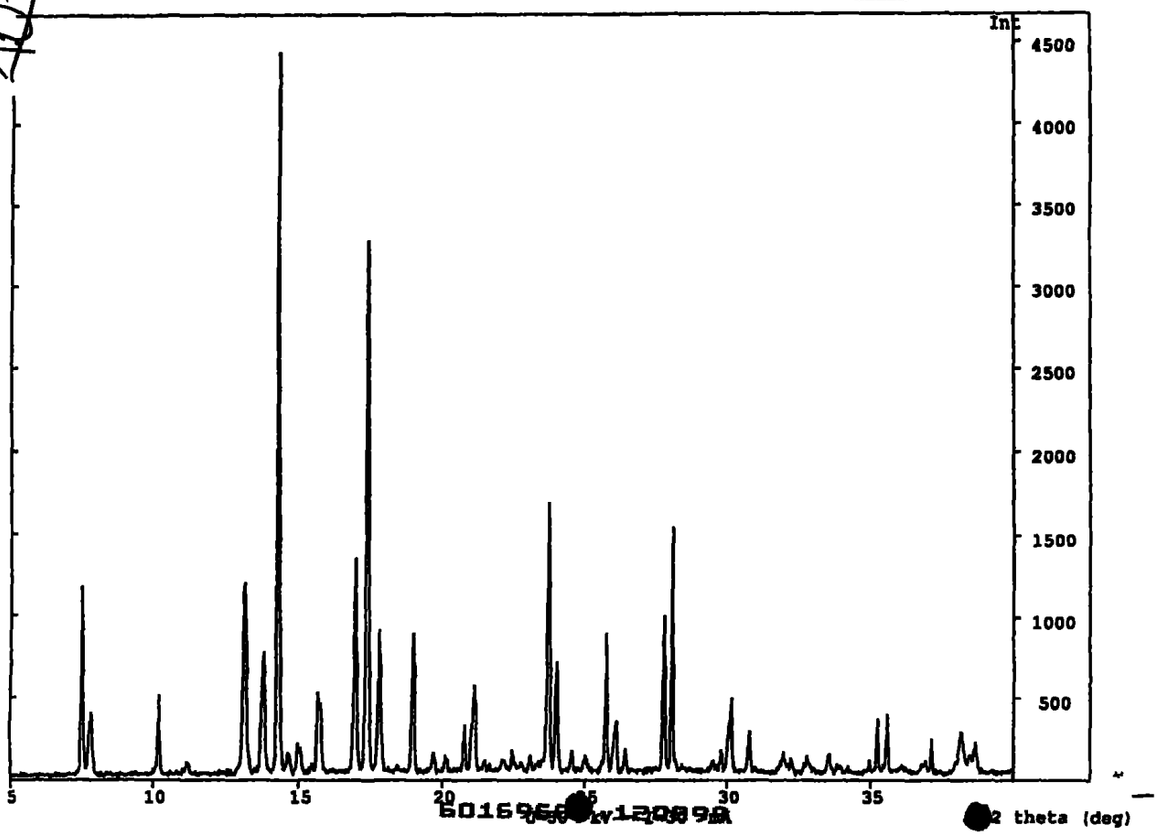

Figure 1-H
acetone solvate

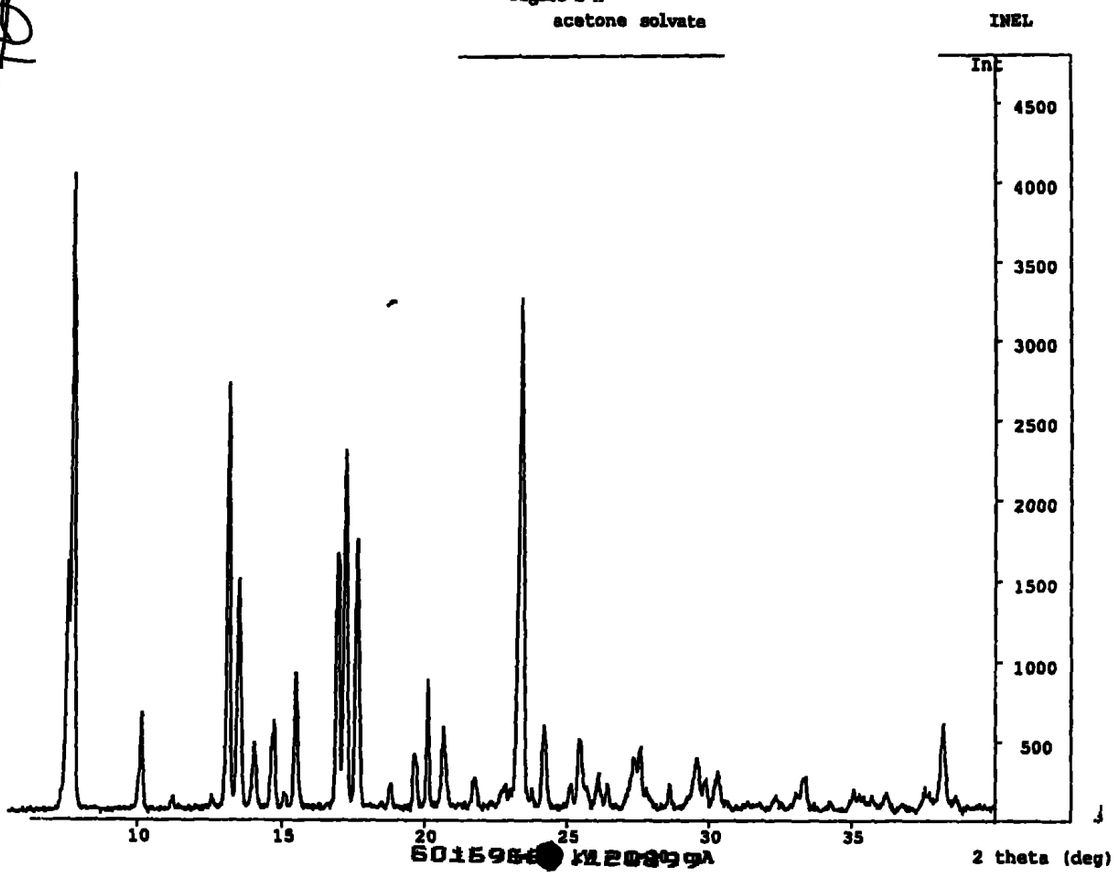

Figure 1-I

toluene solvate

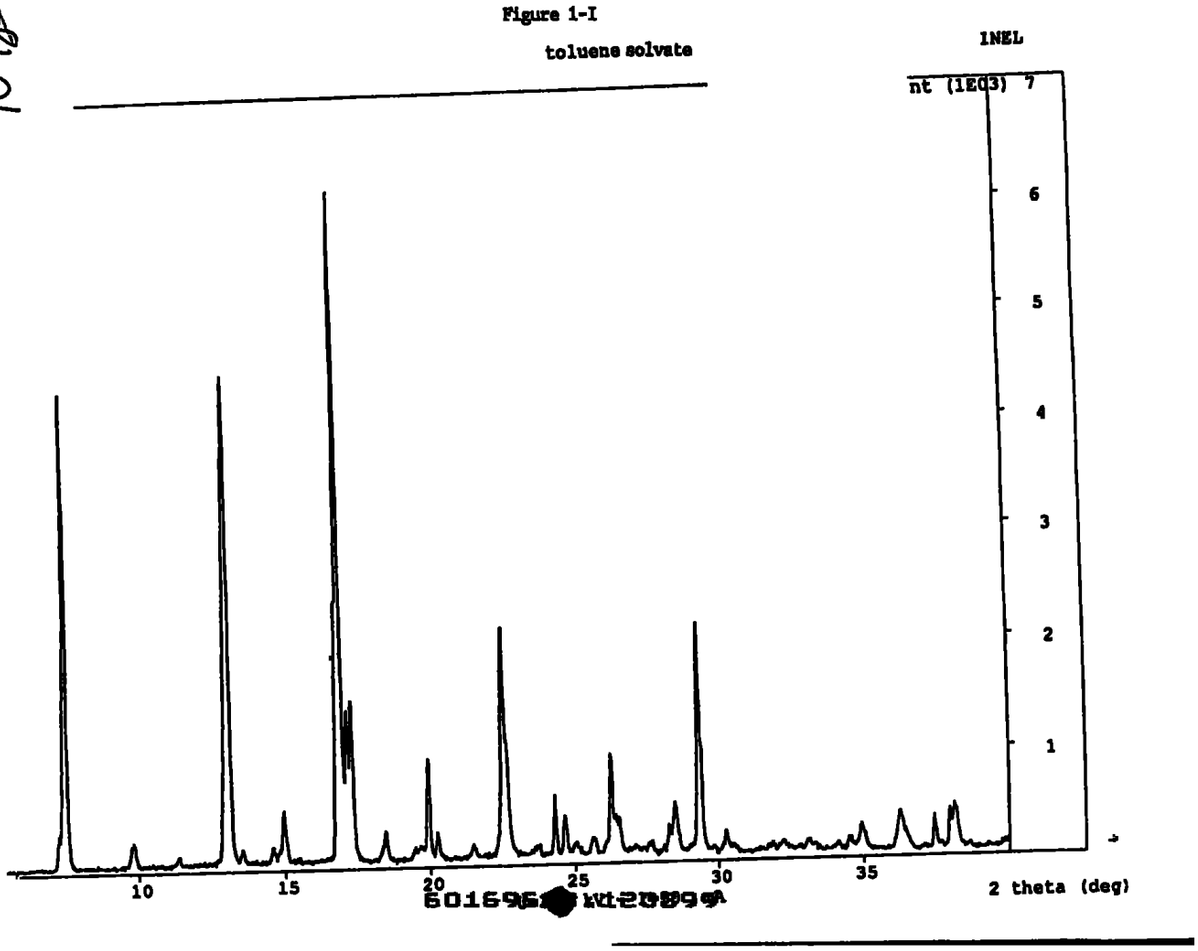

Figure 1-J

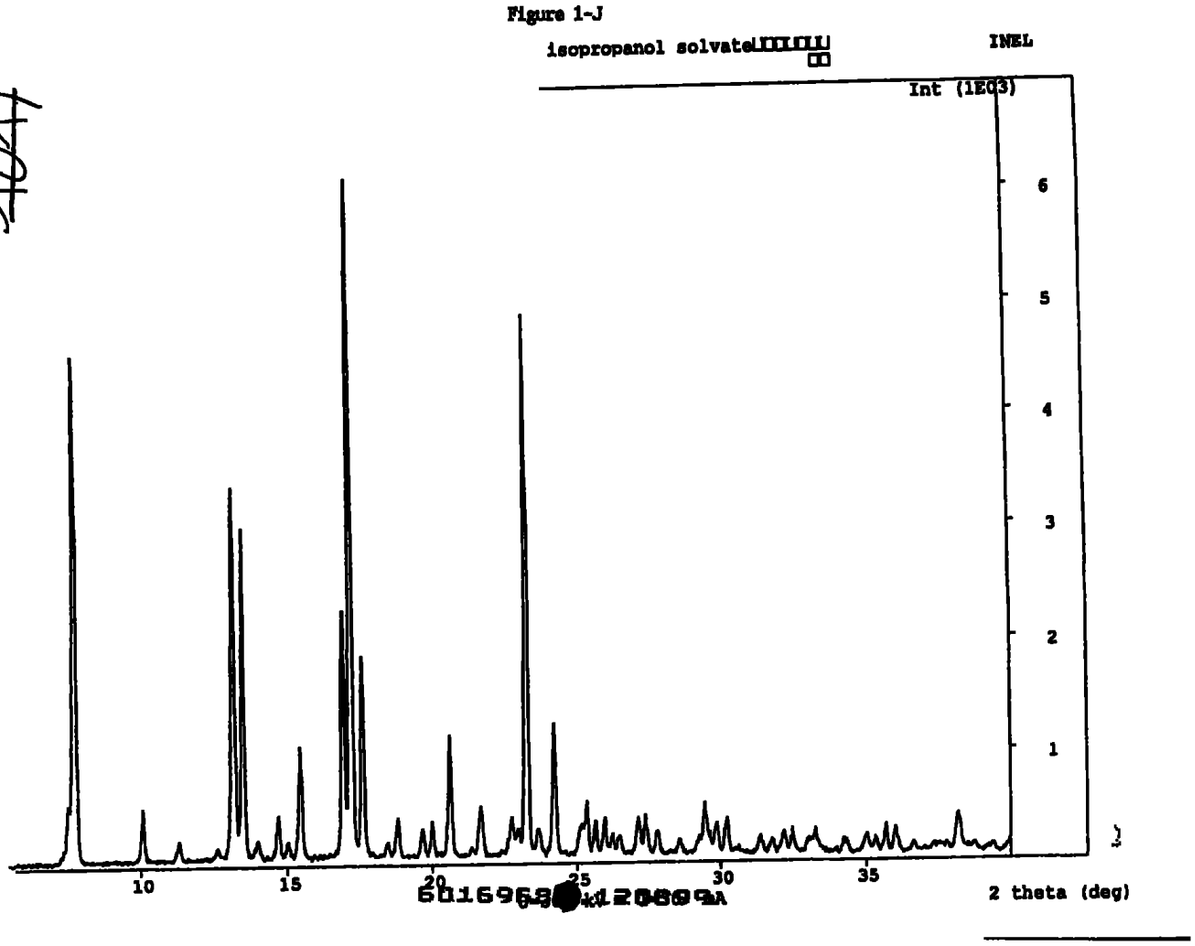

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Figure 1-K

ethanol solvate[illegible]

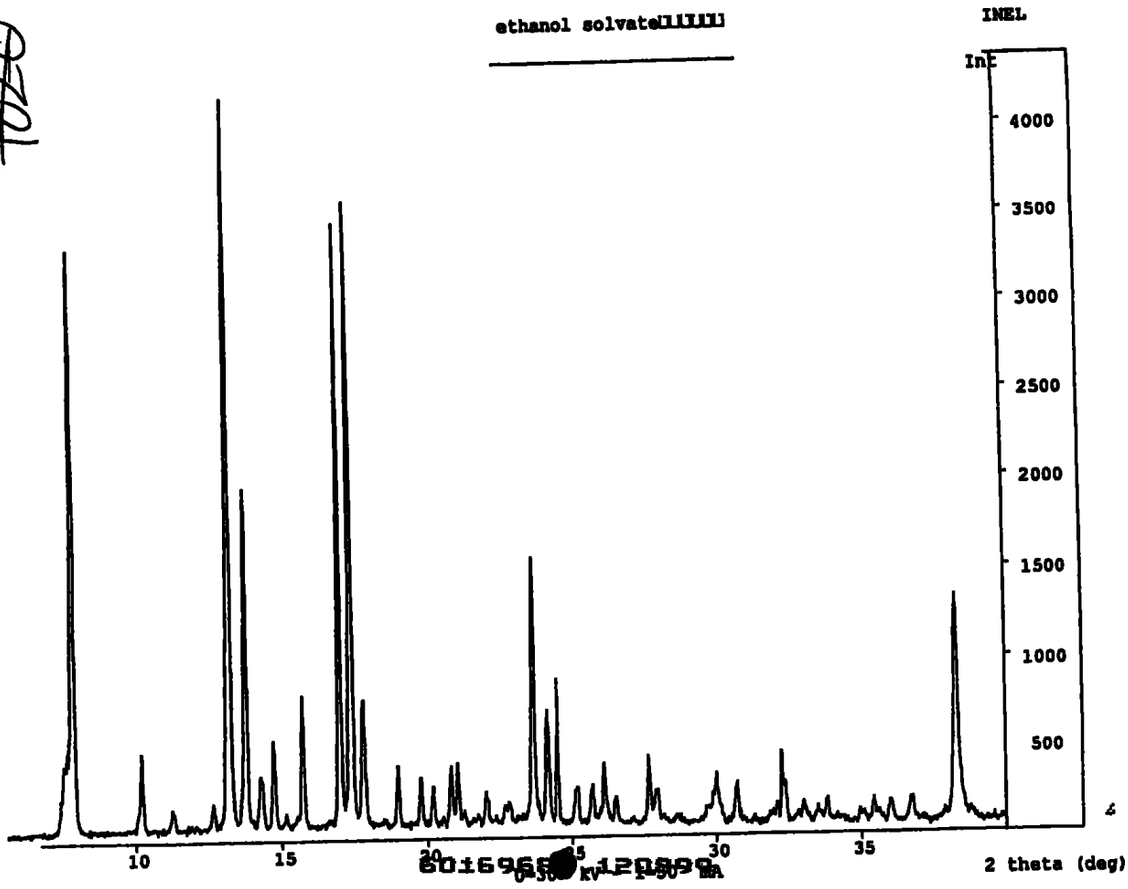


Figure 1-L

isobutyl acetate solvate

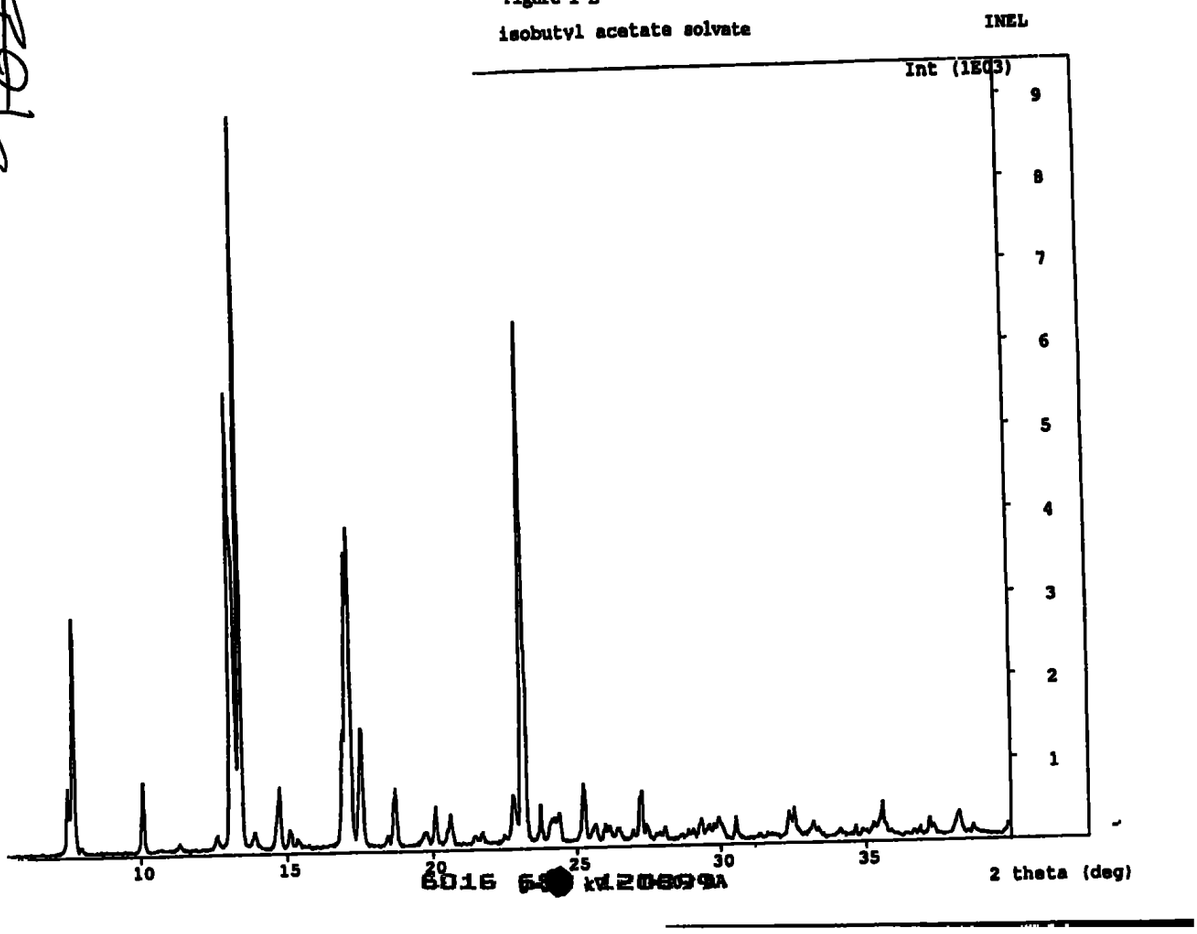
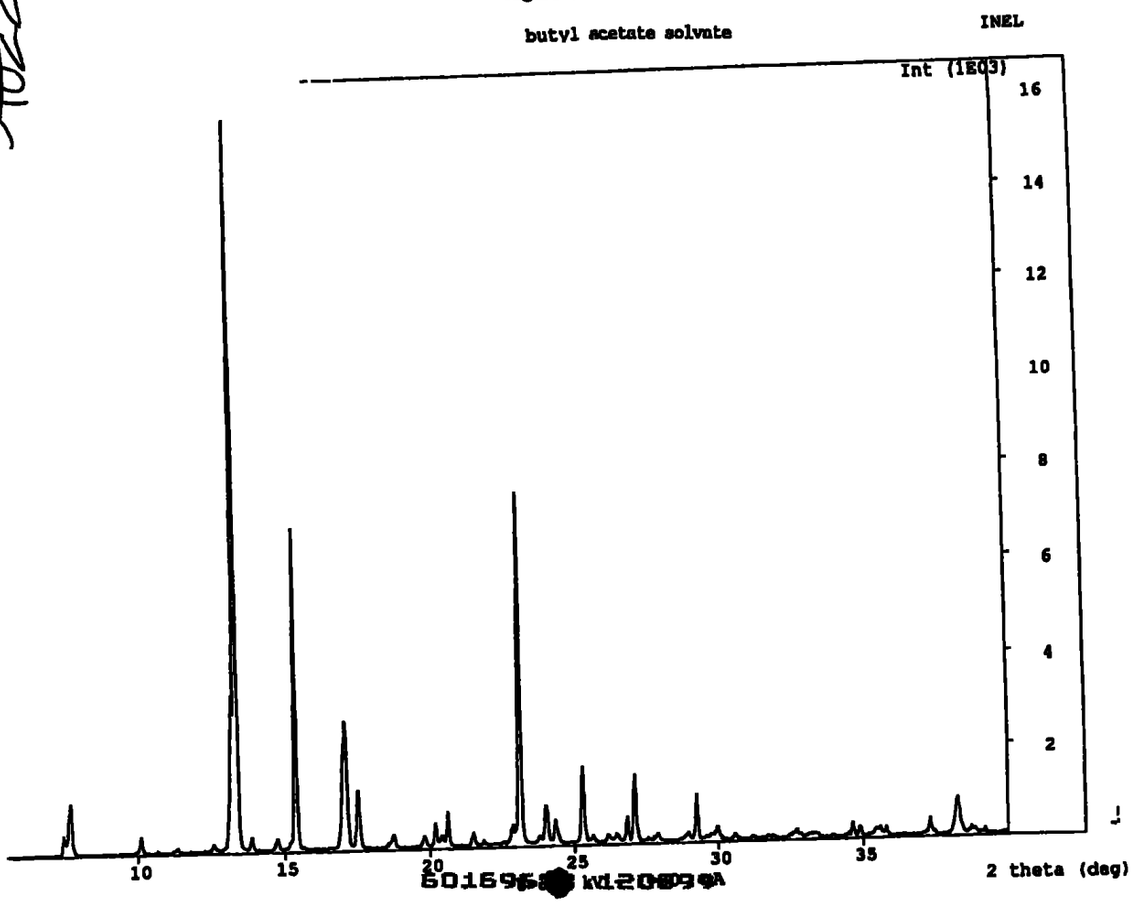


Figure 1-M

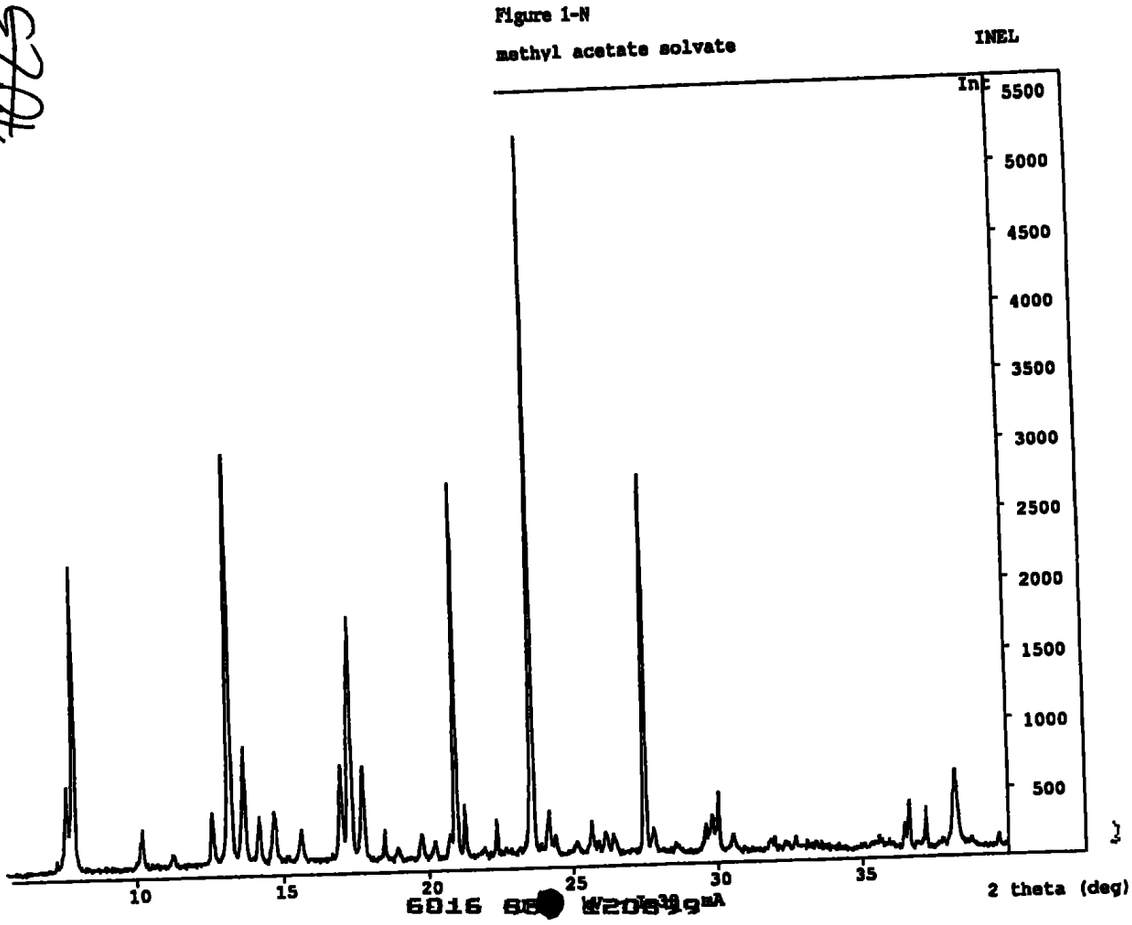
butyl acetate solvate



1023

Figure 1-N

methyl acetate solvate



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1016

Figure 1-0

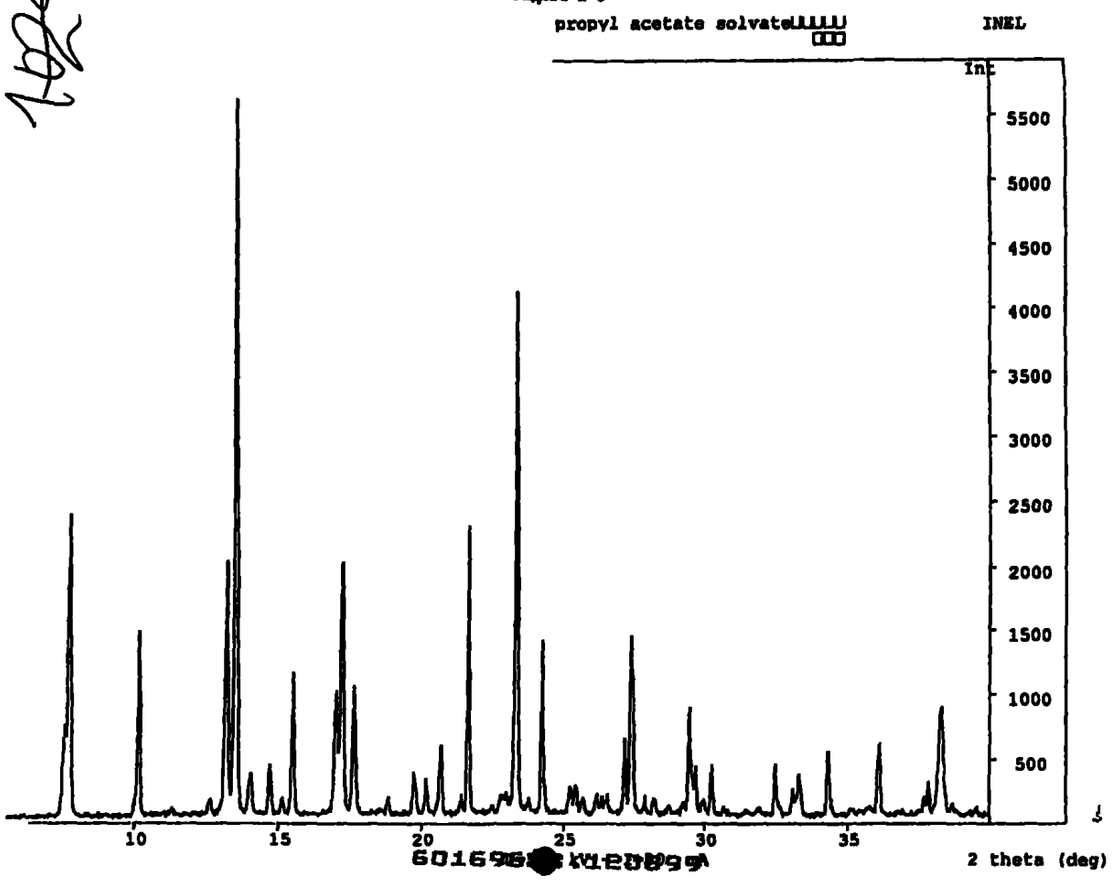

Figure 2-A

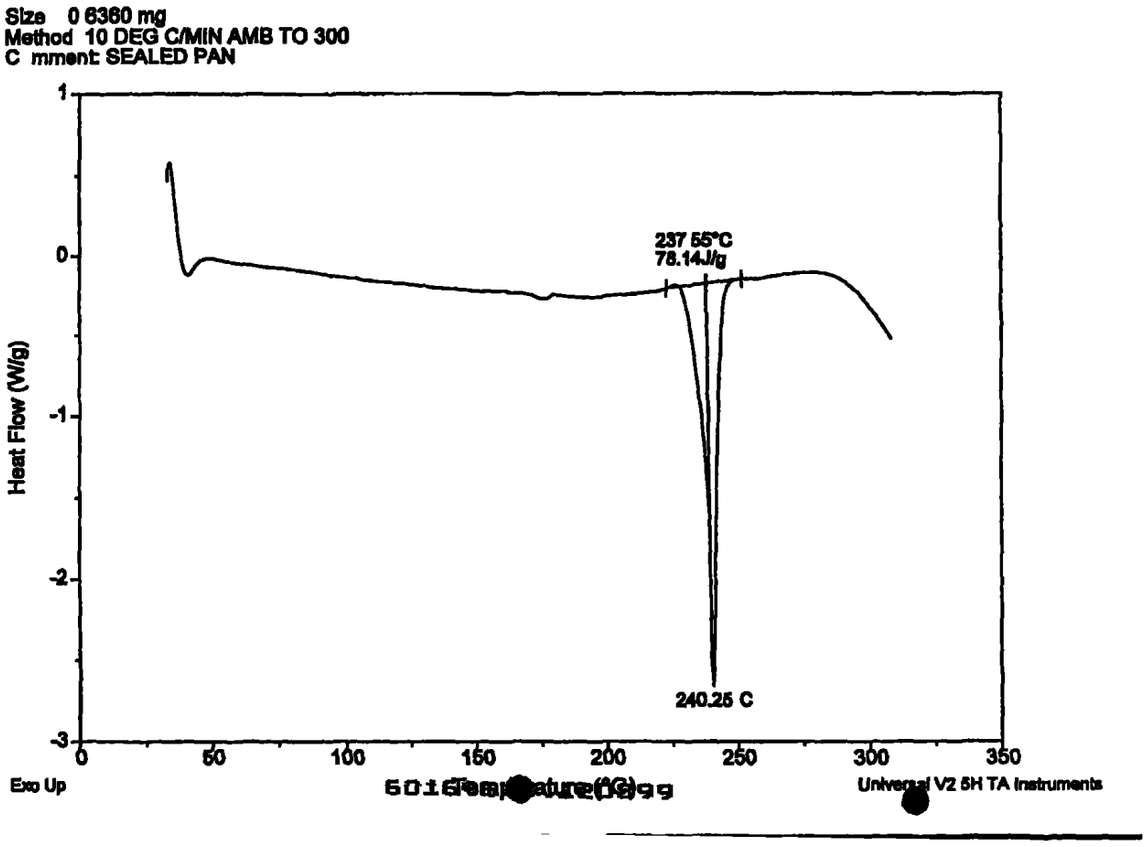
Size 0 6360 mg
Method 10 DEG C/MIN AMB TO 300
C mment: SEALED PAN
237 55°C
78.14J/g
240.25 C
Heat Flow (W/g)
Exo Up
Universal V2 5H TA Instruments

Figure 2-B

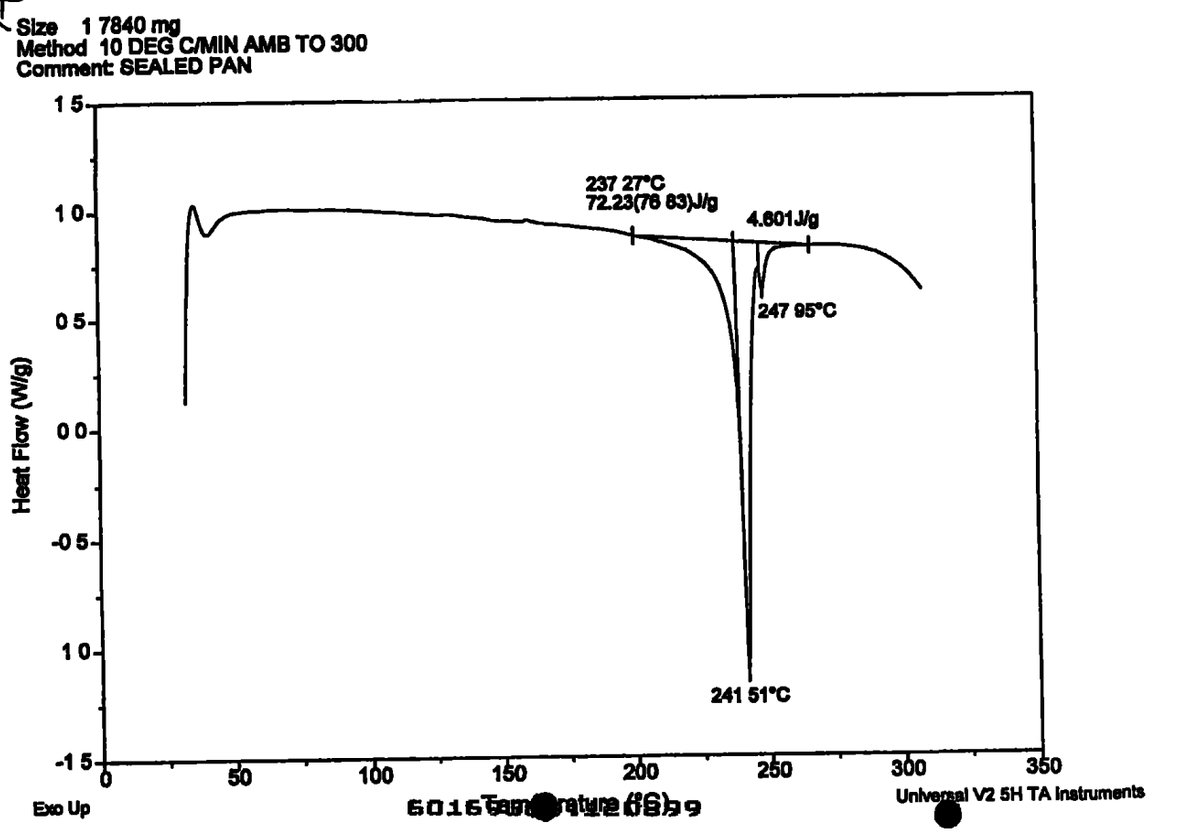
Size 1 7840 mg
Method 10 DEG C/MIN AMB TO 300
Comment SEALED PAN
237 27°C
72.23(76 83)J/g
4.601J/g
247 95°C
241 51°C
Heat Flow (W/g)
Exo Up
Universal V2 5H TA Instruments

Figure 2-C

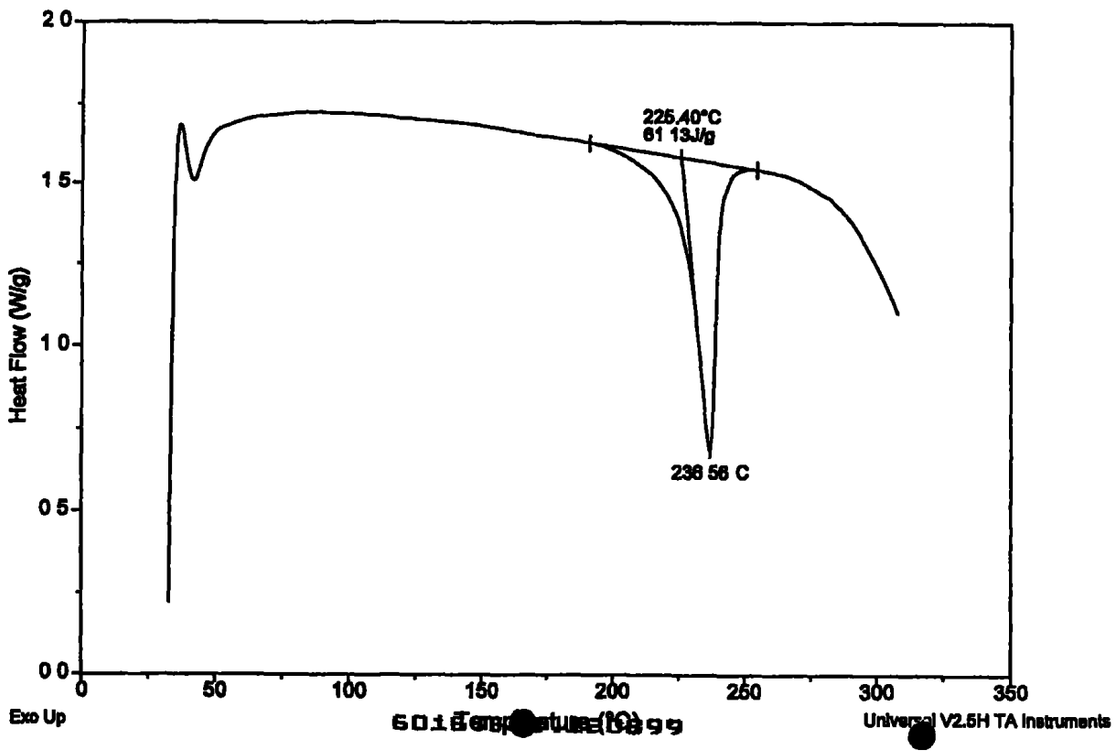
Size 1 4230 mg
Method 10 DEG C/MIN AMB TO 300
Comment: SEALED PAN
2 0
1 5
1 0
0 5
0 0
Heat Flow (W/g)
225.40°C
61 13J/g
236 56 C
0
50
100
150
200
250
300
350
Exo Up
Universal V2.5H TA Instruments

Figure 2-D

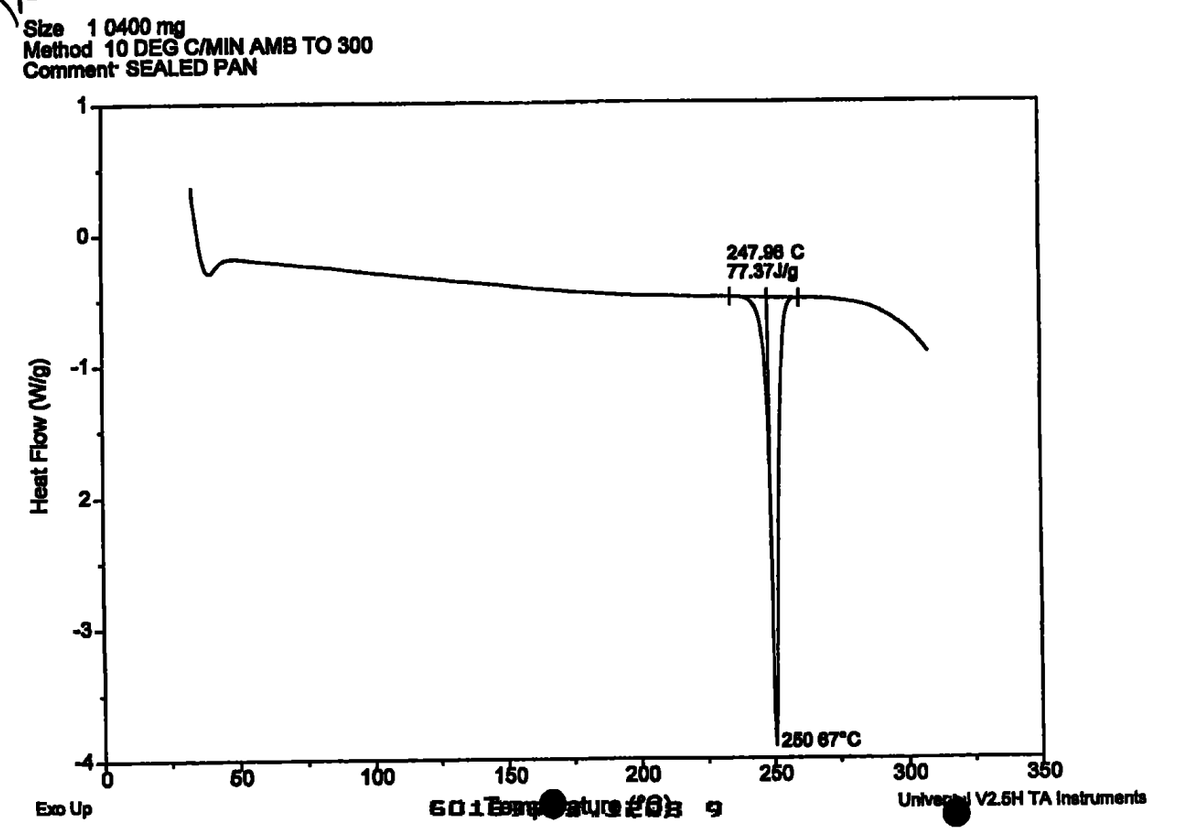
Size 1 0400 mg
Method 10 DEG C/MIN AMB TO 300
Comment SEALED PAN
247.98 C
77.37J/g
250.67°C
Heat Flow (W/g)
Exo Up
Universal V2.5H TA Instruments

Figure 2-E

Sample Weight: 16.300 mg
Comment: N-Propyl Alcohol solvate 1
unnamed.ssn

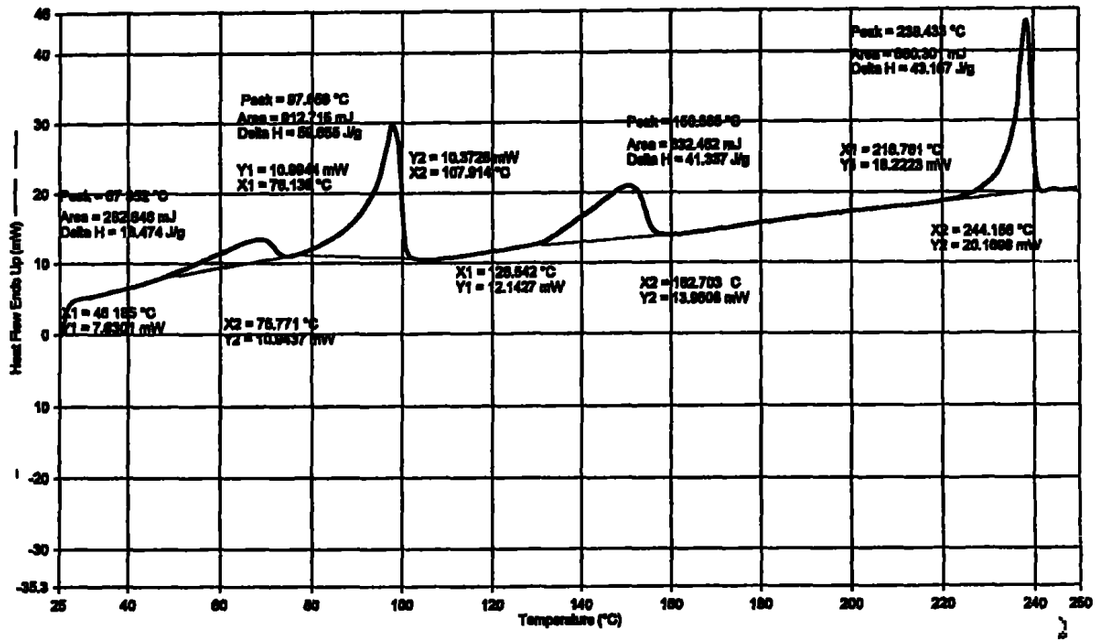

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Figure 2-F

4039.

Sample Weight: 10.400 mg
Comment: THF solvate 1
unwashed pan

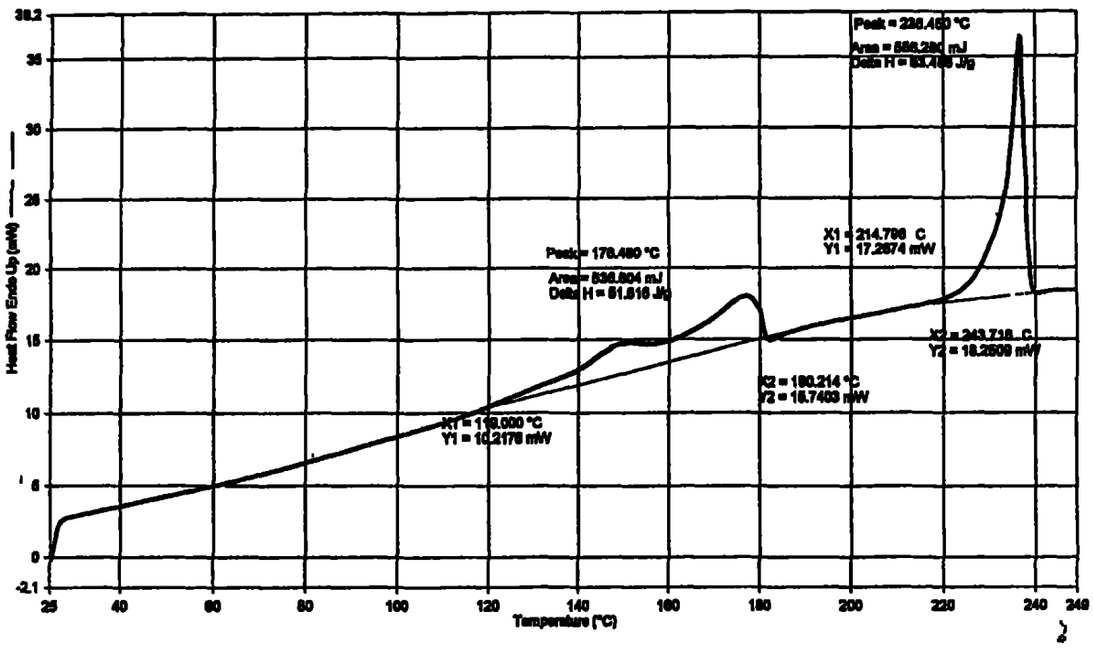

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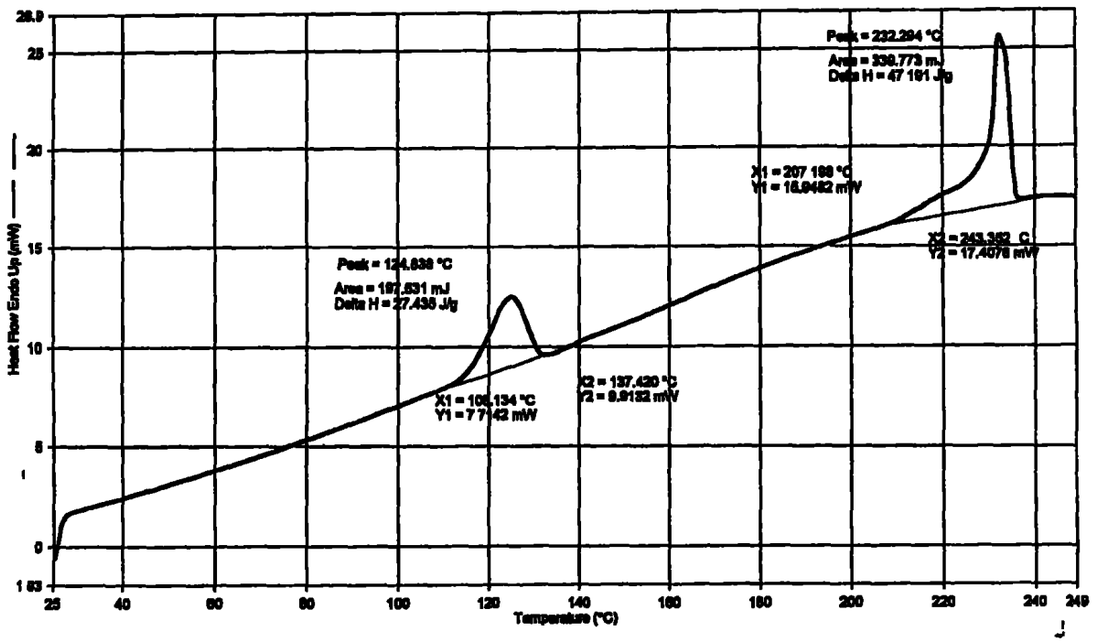
Figure 2-G

1023

Sample Weight: 7.200 mg
Comment: Lot 96K020-F3b
Ethyl propionate solvate
uncrushed pan



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1032

Figure 2-H

Sample Weight: 8.000 mg
Comment: Acetic ACid eclusto 1
unsealed pan

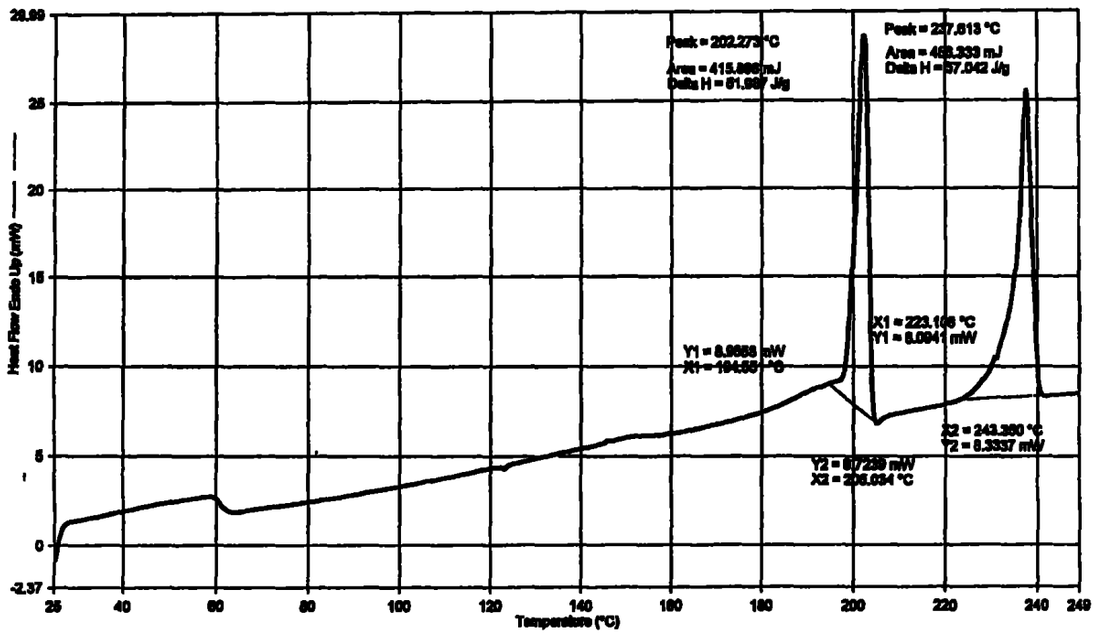

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Figure 2-I

Sample Weight: 12.700 mg
Comment: Chloroform solvate 1
unsealed pan

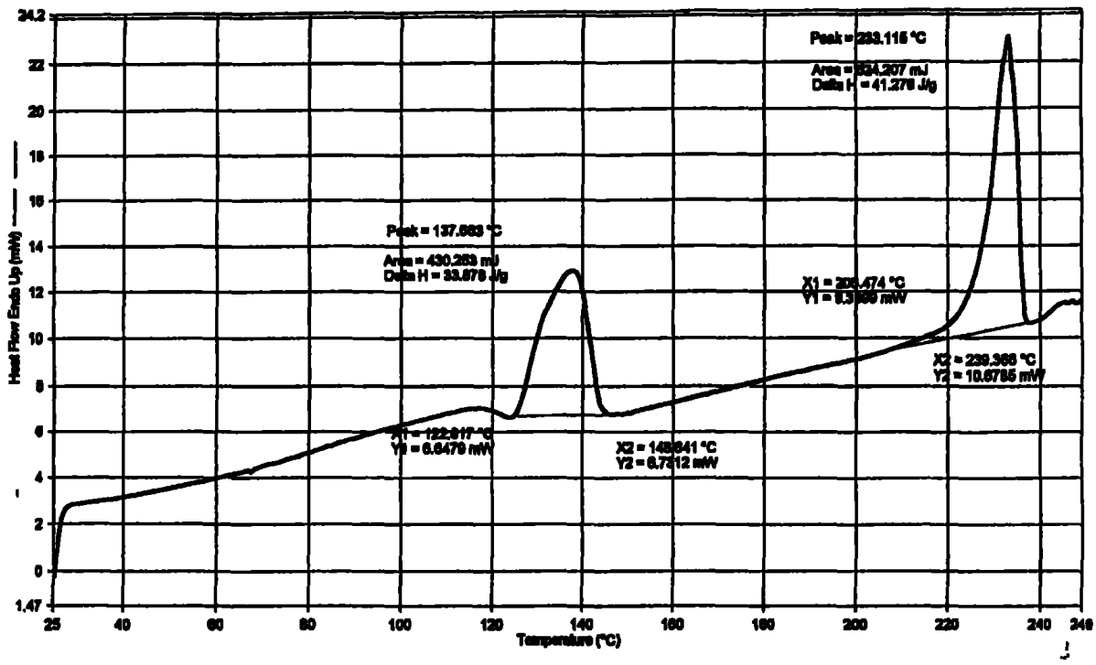

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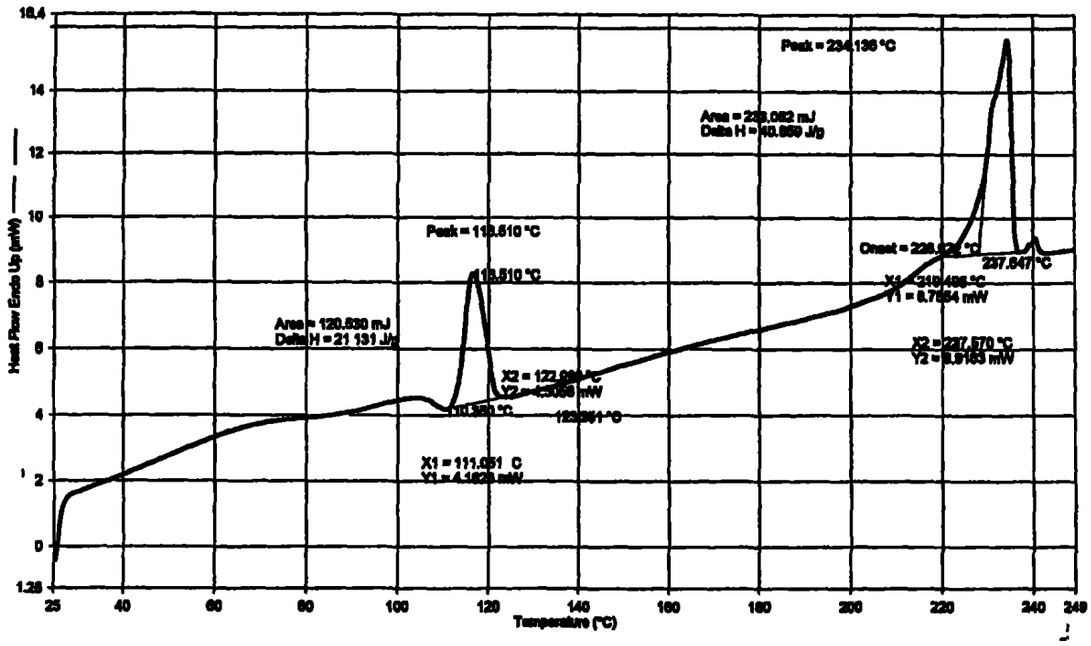
Figure 2-J

1034

Sample Weight: 8.704 mg
Comment: Acetone Solvate



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1027

Figure 2-K

1035

Sample Weight: 8.947 mg
Comment: Toluene Solvate

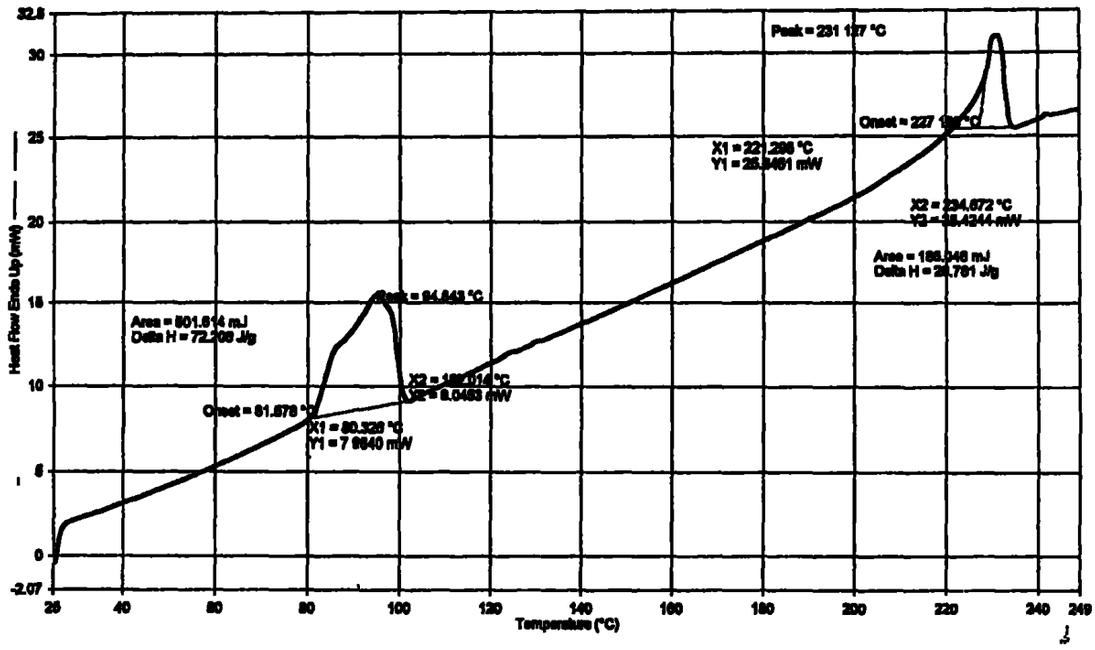

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Figure 2-L

1028

Sample Weight: 8.174 mg
Comment: Isopropanol Solvate

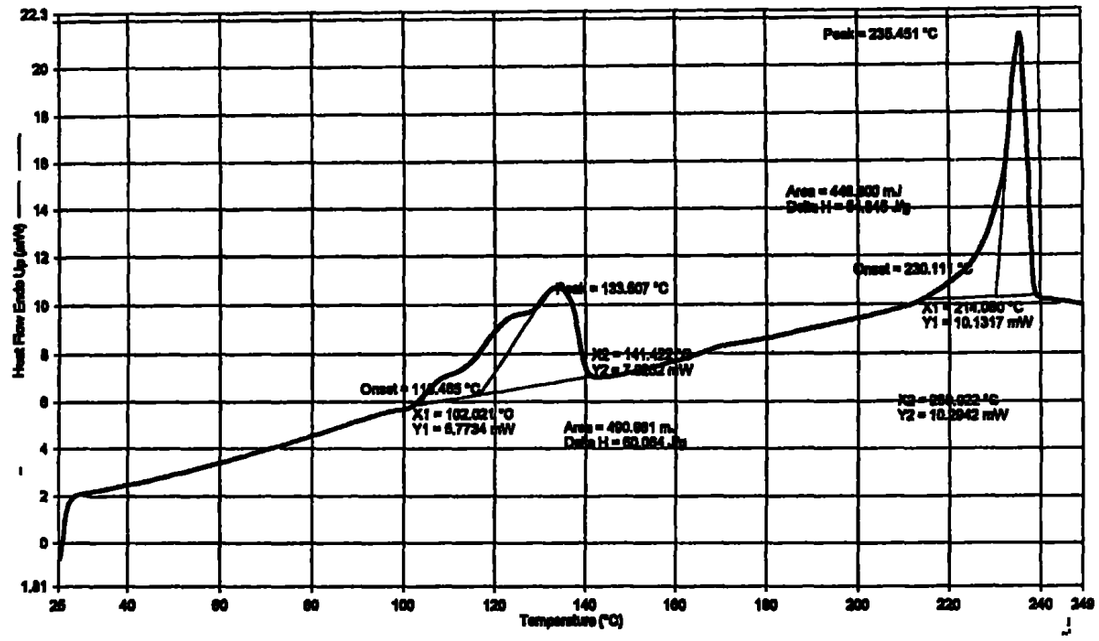

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Figure 2-H

Sample Weight: 6.122 mg
Comment:
ethanol solvate
10 °C/min sealed pan

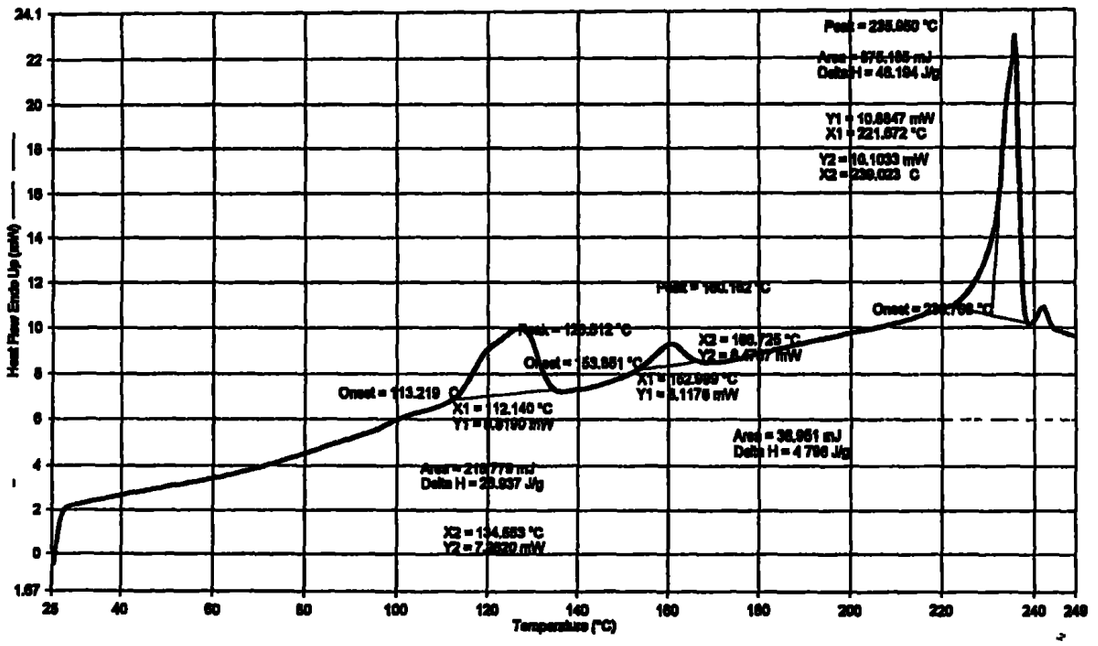

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Figure 2-N

1033.

Sample Weight: 6.640 mg
Comment: t-butyl acetate solvate
10 °C/min sealed pan

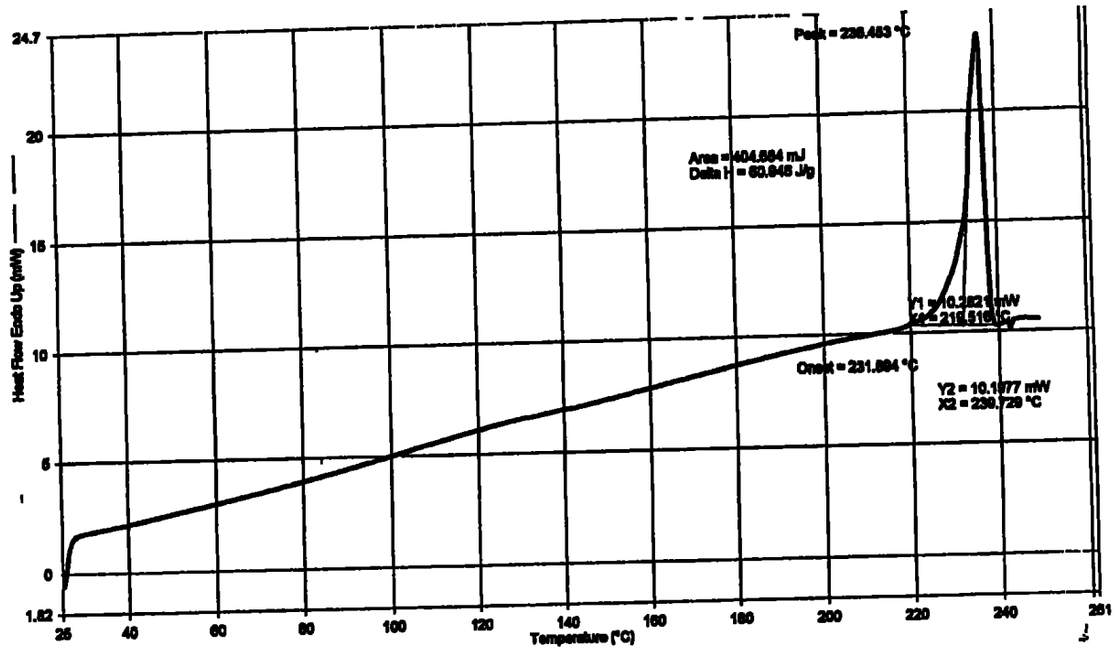

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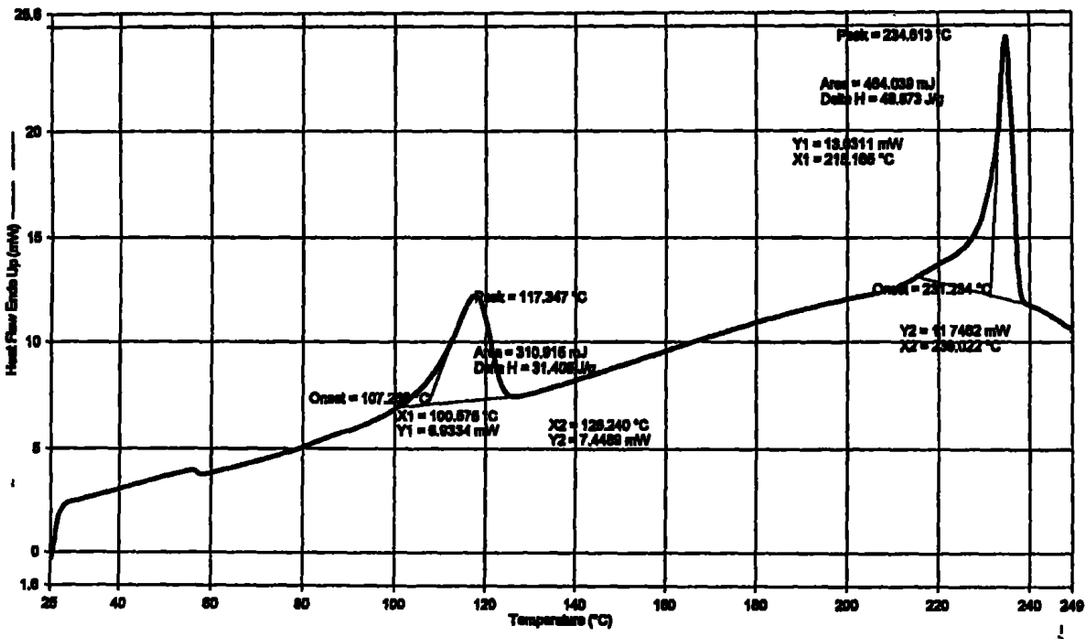
Figure 2-0

Sample Weight: 6.900 mg
Comment: Isobutyl acetate solvate
10 °C/min sealed pan



1040

Figure 2-P

Sample weight: 0.UUU mg
Comment: butyl acetate solvate
10 °C/min sealed pan

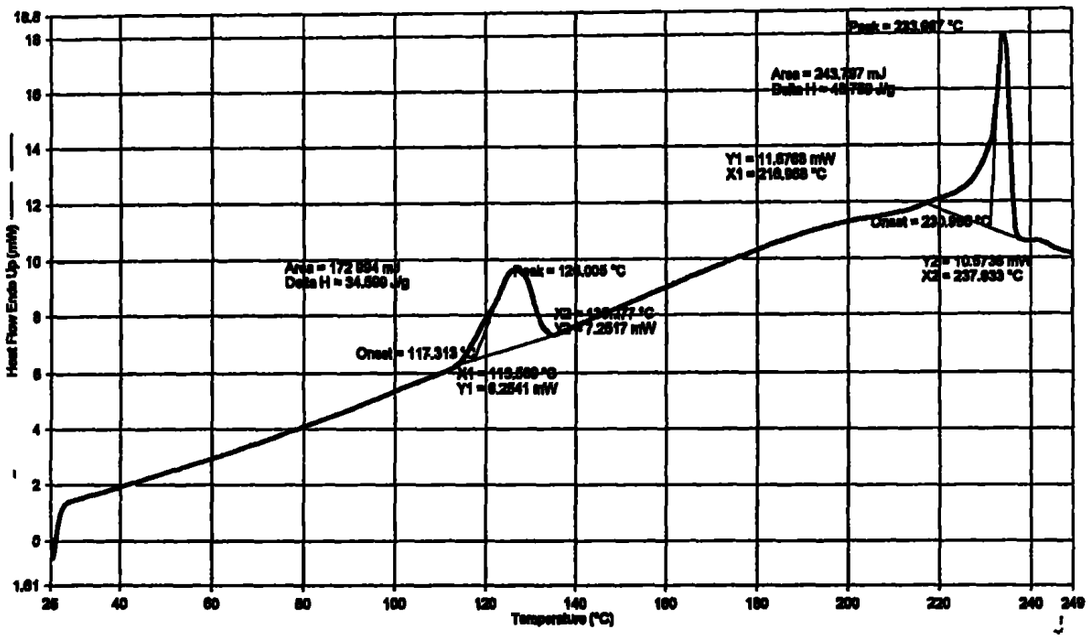


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Figure 2-Q

1044

Sample Weight: 5.536 mg
Comment:
methyl acetate solvate 1
10 °C/min sealed pan

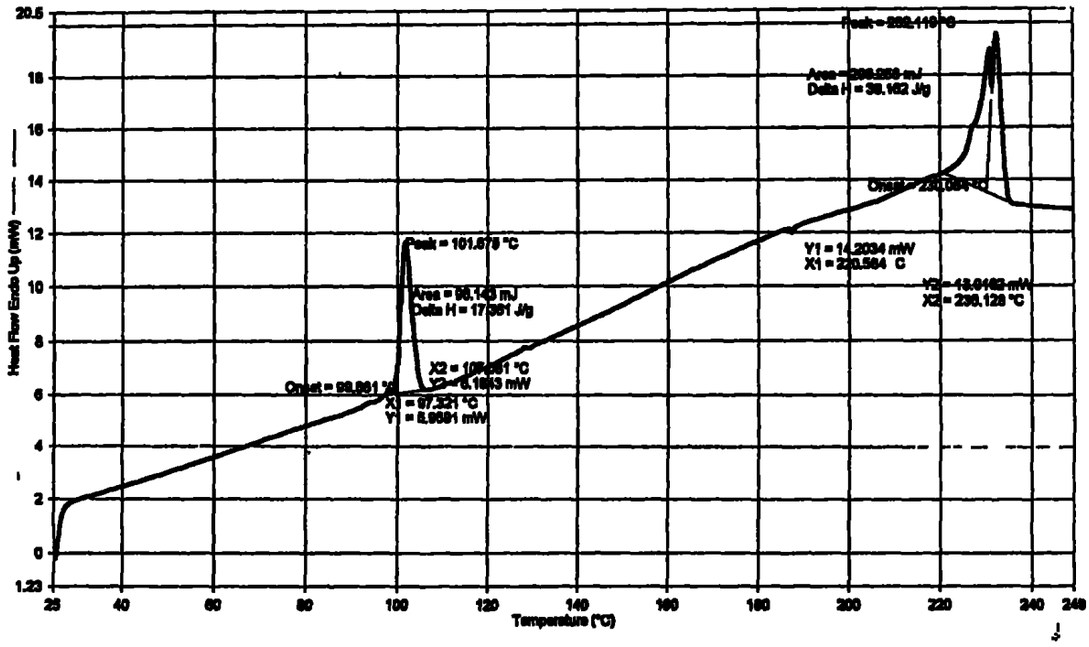

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Figure 2-R

Sample Weight: 6.074 mg
Comment:
propyl acetate solvate 1
10 °C/min sealed pan

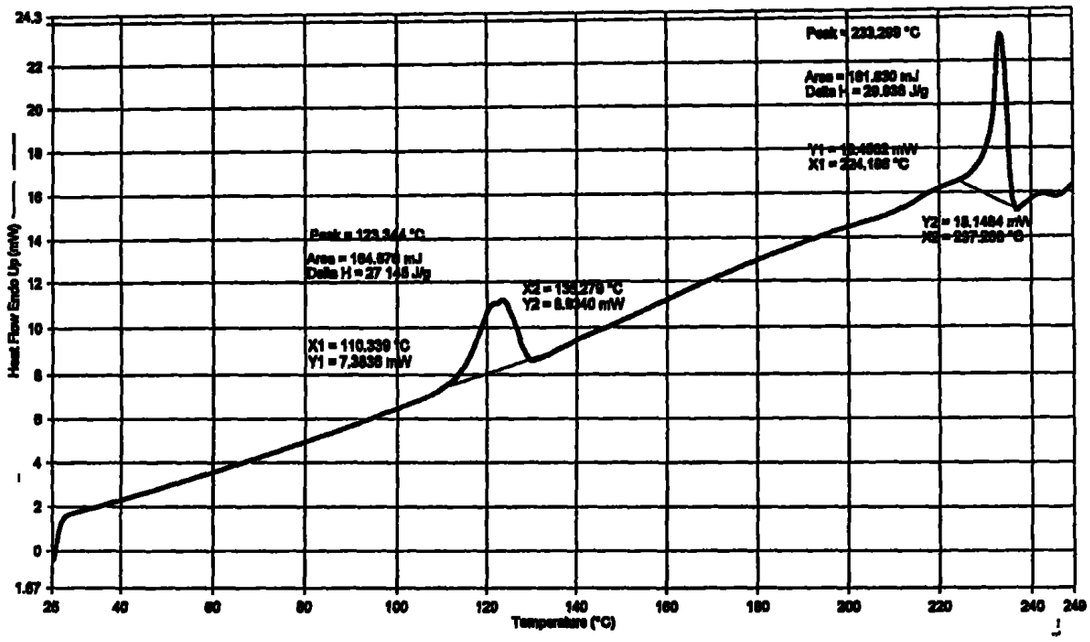

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Figure 2-S

n-butanol solvate

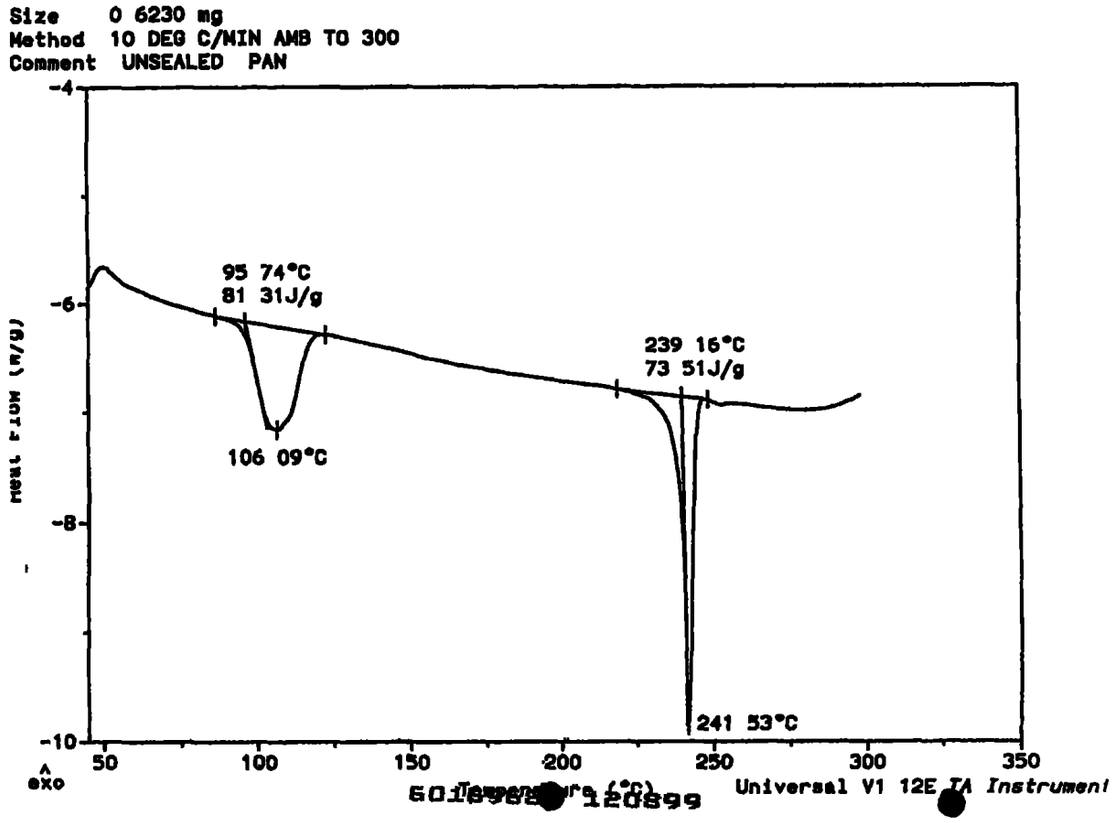

Figure 2-T

n-octanol solvate

Size 0 5220 mg
Method 10 DEG C/MIN AMB TO 300
Comment UNSEALED PAN

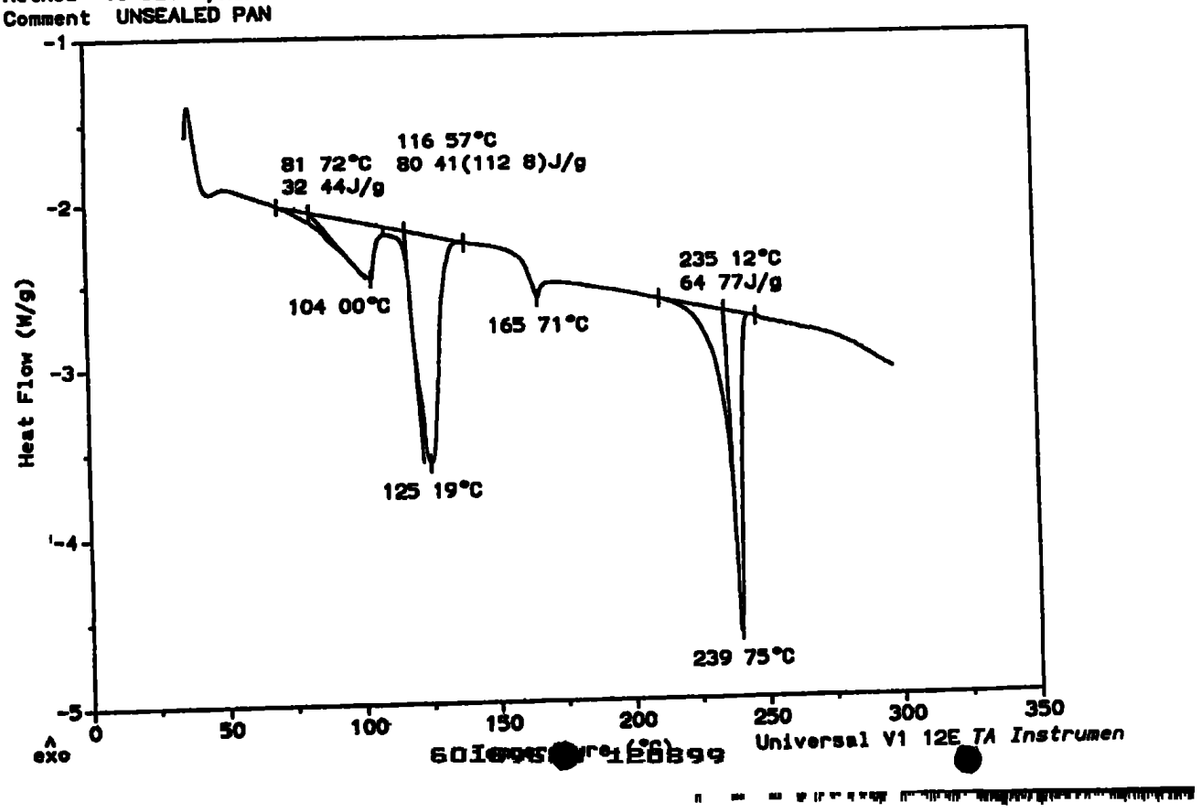

Figure 3-A

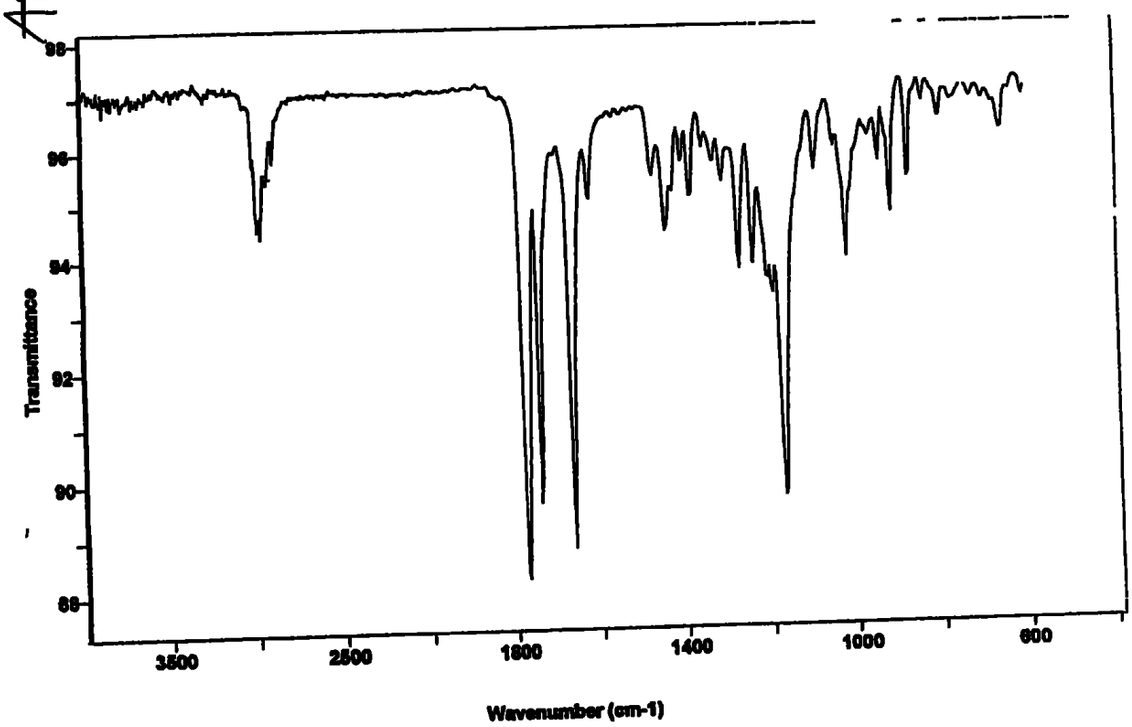

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Figure 3-B

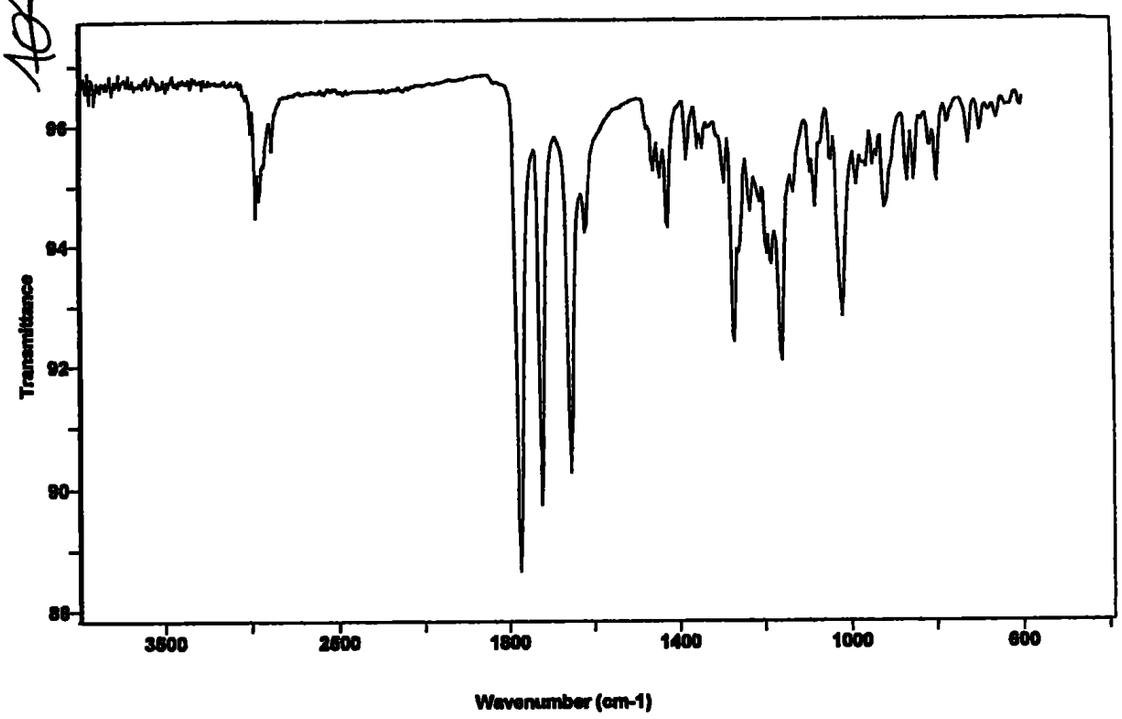

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Figure 3-C

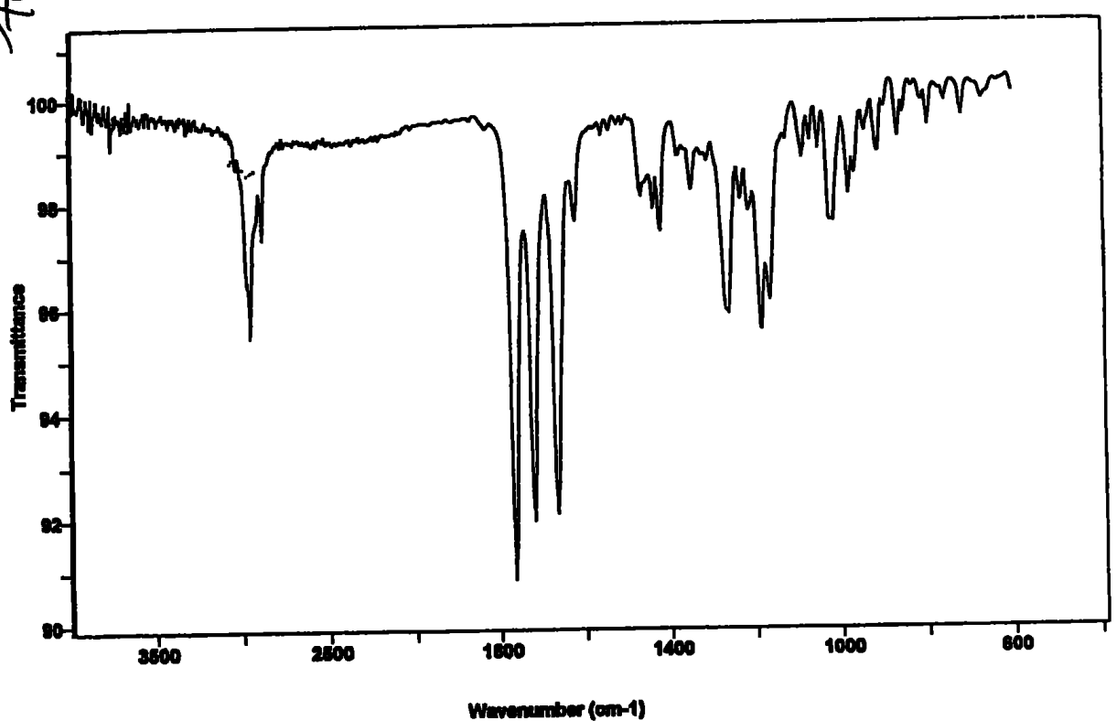

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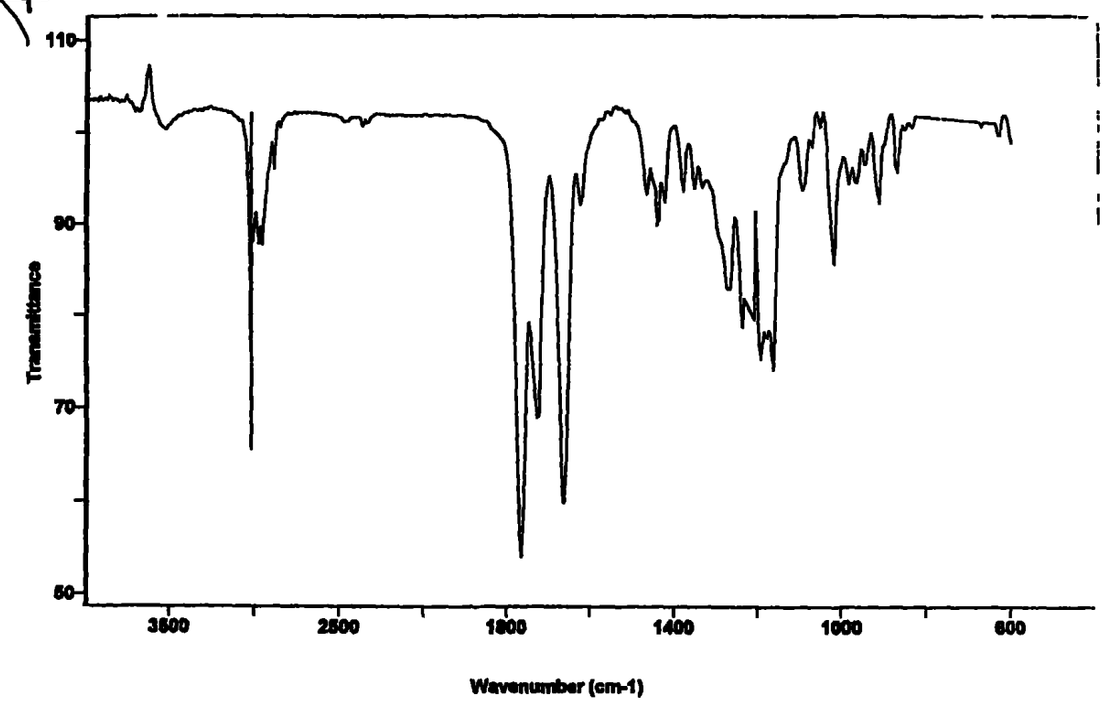
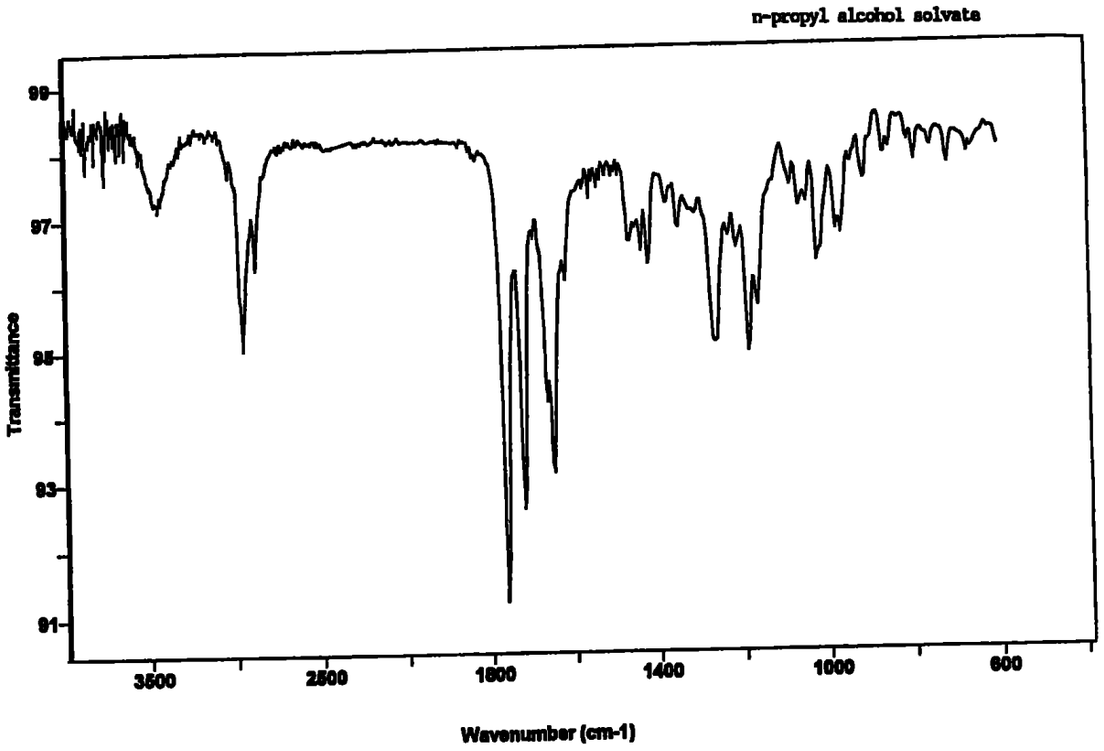
Figure 3-D



601696[redacted]3 120899

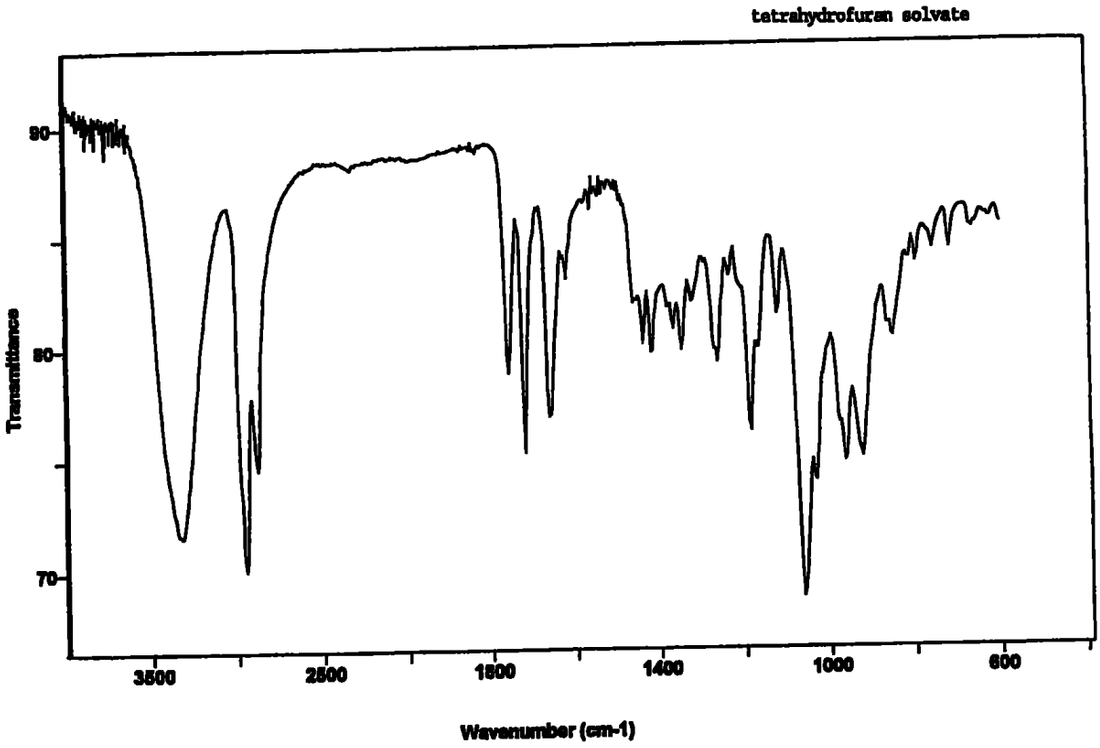
1049

Figure 3-E



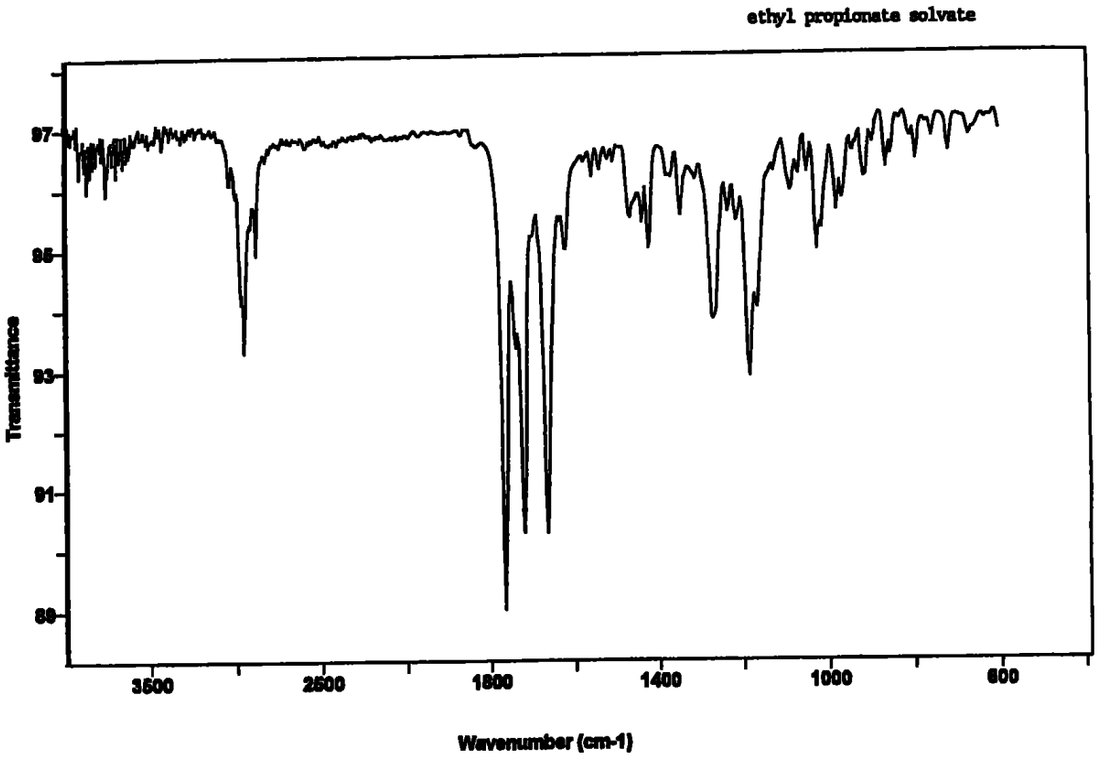
601696[redacted] 120899

Figure 3-F



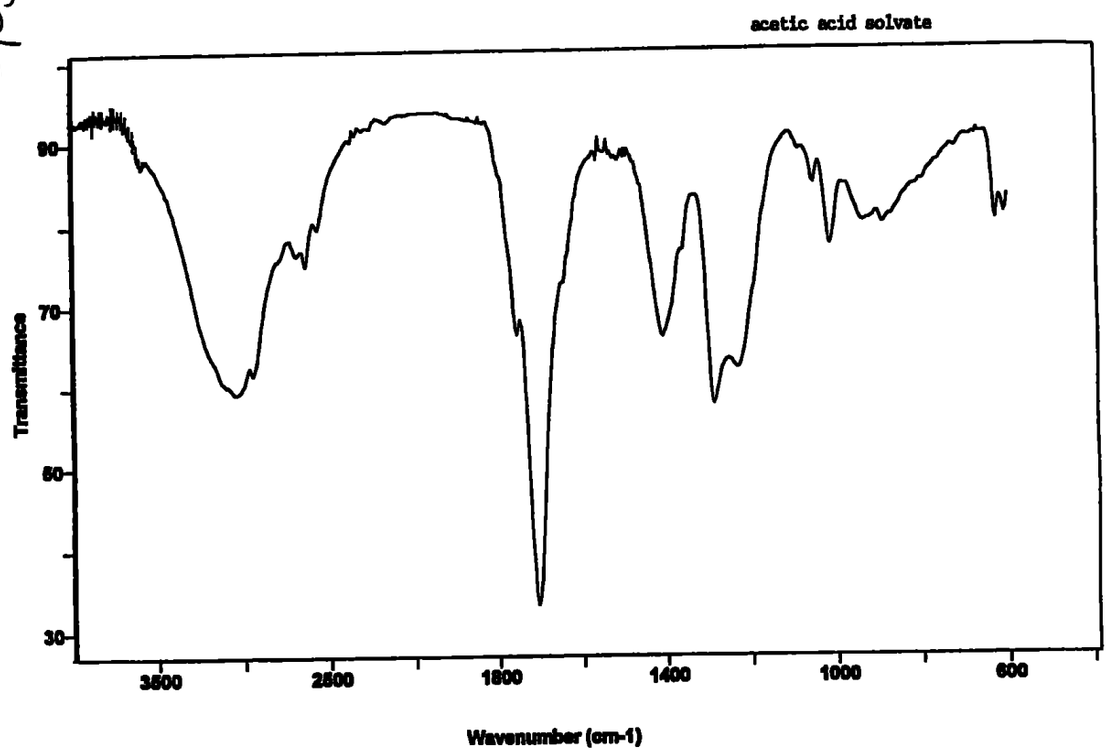
601696[redacted] 120899

Figure 3 G



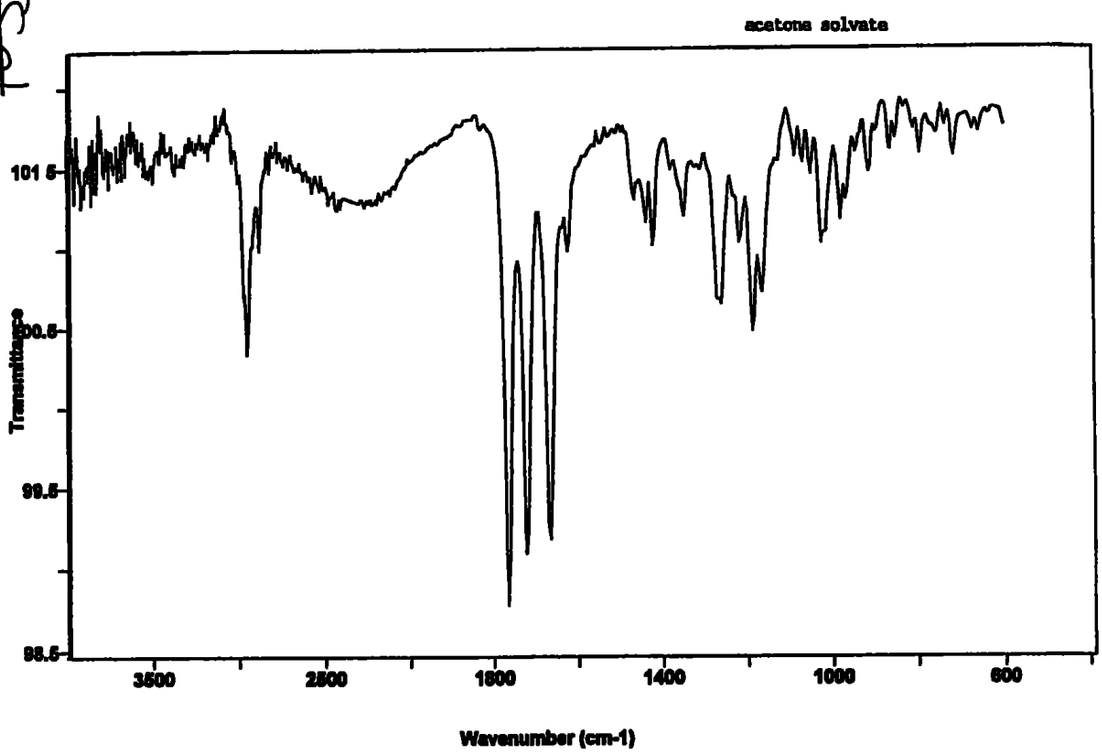
601696[redacted] 1208

Figure 3-H



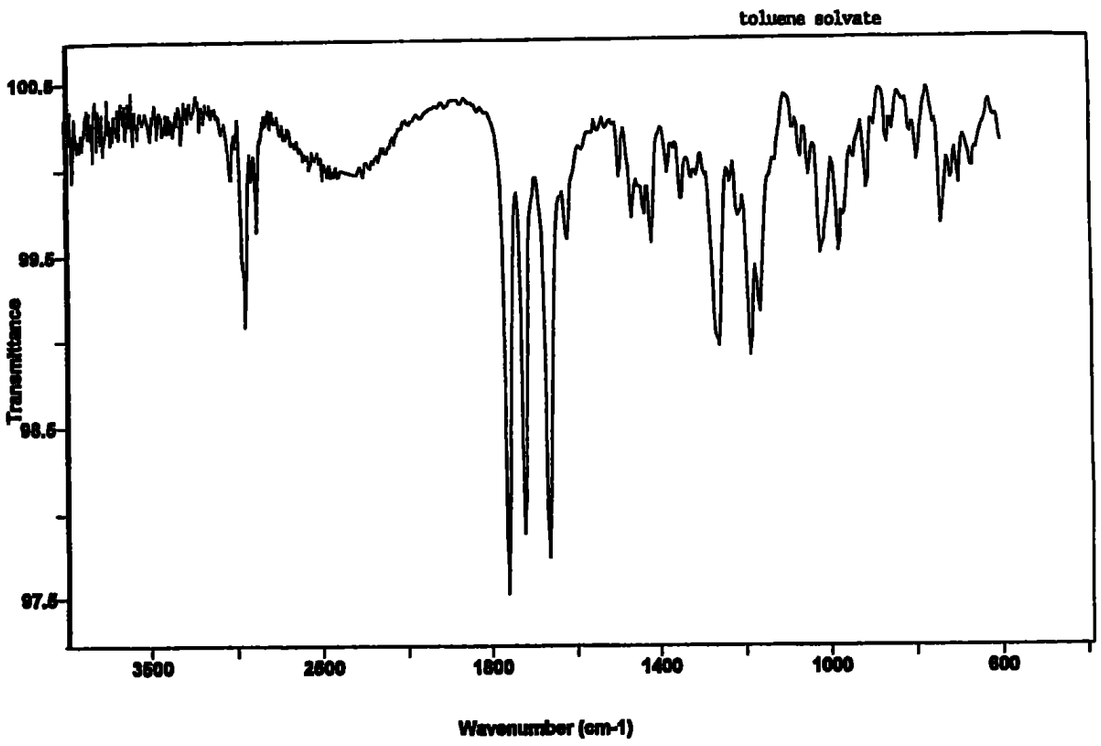
6016 6[redacted] 120899

Figure 3-I



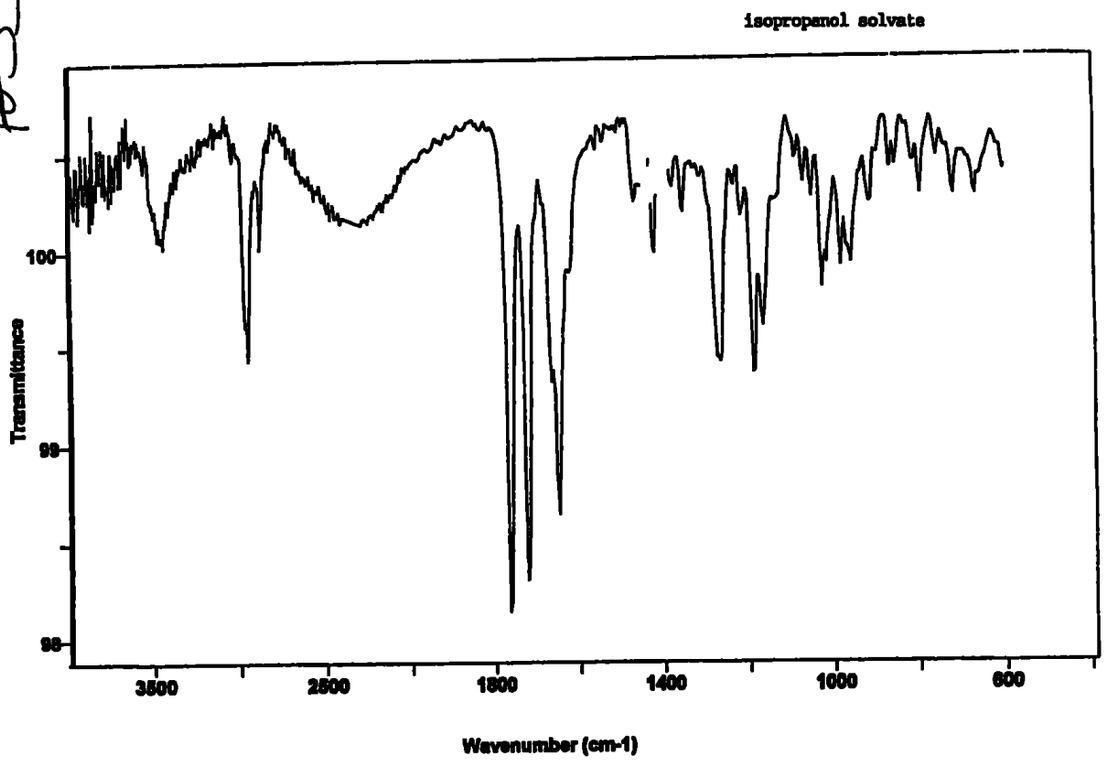
6 1696[illegible]3 120899

Figure 3-J



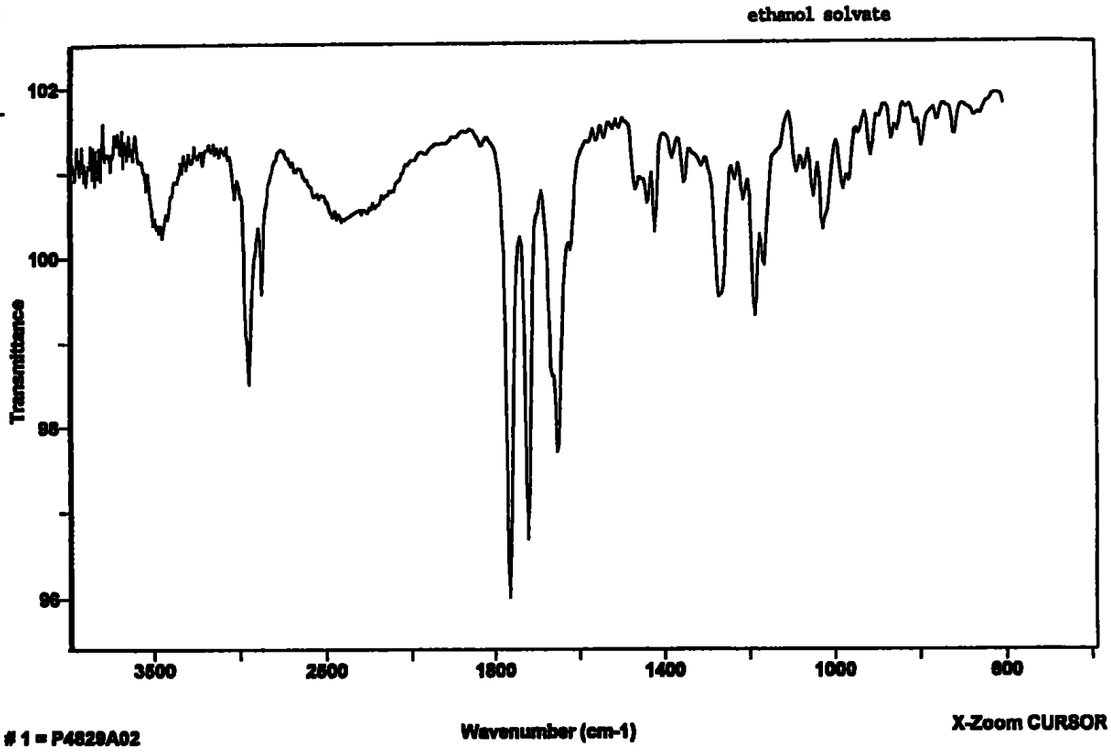
601696[redacted]3.120899

Figure 3-K



6016 [redacted]3 1208 9

Figure 3-L



601696[redacted]3 120899

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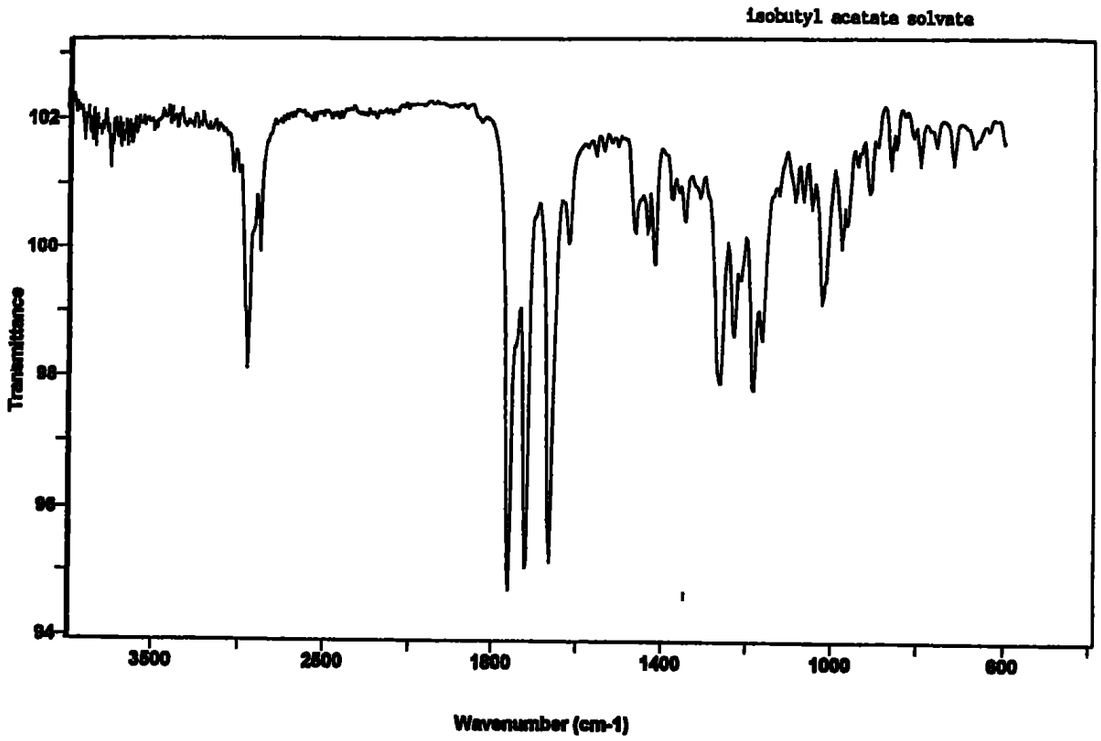
Figure 3-M



601696[redacted]3 1208 9

Figure 3-N

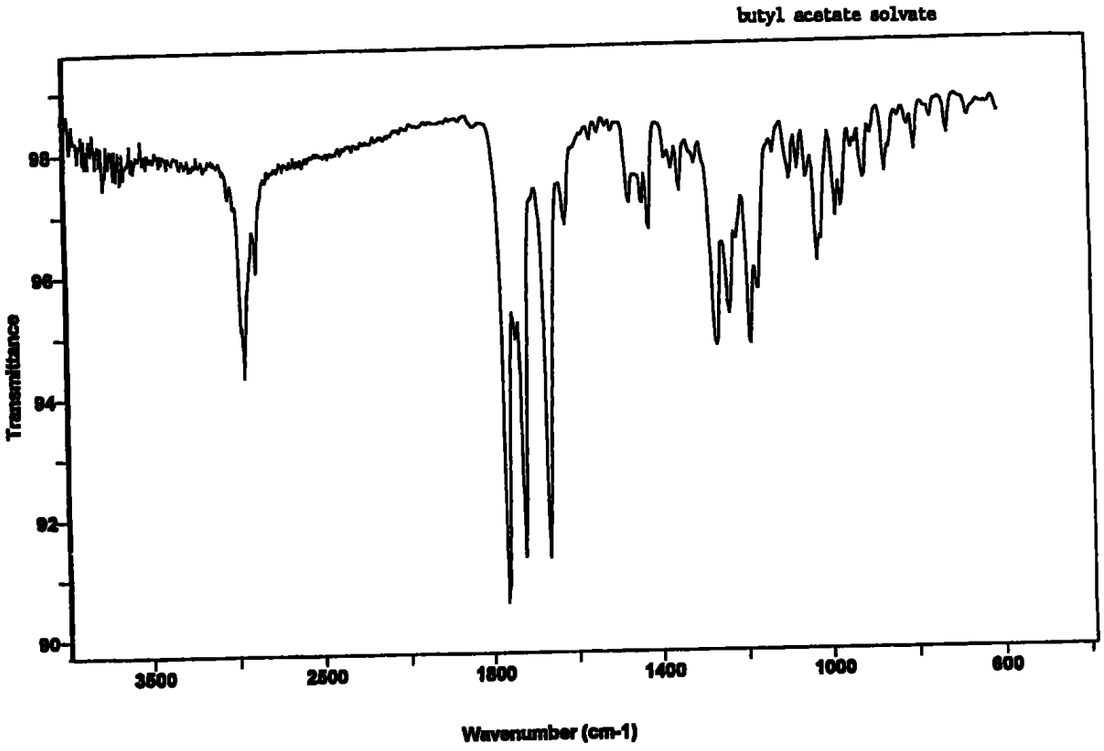


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Figure 3-0

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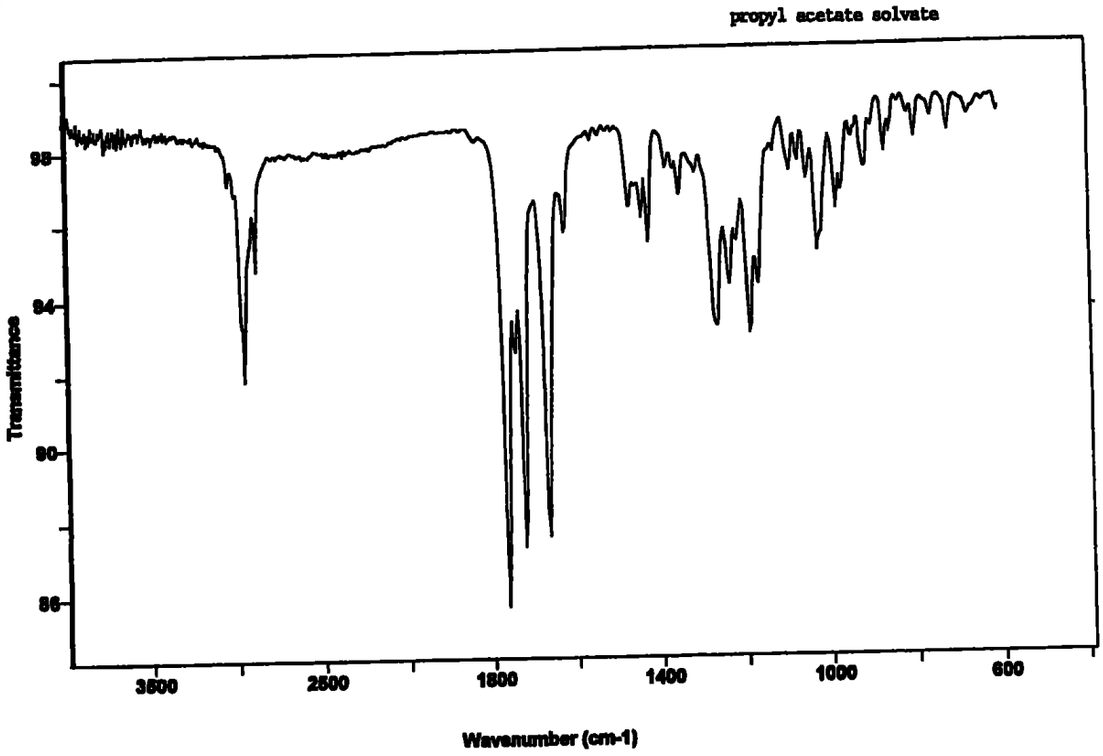

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Figure 3-P

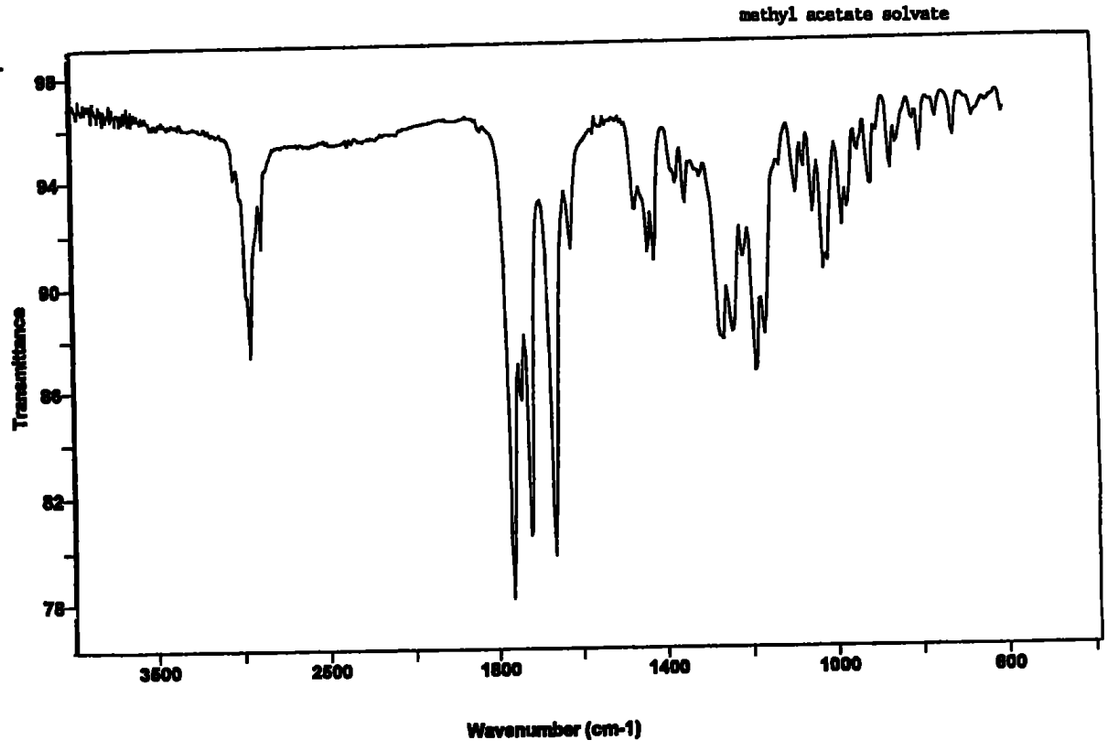

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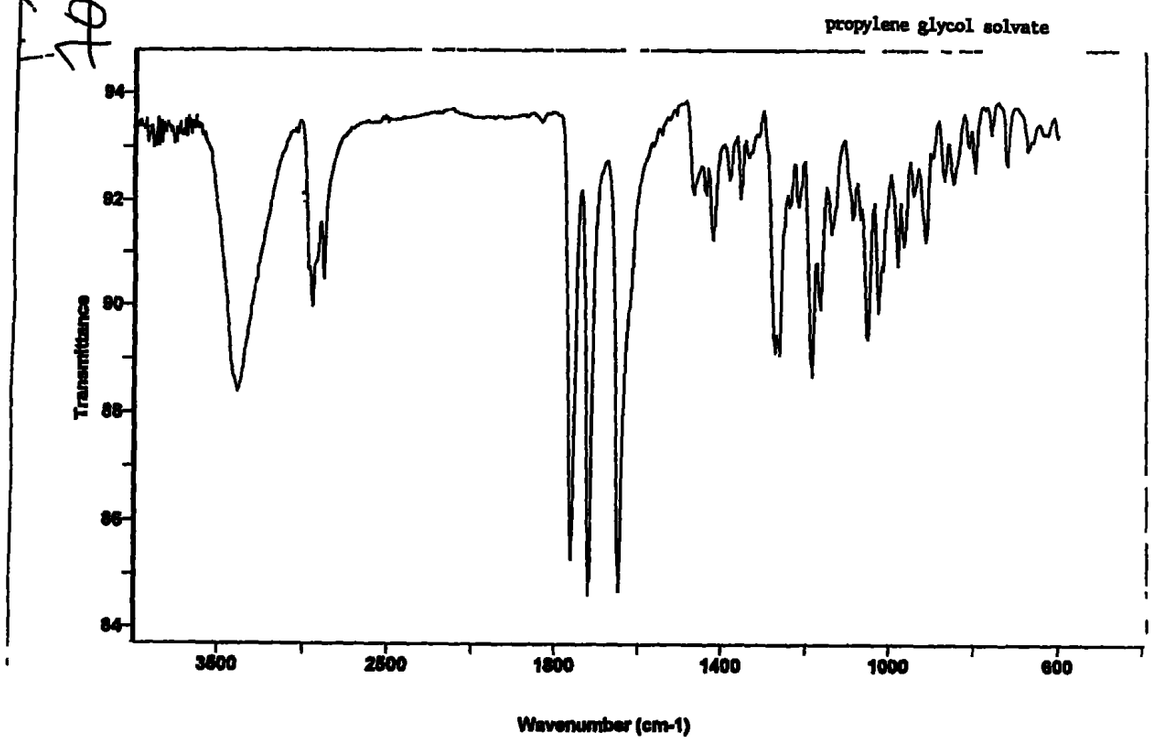
Figure 3-Q



601696[illegible] 120899

Figure 3-R

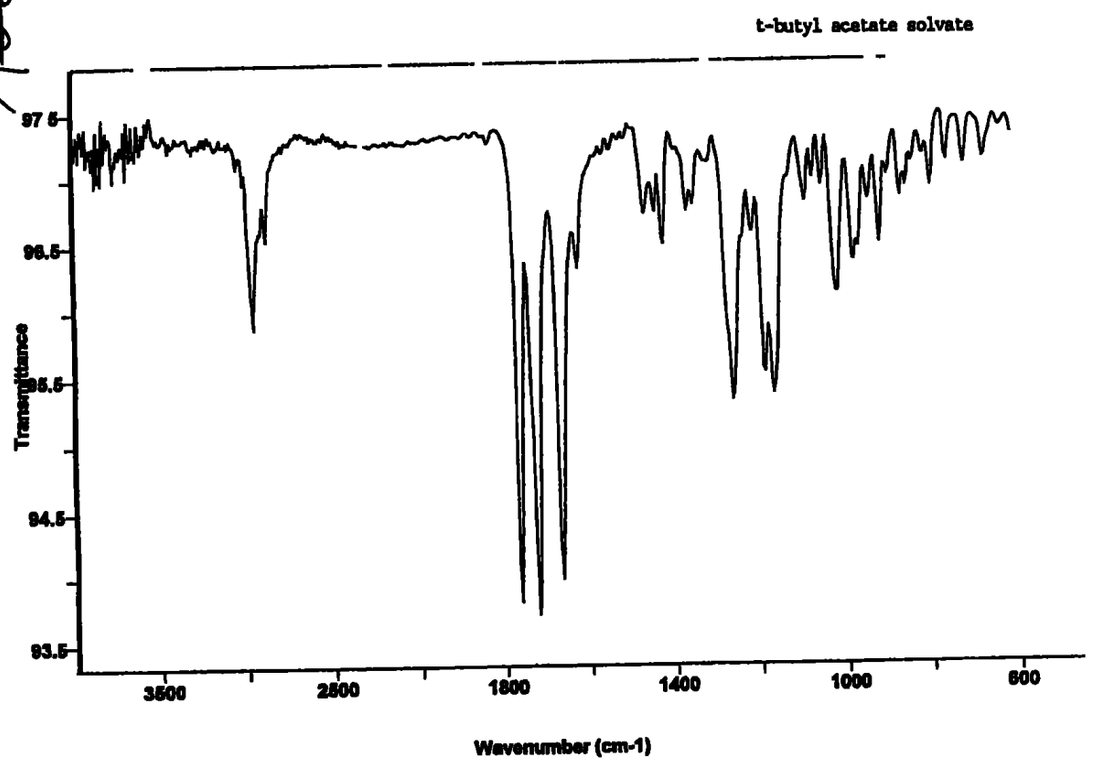


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Figure 4

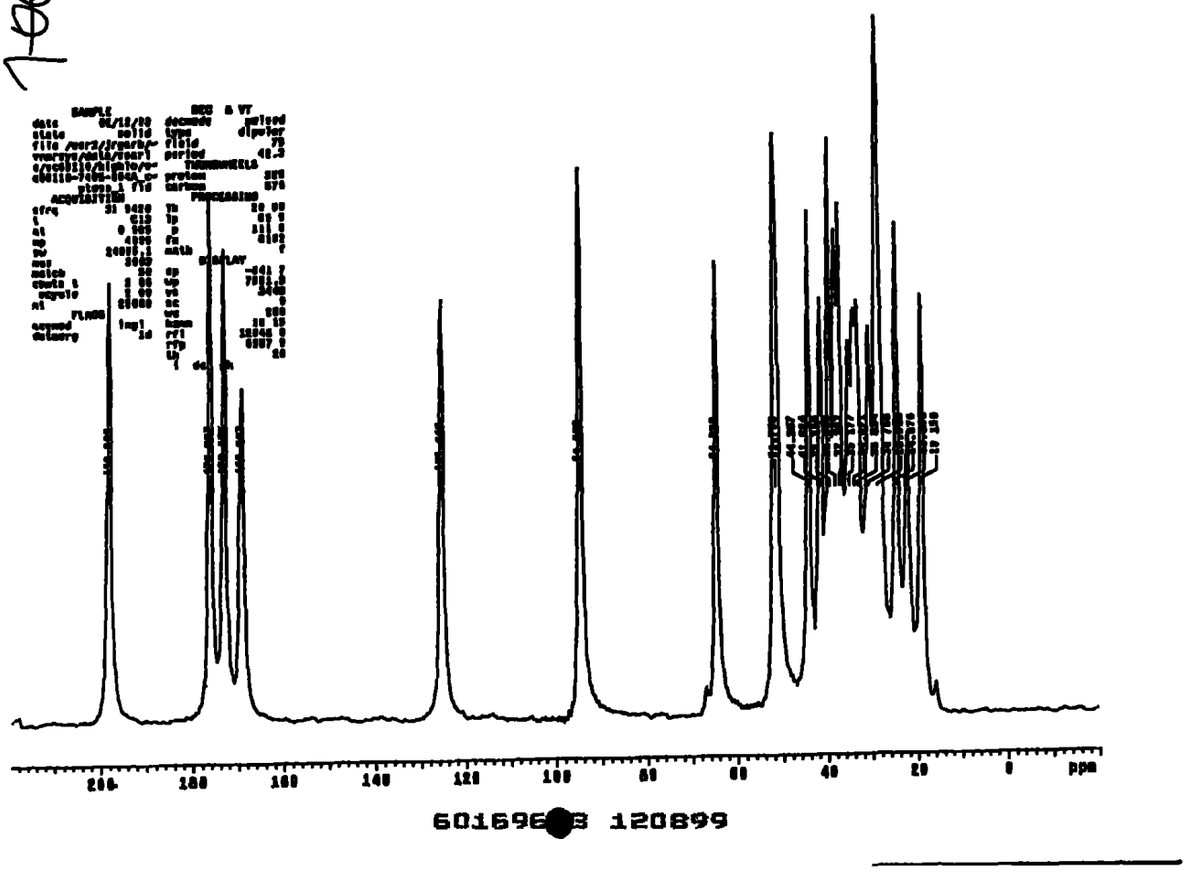


Figure 5

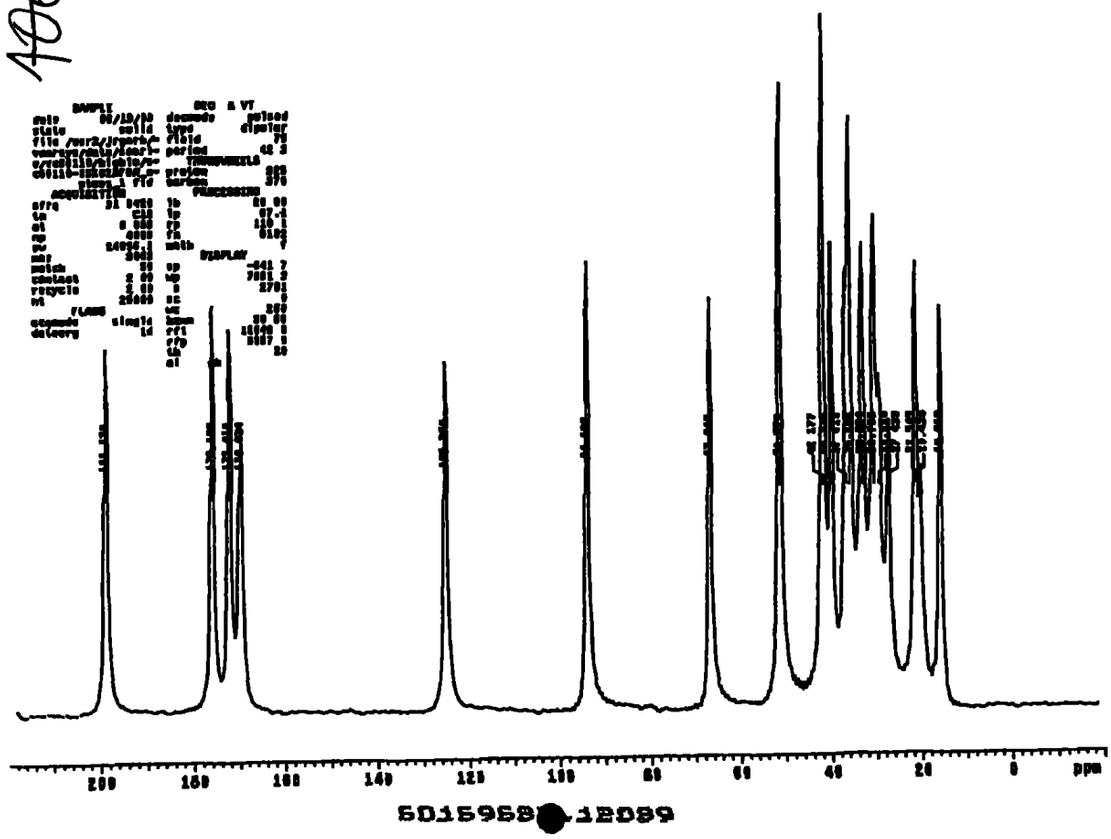
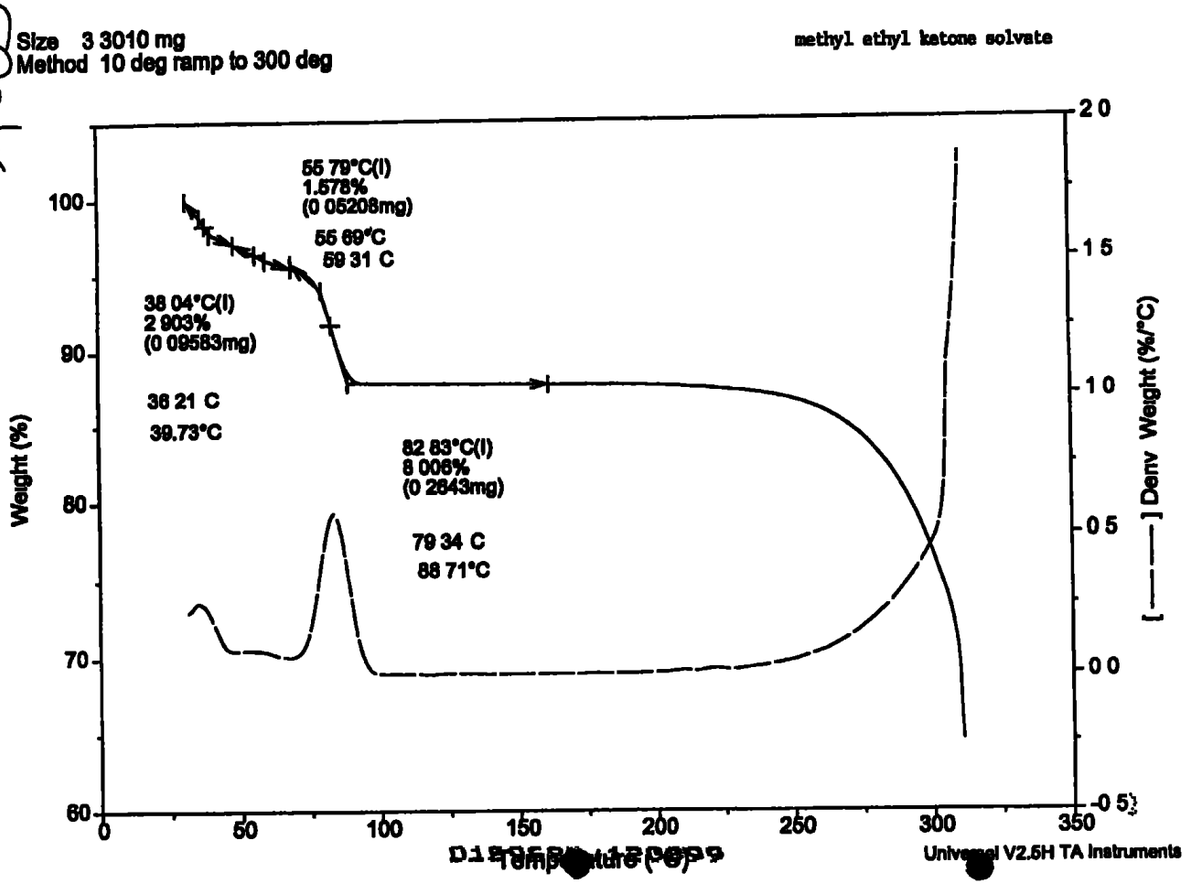


Figure 6-A

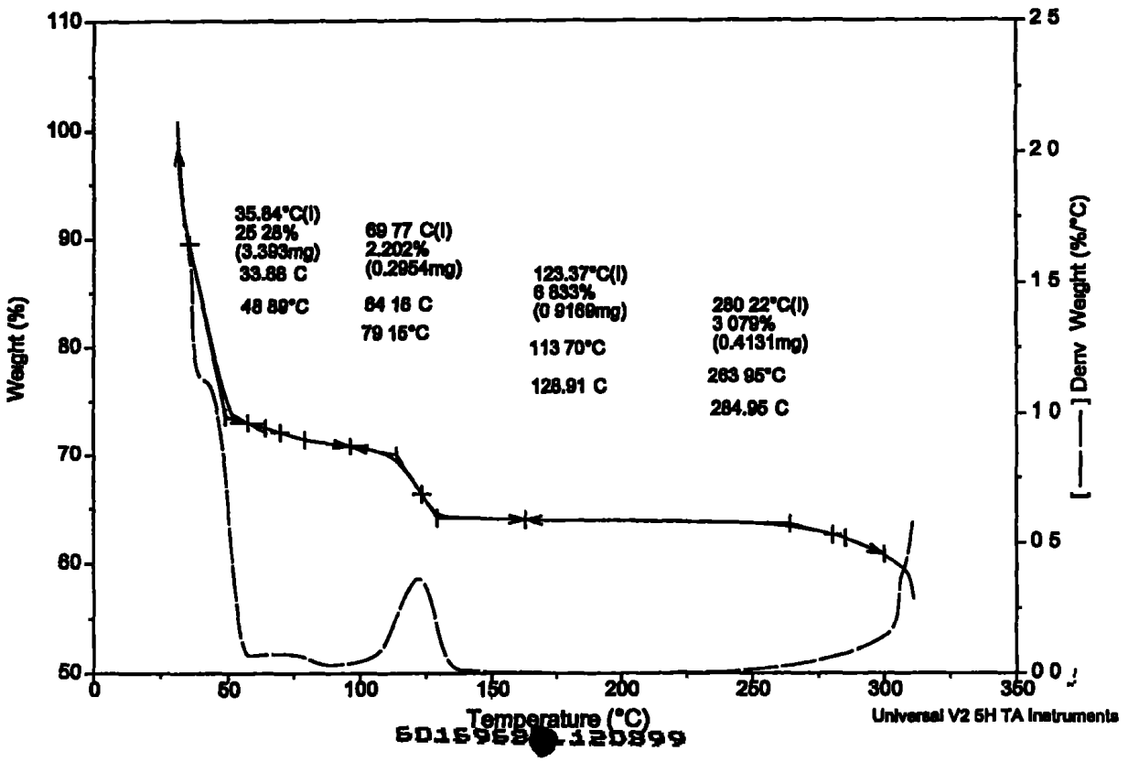

Size 3 3010 mg
Method 10 deg ramp to 300 deg
methyl ethyl ketone solvate
55 79°C(I)
1.578%
(0 05208mg)
55 69°C
59 31 C
38 04°C(I)
2 903%
(0 09583mg)
36 21 C
39.73°C
82 83°C(I)
8 006%
(0 2643mg)
79 34 C
88 71°C
Weight (%)
[——] Deriv Weight (%/°C)
Temperature (°C)
Universal V2.5H TA Instruments

7666

Figure 6 B

Size 13.4190 mg
Method 10 deg ramp to 300 deg

n-propyl alcohol solvate



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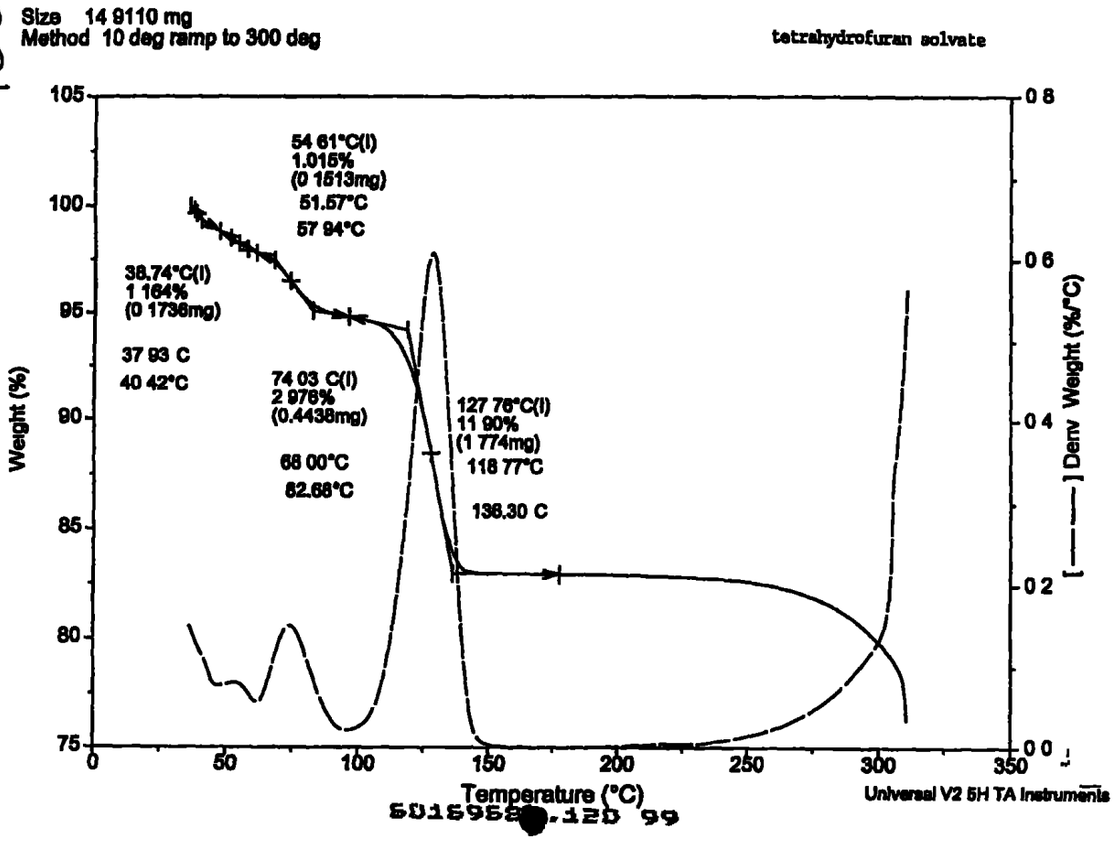
1067

Figure 6-C

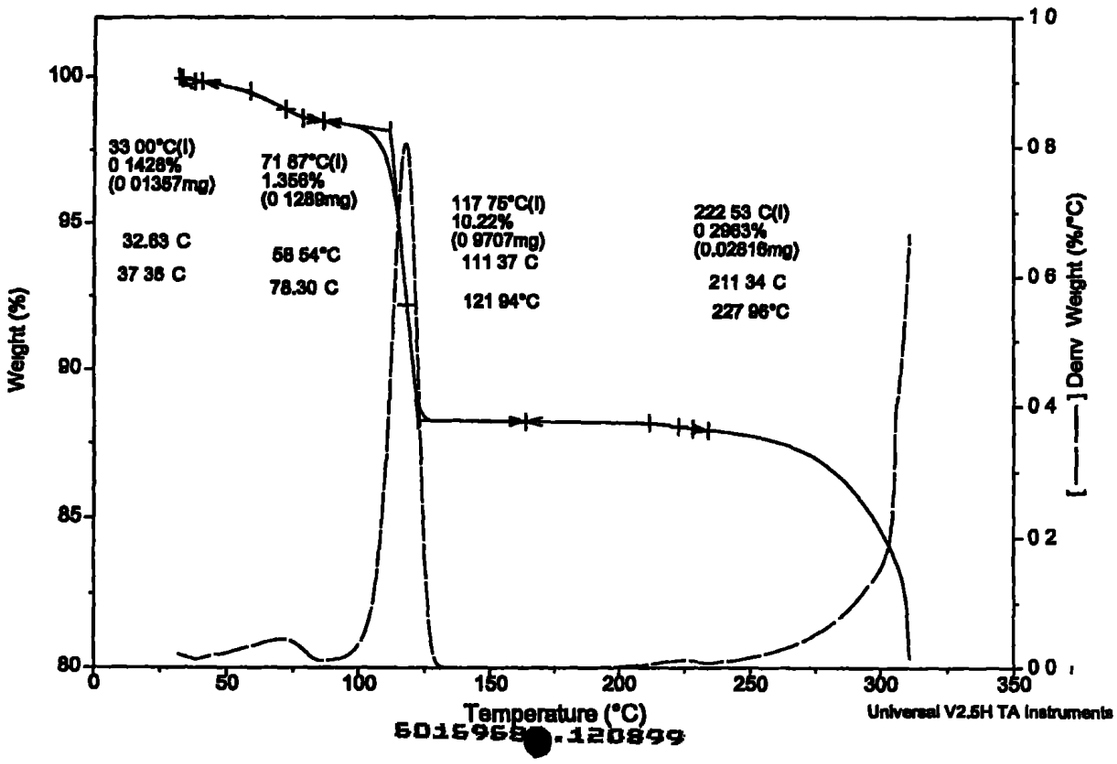


1060

Figure 6-D

Size 9 5030 mg
Method 10 deg ramp to 300 deg

ethyl propionate solvate



5015958.120899

Figure 6 E

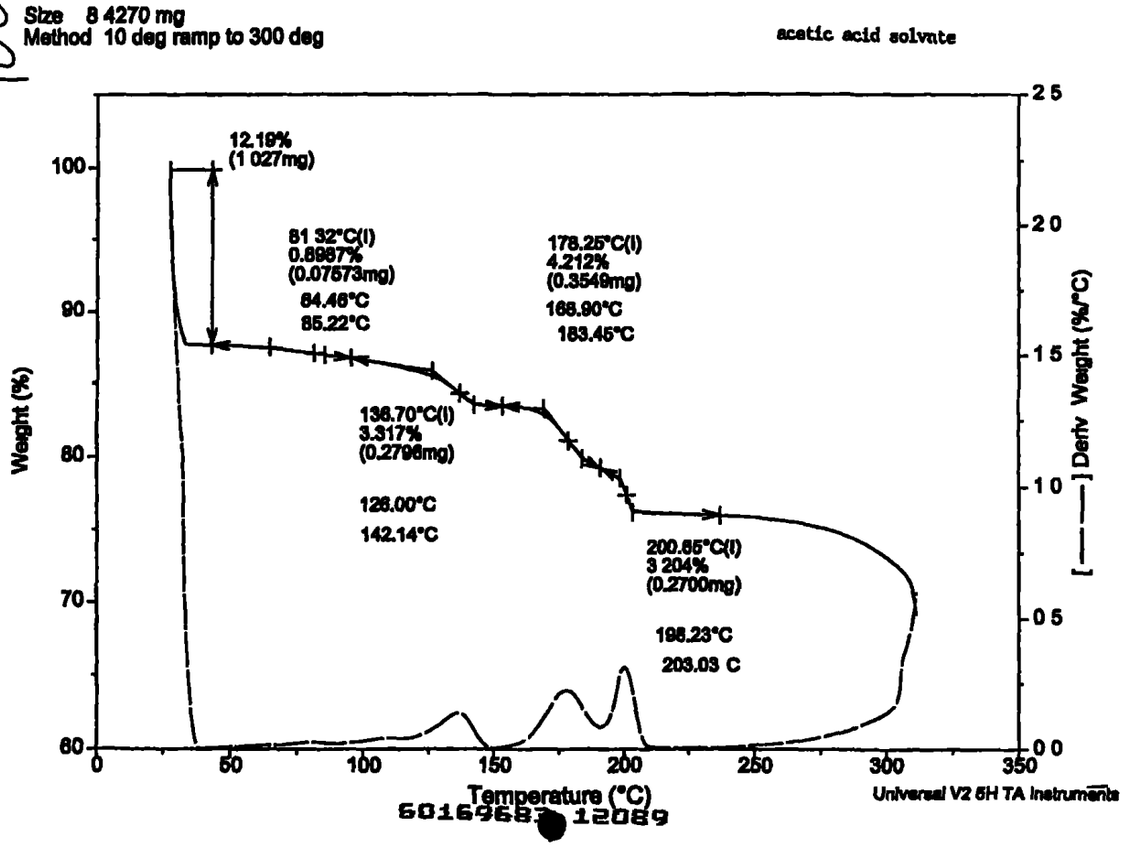
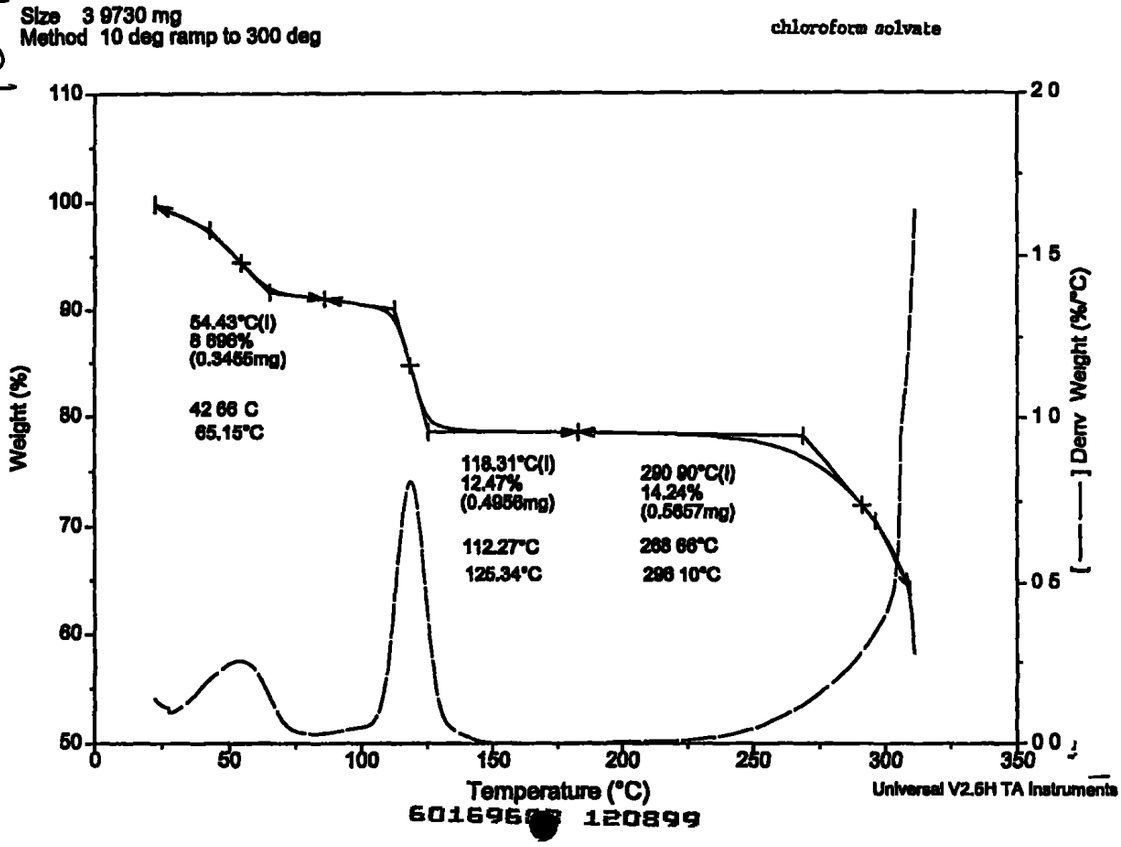

Size 8 4270 mg
Method 10 deg ramp to 300 deg
acetic acid solvate
12.19%
(1 027mg)
81 32°C(I)
0.8987%
(0.07573mg)
84.46°C
85.22°C
178.25°C(I)
4.212%
(0.3549mg)
168.90°C
183.45°C
136.70°C(I)
3.317%
(0.2796mg)
126.00°C
142.14°C
200.65°C(I)
3 204%
(0.2700mg)
198.23°C
203.03 C
Weight (%)
[——] Deriv Weight (%/°C)
Temperature (°C)
Universal V2 6H TA Instruments

Figure 6-F


Size 3 9730 mg
Method 10 deg ramp to 300 deg
chloroform solvate
54.43°C(I)
8 696%
(0.3456mg)
42 66 C
65.15°C
118.31°C(I)
12.47%
(0.4956mg)
112.27°C
125.34°C
290 90°C(I)
14.24%
(0.5657mg)
288 66°C
296 10°C
Weight (%)
[——] Deriv Weight (%/°C)
Temperature (°C)
Universal V2.6H TA Instruments
120899

1074

Figure 6-G

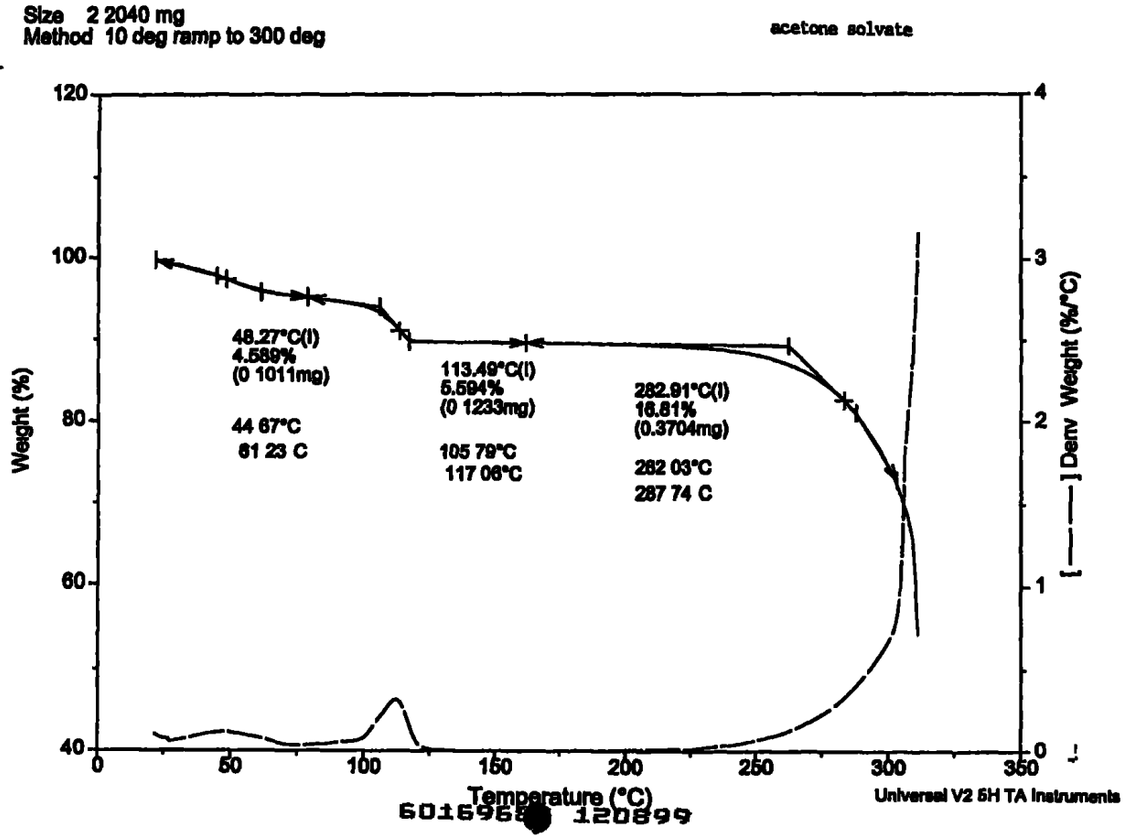


Figure 6-H

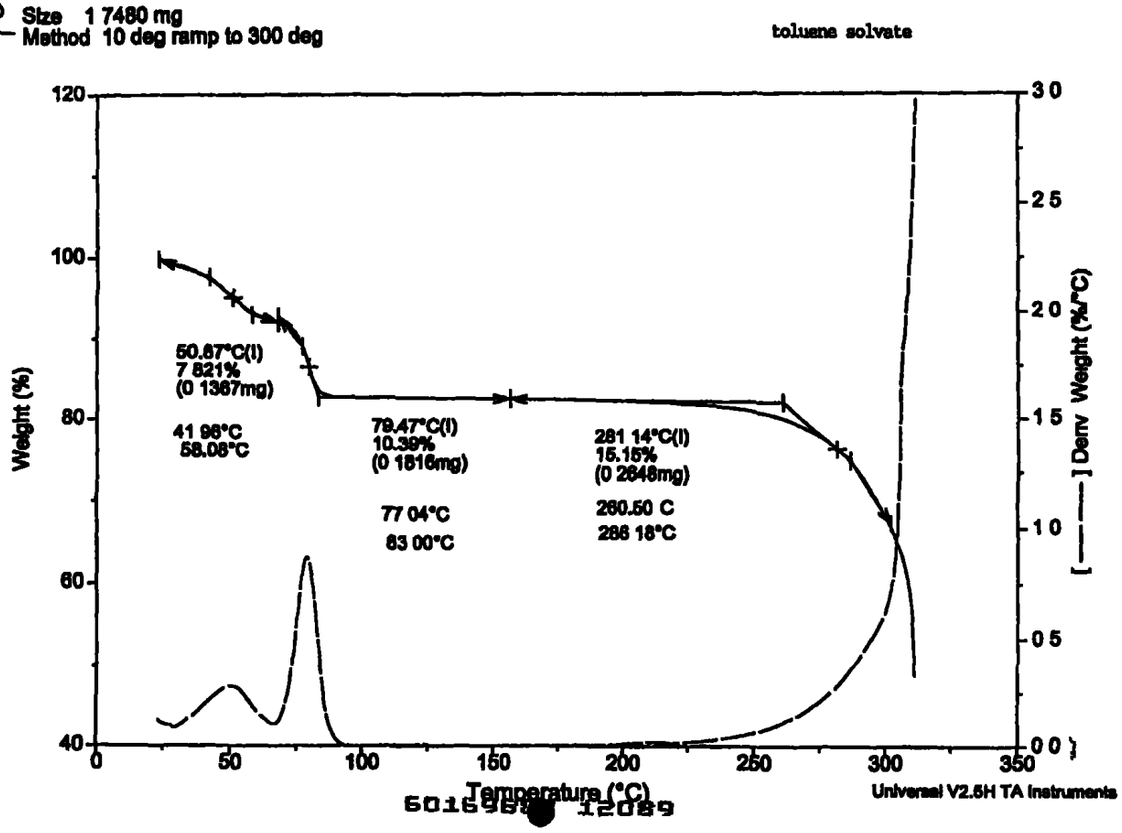

Size 1 7480 mg
Method 10 deg ramp to 300 deg
toluene solvate
50.67°C(I)
7 821%
(0 1367mg)
41 96°C
58.08°C
79.47°C(I)
10.39%
(0 1816mg)
77 04°C
83 00°C
281 14°C(I)
15.15%
(0 2648mg)
260.50 C
286 18°C
Weight (%)
[——] Deriv Weight (%/°C)
Temperature (°C)
Universal V2.5H TA Instruments

Figure 6-I

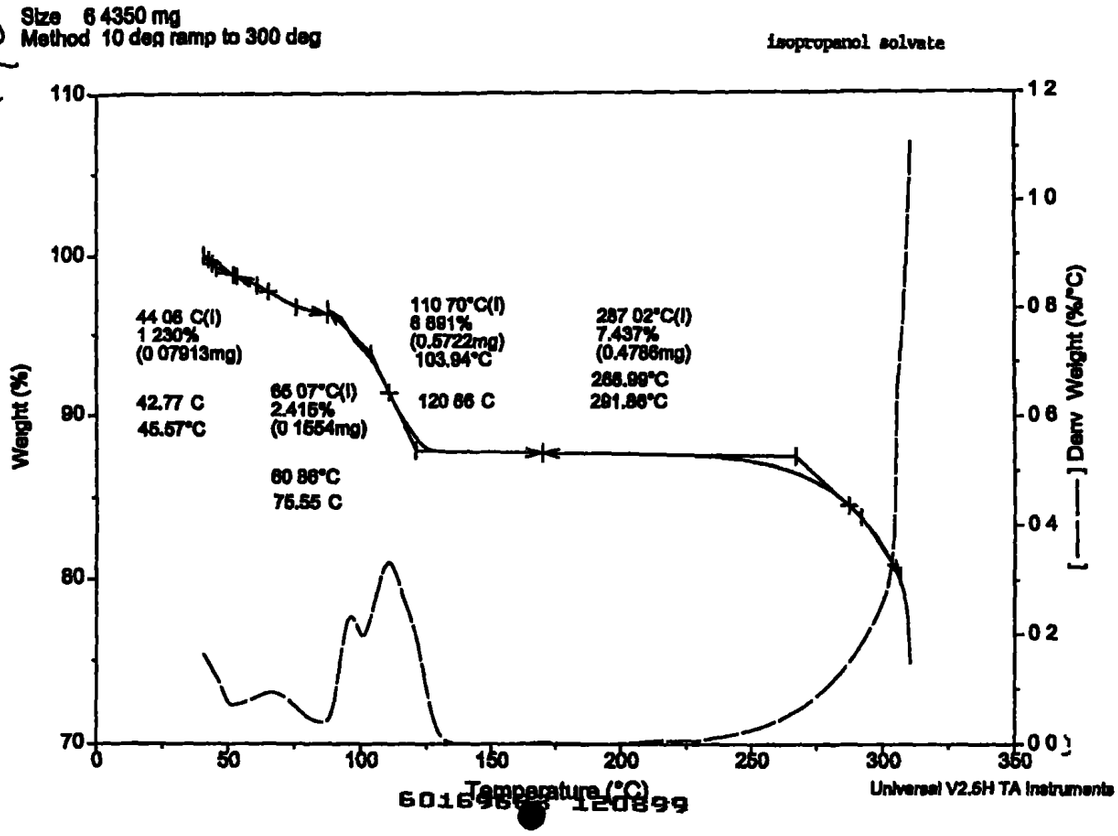

Size 6 4350 mg
Method 10 deg ramp to 300 deg
isopropanol solvate
44 06 C(I)
1 230%
(0 07913mg)
42.77 C
45.57°C
66 07°C(I)
2.415%
(0 1554mg)
60 86°C
75.55 C
110 70°C(I)
6 891%
(0.5722mg)
103.94°C
120 86 C
287 02°C(I)
7.437%
(0.4786mg)
288.99°C
291.86°C
Weight (%)
[——] Deriv Weight (%/°C)
Temperature (°C)
Universal V2.5H TA Instruments

Figure 6-J

Size 6 1160 mg
Method 10 deg ramp to 300 deg

ethanol solvate

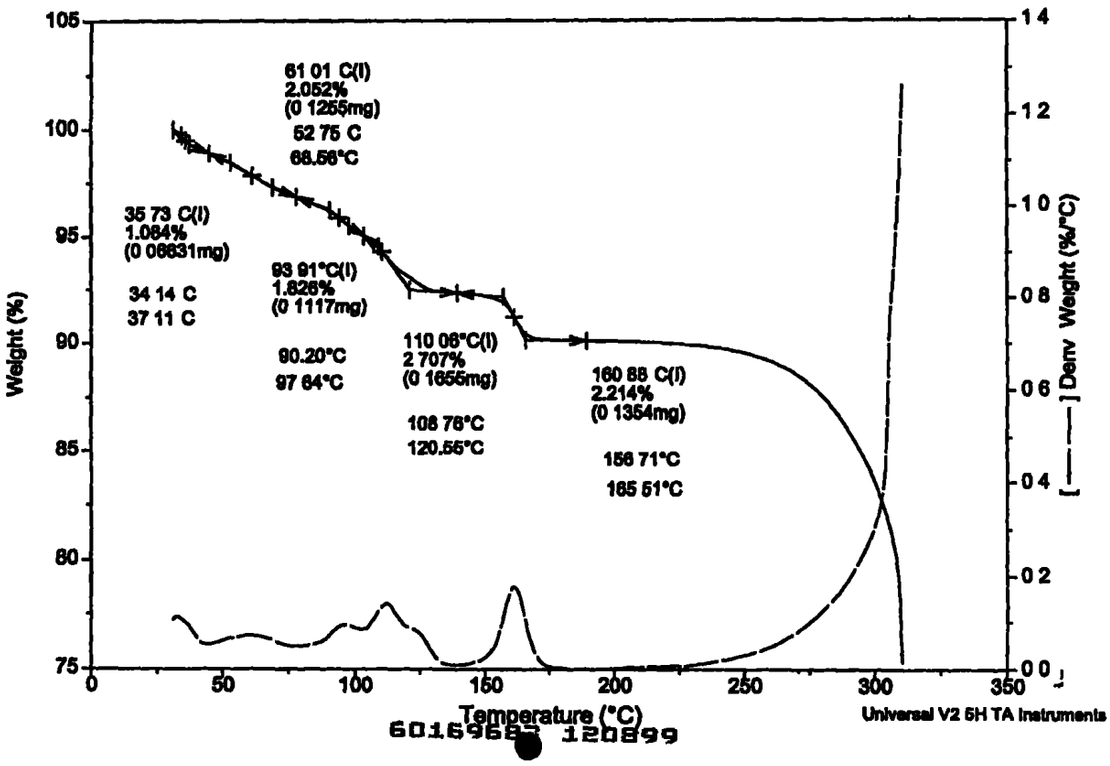


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Figure 6-K

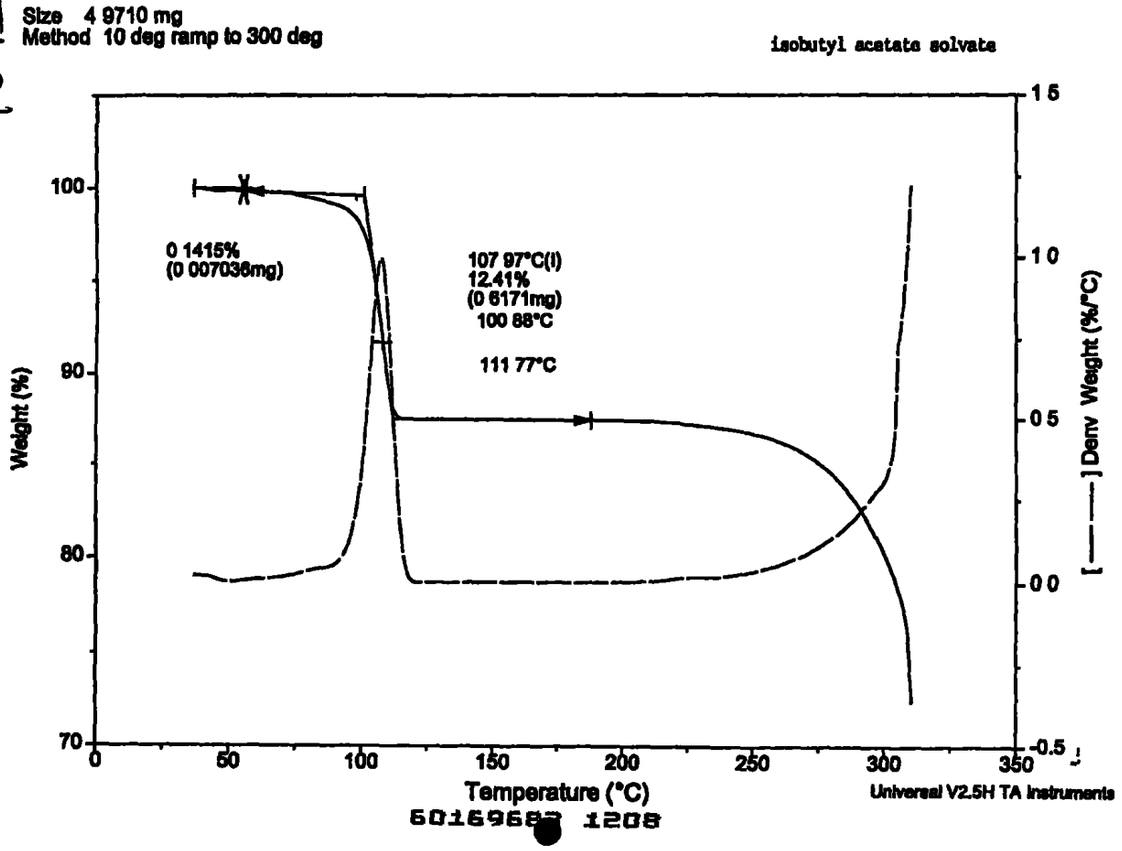

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Figure 6-L

Size 2 3240 mg
Method 10 deg ramp to 300 deg

butyl acetate solvate

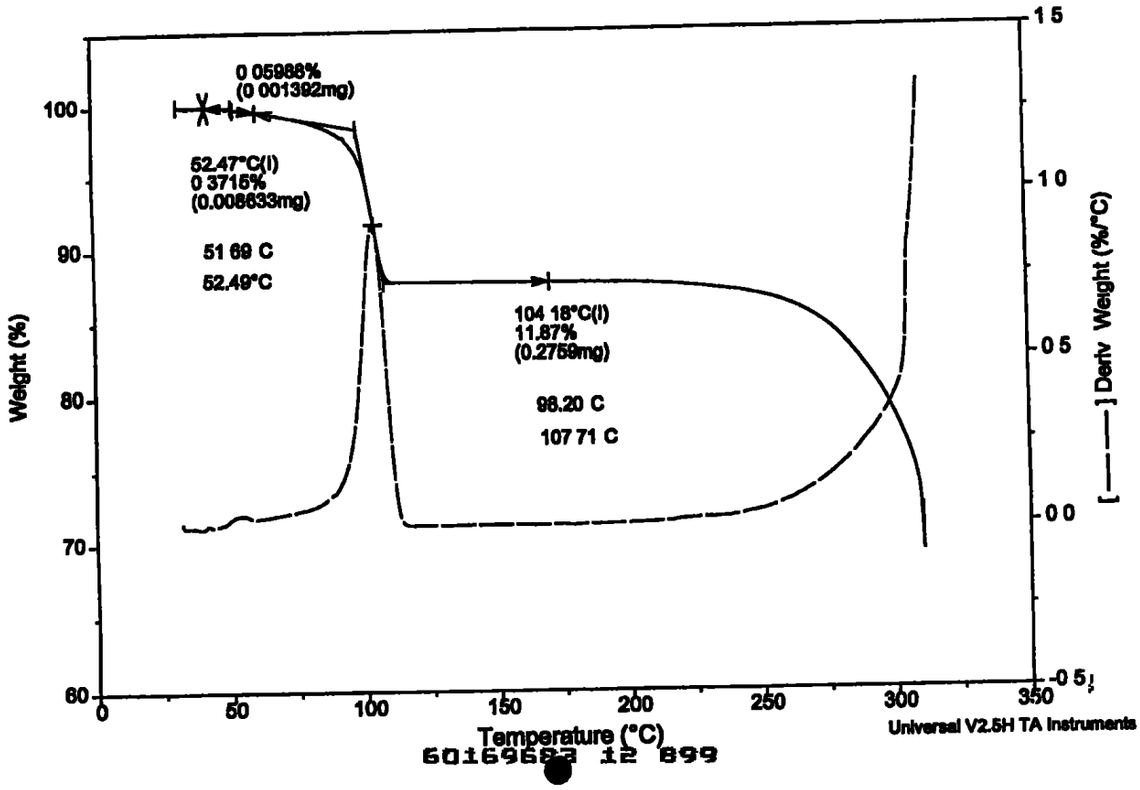

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Figure 6-M

Size 2 7750 mg
Method 10 deg ramp to 300 deg

methyl acetate solvate

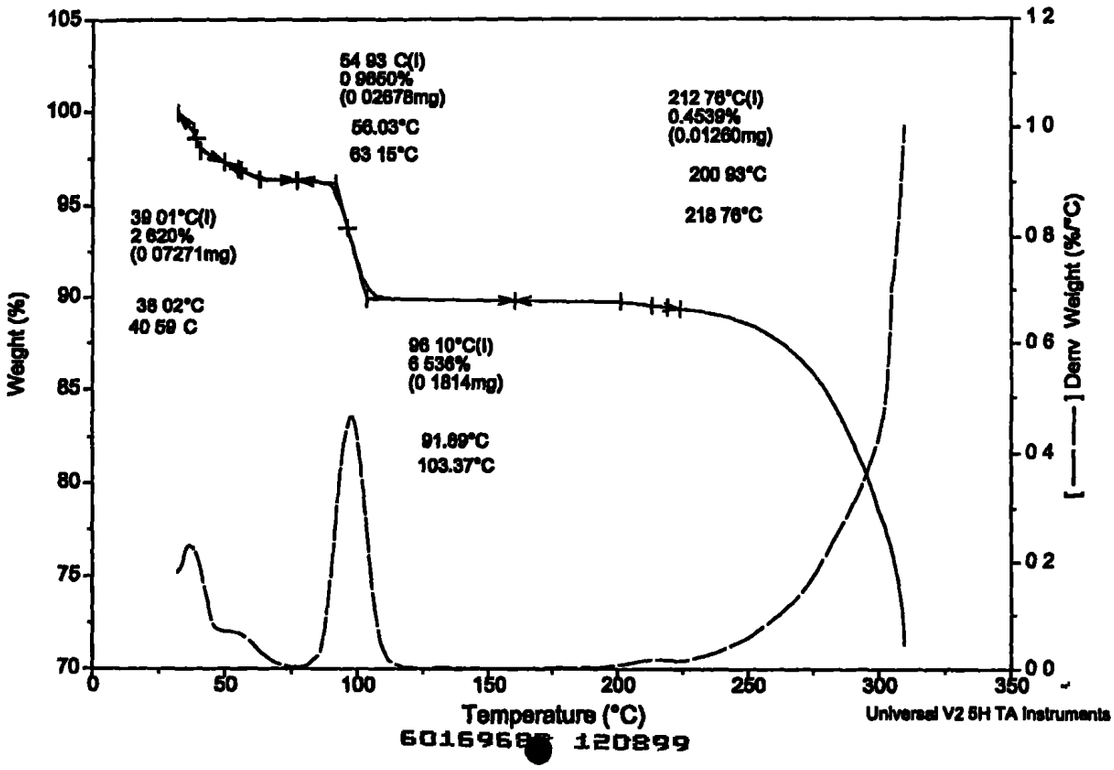

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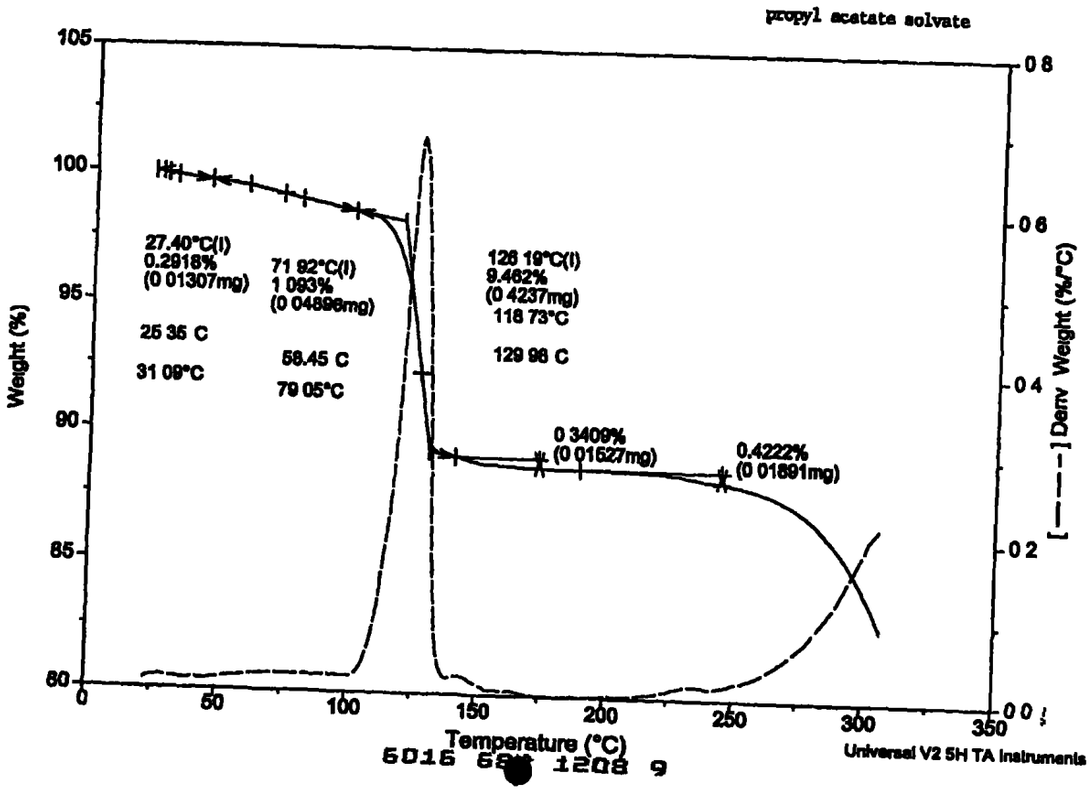
Figure 6-N



5016 6[redacted] 1208 9

Figure 6-0

propylene glycol solvate

Size 2 9590 mg
Method TG 10D/M TO 300

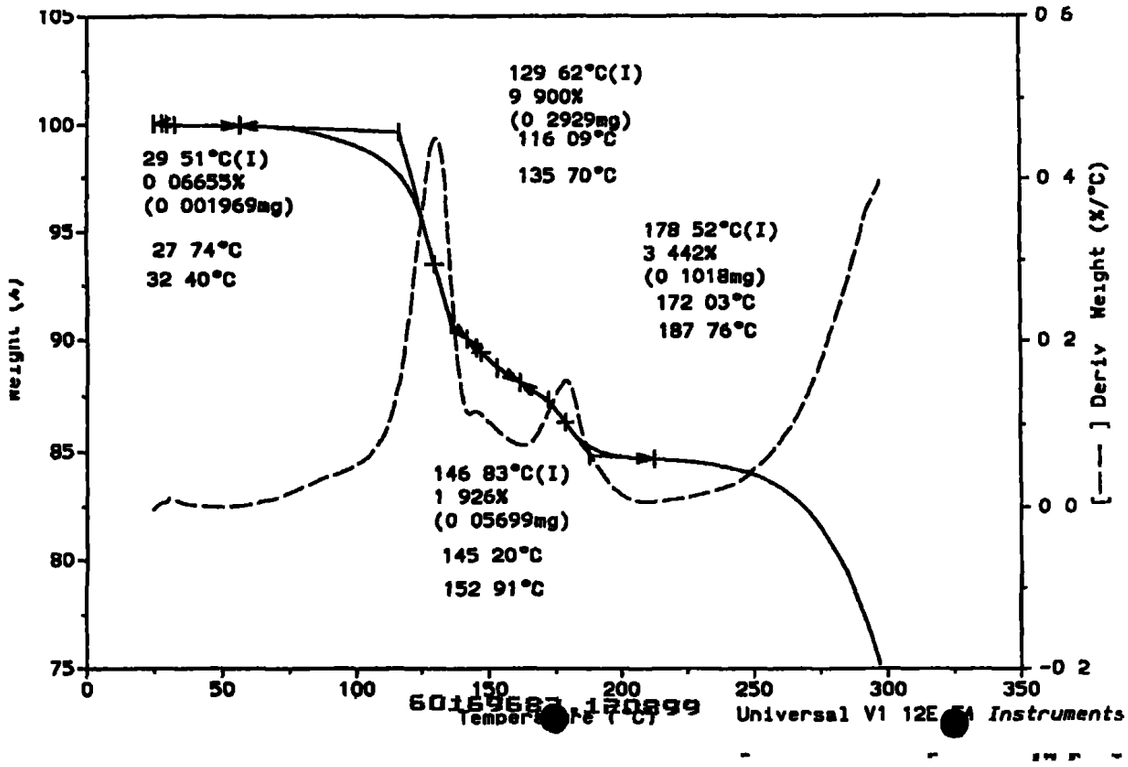
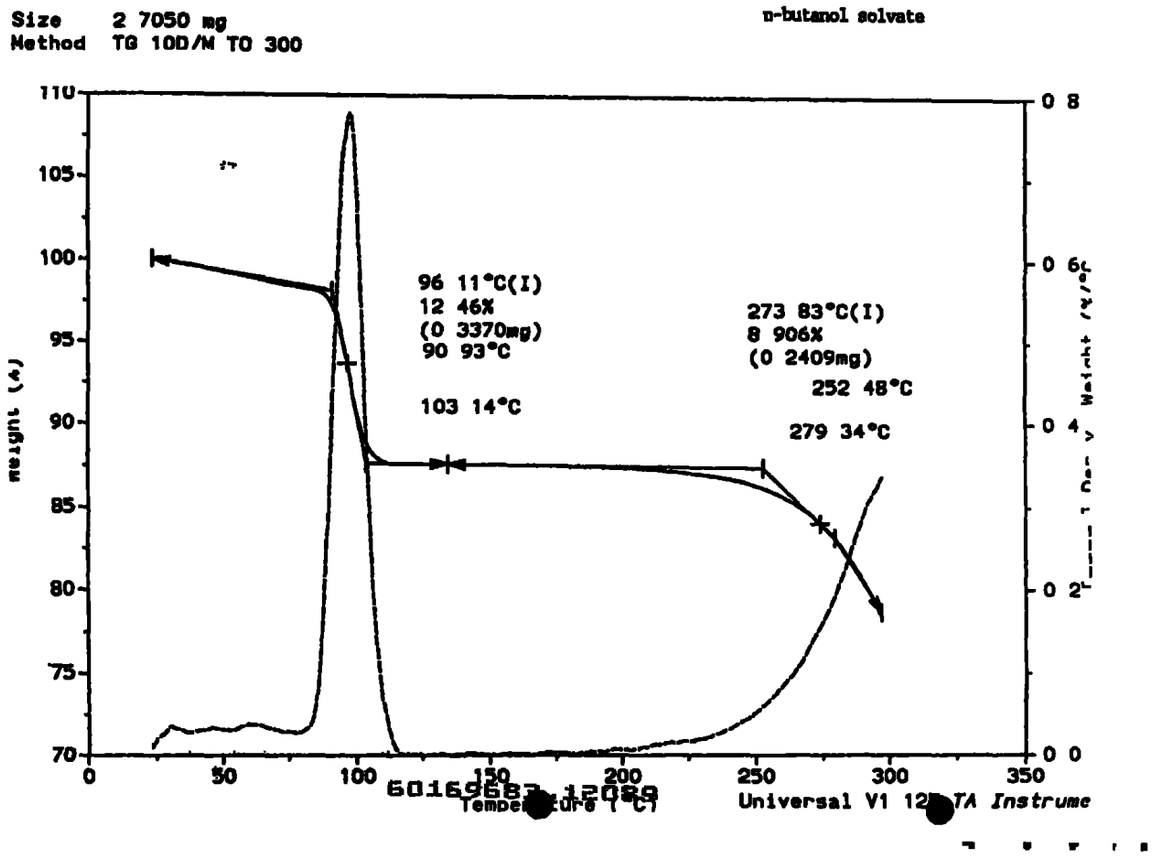


Figure 6-P


Size 2 7050 mg
Method TG 10D/M TO 300
n-butanol solvate
96 11°C(I)
12 46%
(0 3370mg)
90 93°C
103 14°C
273 83°C(I)
8 906%
(0 2409mg)
252 48°C
279 34°C
Weight (%)
Temperature (°C)
Universal V1 12 TA Instrume

1084

Figure 6-Q

Size 2 2420 mg
Method TG 10D/M TD 300

n-octanol solvate

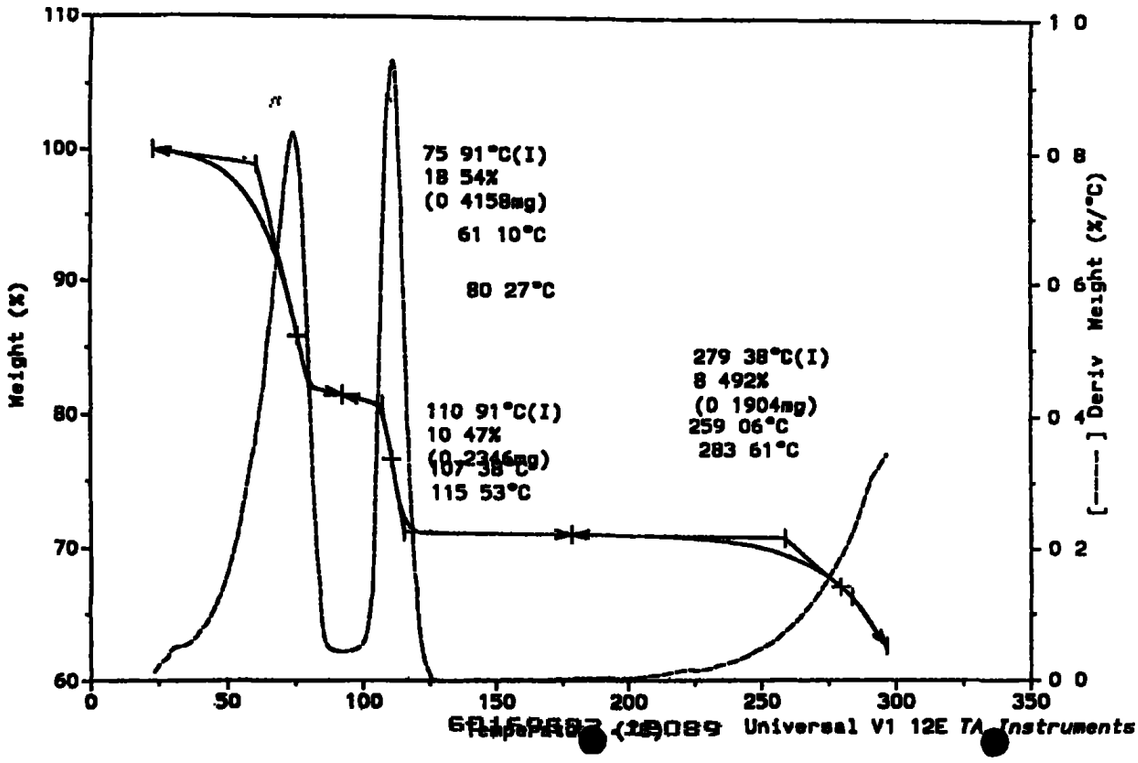


Figure 6-R

Size 2 8630 mg
Method TG 10D/M TO 300

t-butyl acetate solvate

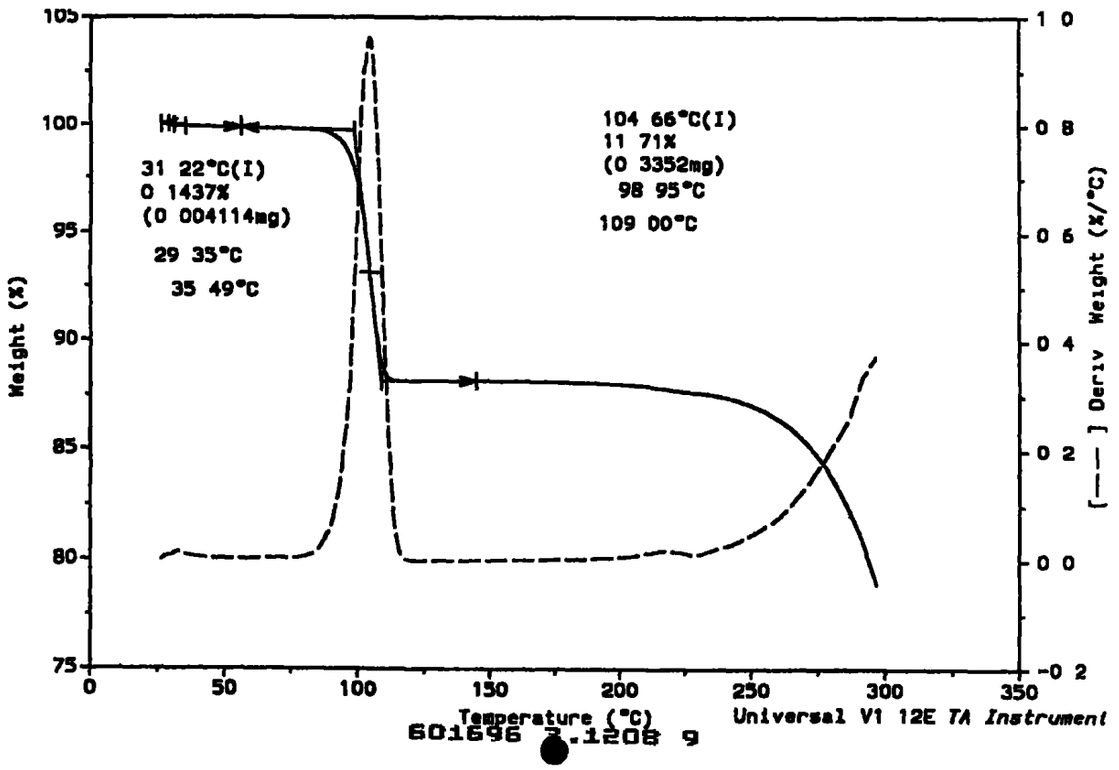


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Figure 7

Figure 8

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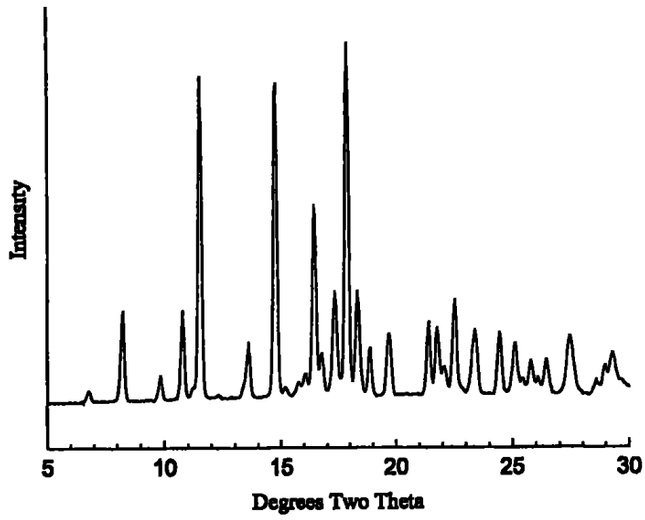

Figure 9

6016 683 120899

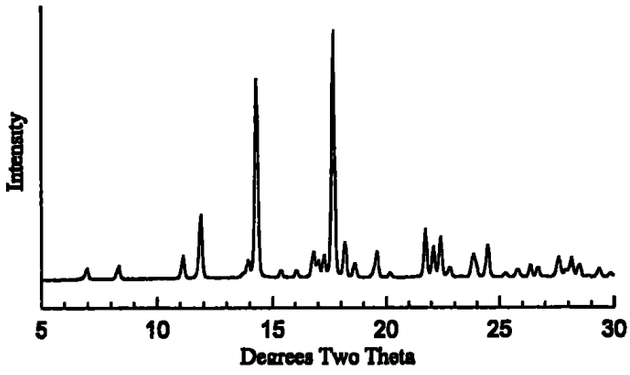

Figure 10

6016 683 120899

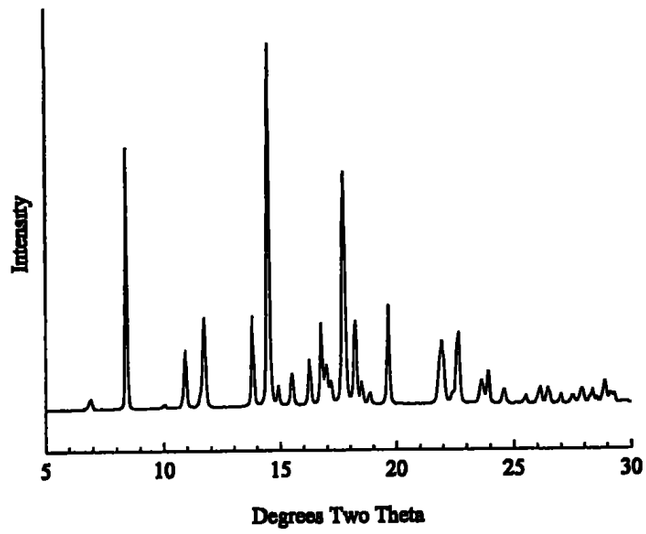

Figure 11

60169683 120899

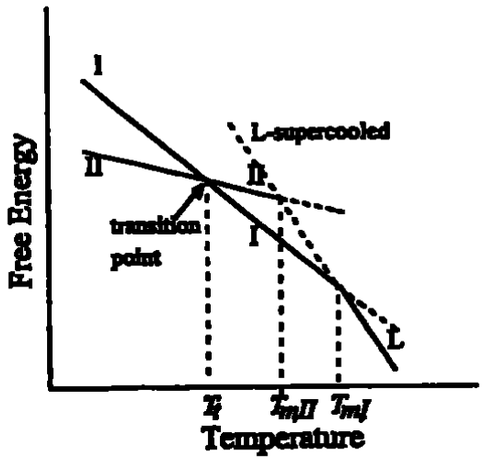

Figure 12:

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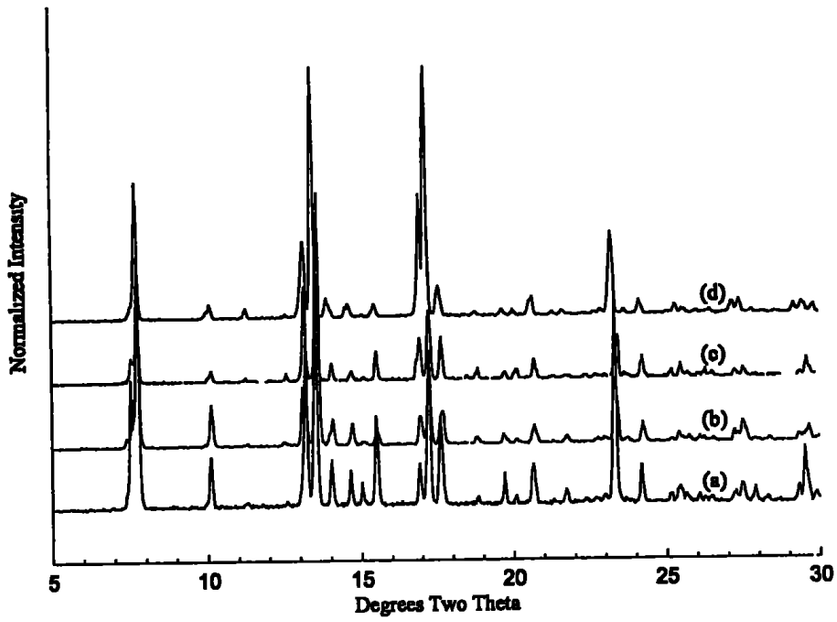

Figure 13

6016 683 12089

A0287

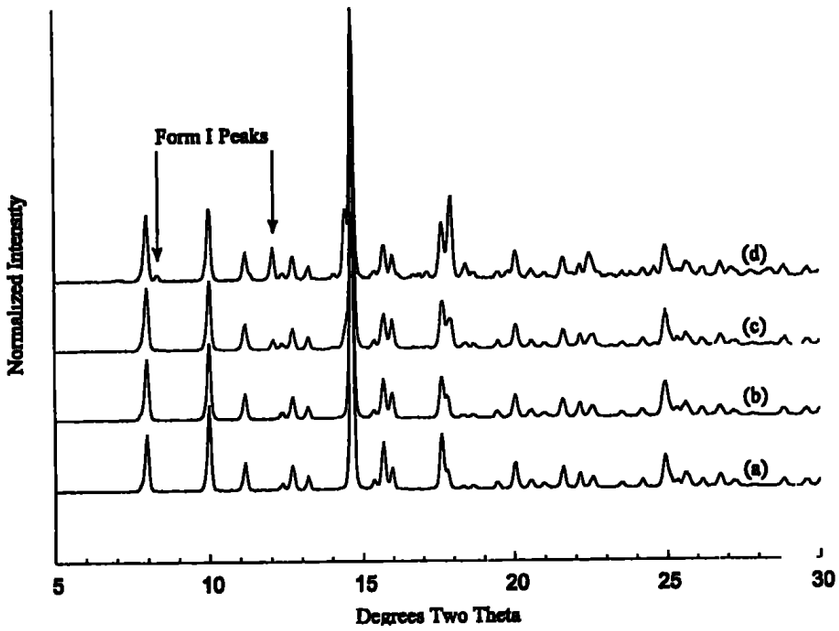

Figure 14

6016 683 120899

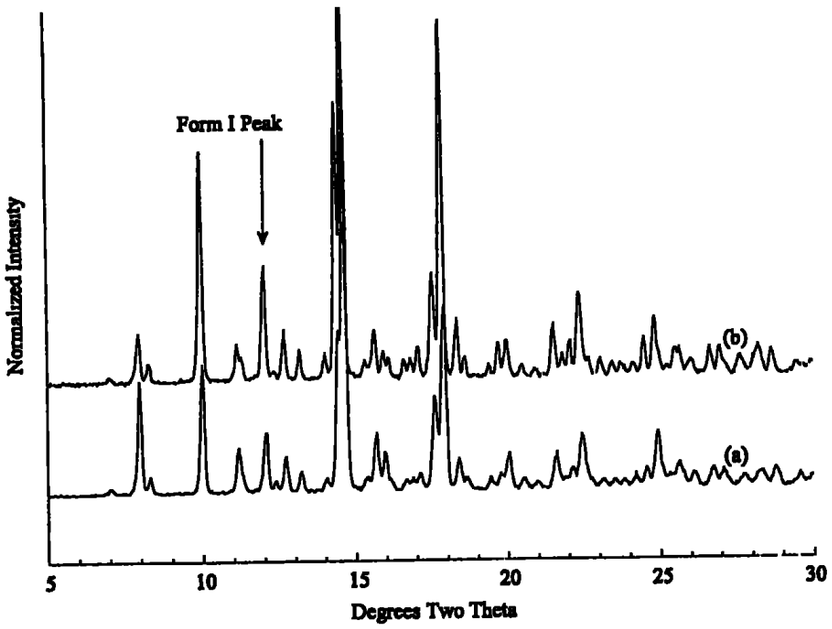

Figure 15

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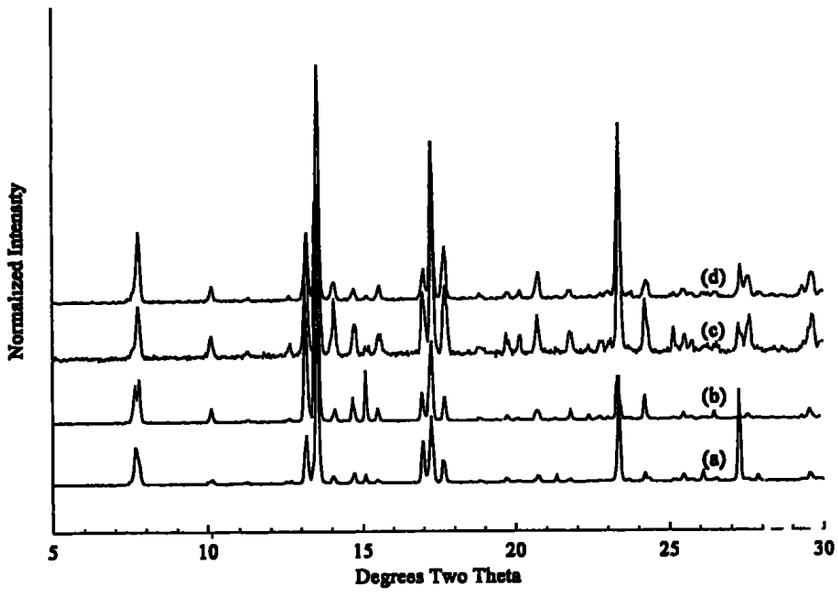


Figure 16

60169683 1208

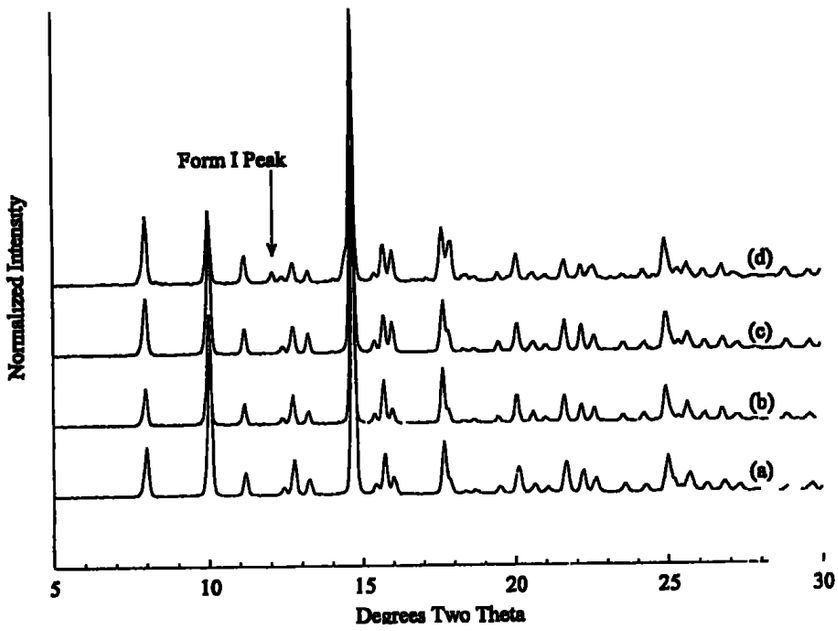
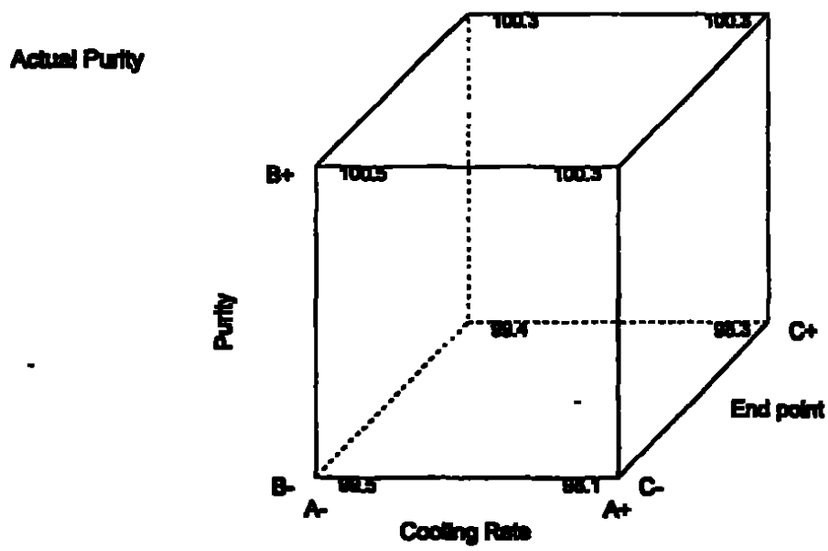


Figure 17

60169 83 1208 9

Figure 18



6016 683 120899

Figure 19

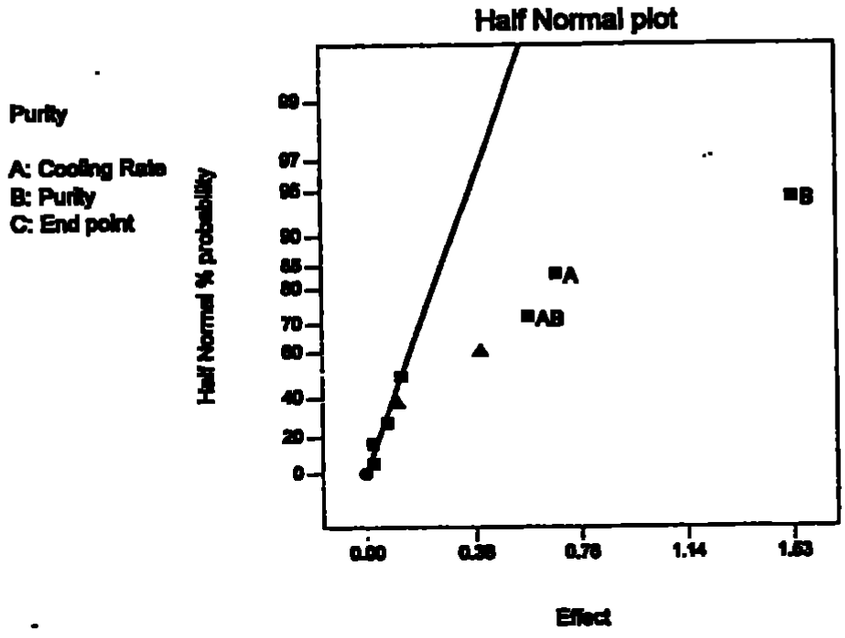


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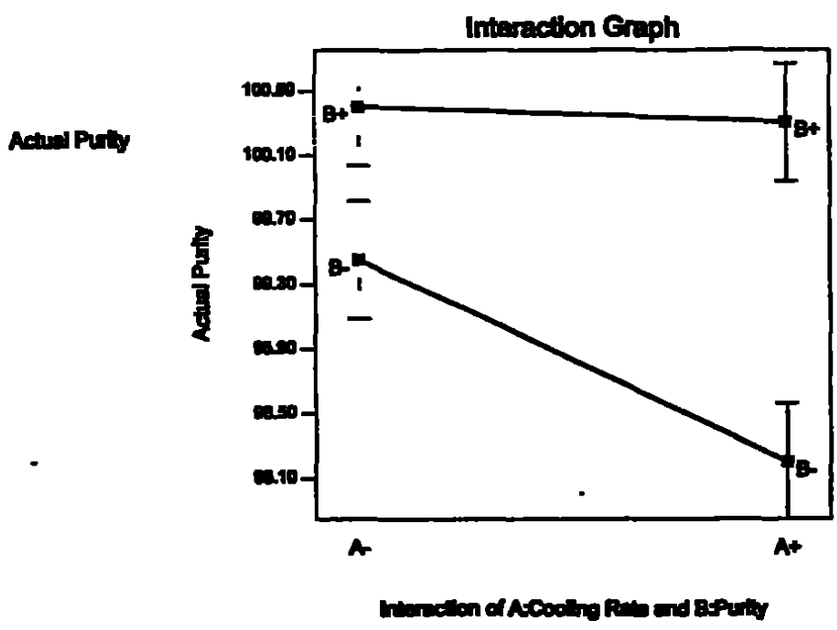
Figure 20



6016 683.12089

5601

Figure 21

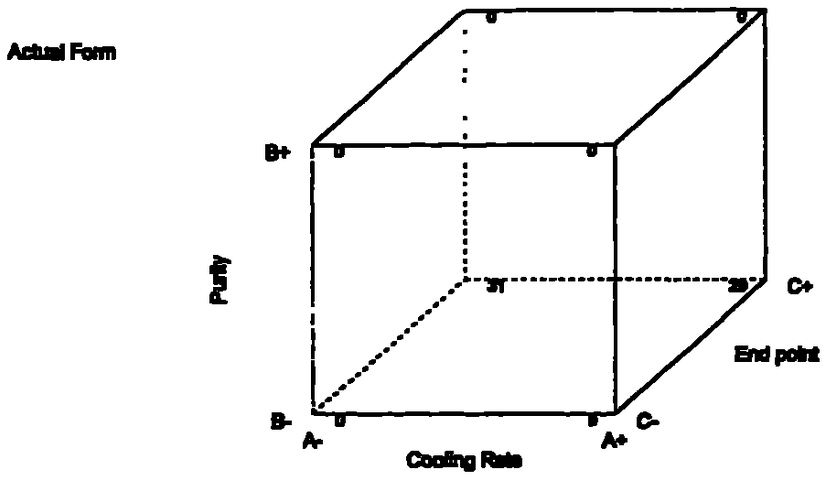


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Figure 22

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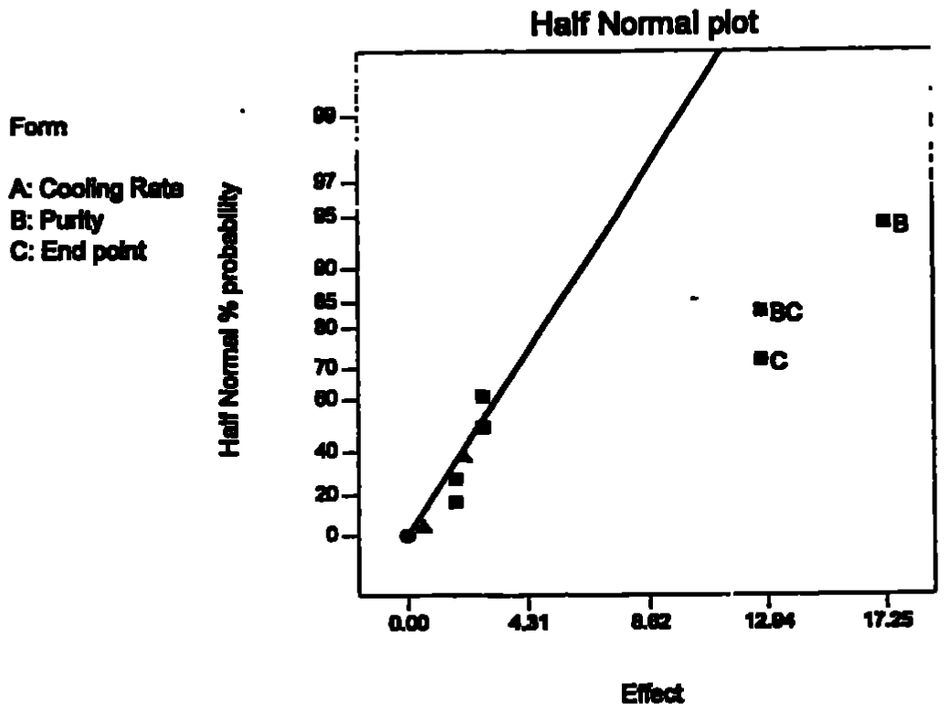

6016 683-1208969

Figure 23

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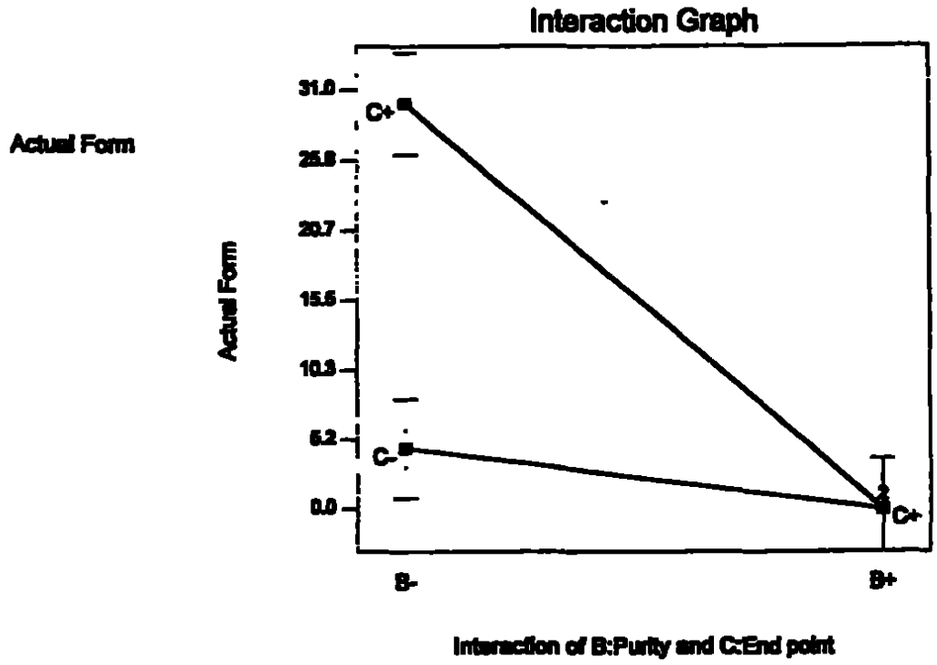

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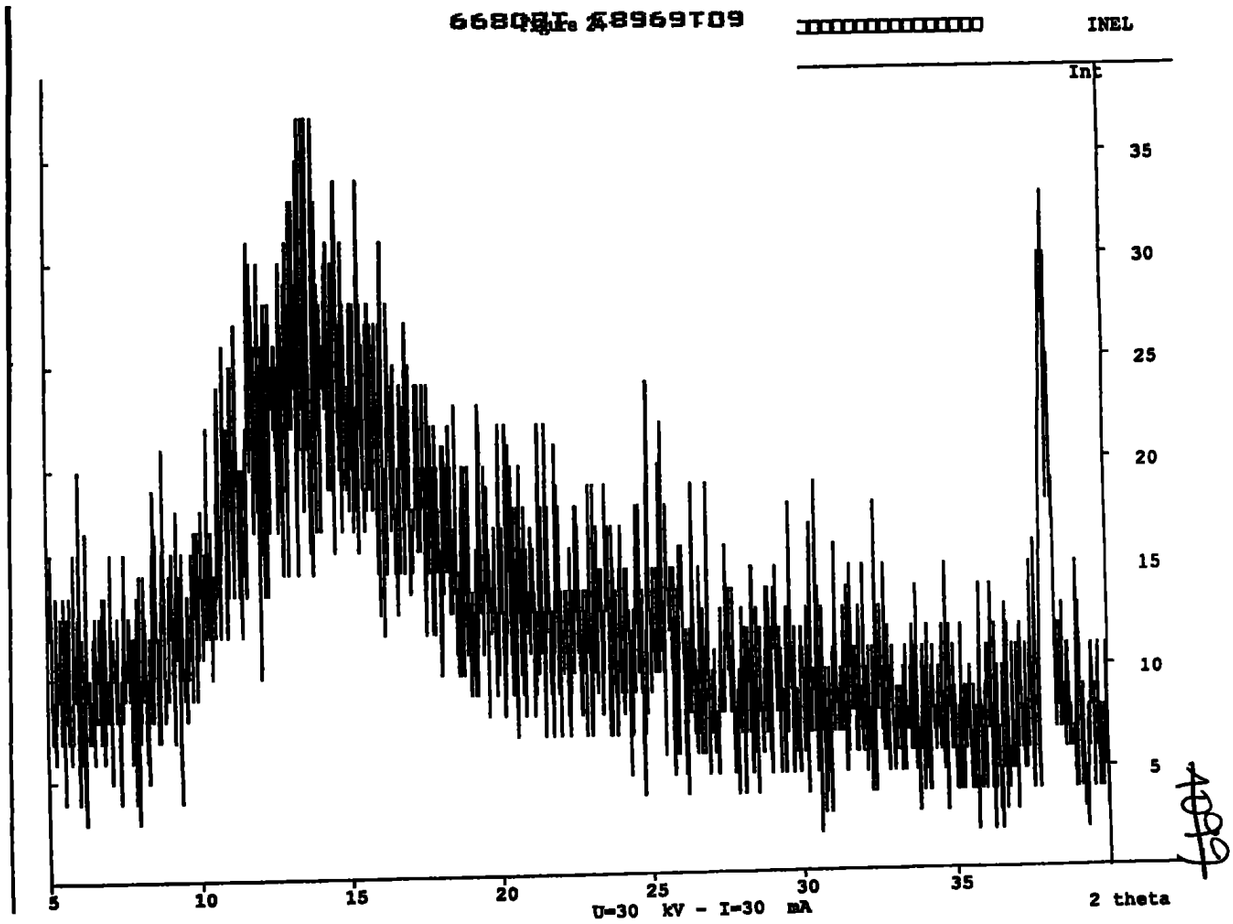

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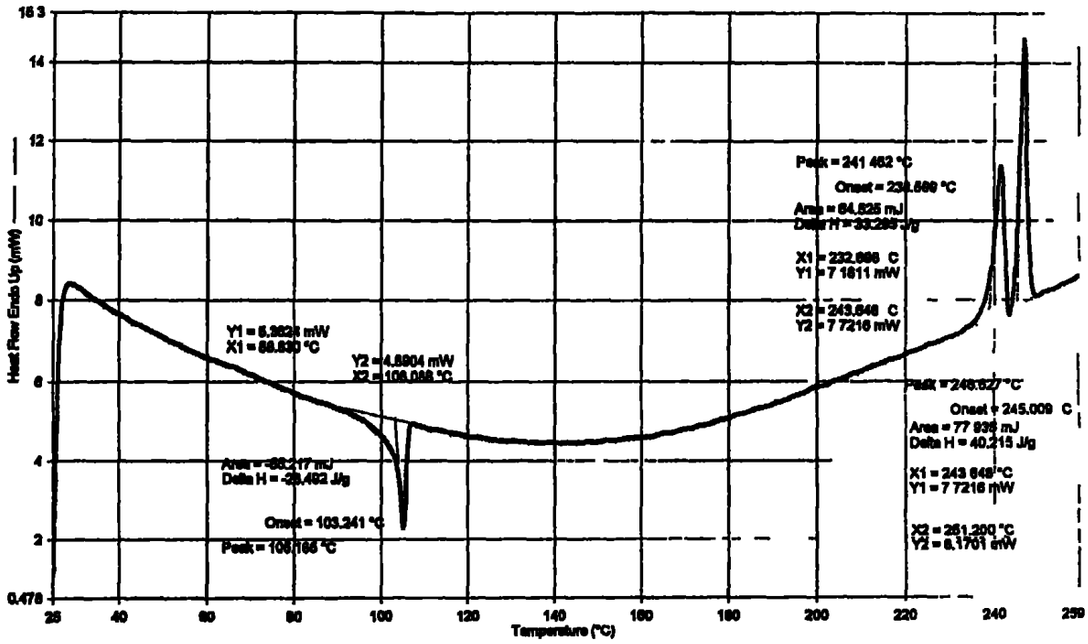
Figure 2

Sample Weight: 1.938 mg
Comment: Epirenone
Amorphous by freeze dry in water



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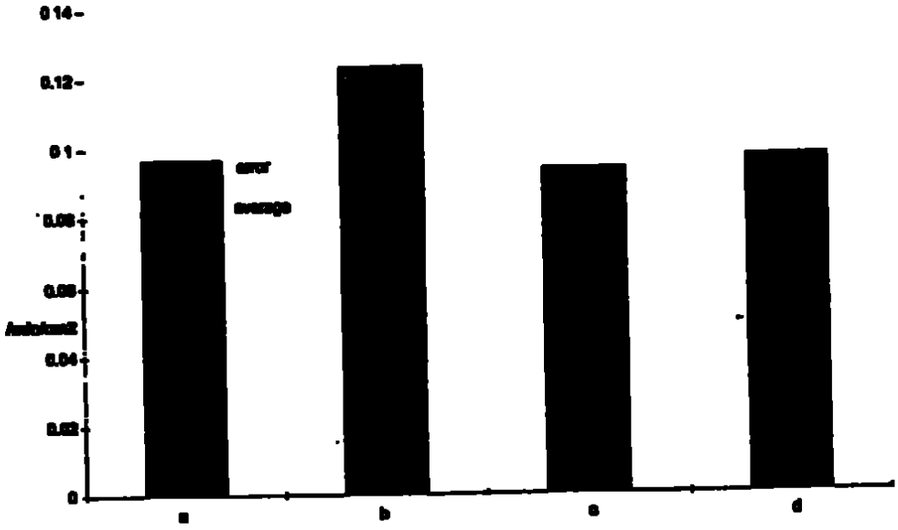

Figure 26:

60169 83.12089

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LOS ESTADOS UNIDOS DE AMERICA

A TODOS AQUELLOS A QUIENES LA PRESENTE CONCIERNE:
DEPARTAMENTO DE COMERCIO DE LOS ESTADOS UNIDOS

Oficina de Patentes y Marcas de los Estados Unidos

30 de diciembre de 2000

LA PRESENTE ES PARA CERTIFICAR QUE LA ANEXA A ESTA ES UNA COPIA FIEL DE LOS REGISTROS DE LA OFICINA DE PATENTES Y MARCAS DE LOS ESTADOS UNIDOS DE LOS DOCUMENTOS DE LA SOLICITUD DE PATENTE IDENTIFICADA MAS ADELANTE QUE SATISFACEN LOS REQUISITOS PARA QUE SE OTORGUE UNA FECHA DE PRIORIDAD BAJO 35 USC 111..

SOLICITUD NUMERO: 60/169,683

FECHA DE PRESENTACION: ***8 de diciembre de 1999***

Por la Autoridad del
COMISIONADO DE PATENTES Y MARCAS

Firmado por

T. LAWRENCE

Funcionario Certificador

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REIVINDICACIONES

1. La forma cristalina de Forma II de la eplerenona.

2. Una forma de acuerdo a la reivindicación 1 que tiene un patrón de difracción de polvos en rayos X con un pico de 8.0 ± 0.2 grados dos theta.

3. Una forma de acuerdo a la reivindicación 1 que tiene un patrón de difracción de polvos en rayos X con un pico de 8.0 ± 0.2, 12.4 ± 0.2, y 13.3 ± 0.2 grados dos tetha.

4. Una forma de acuerdo a la reivindicación 1 que tiene un patrón de difracción de polvos en rayos X sin un pico significante a 12.0 ± 0.2.

5. Una forma de acuerdo a la reivindicación 1 que tiene un patrón de difracción de polvos en rayos X substancialmente como se muestra en la figura 1-B.

6. Una forma de acuerdo a la reivindicación 1 que tiene un rango de fusión de entre 223°C hasta 234°C.

7. Una forma de acuerdo a la reivindicación 1 que tiene un rango de fusión de entre 237°C hasta 242°C.

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8. Una forma de acuerdo a la reivindicación 1 que tiene un espectro infrarrojo con un pico en 1724 cm^{-1}.

9. Una forma de acuerdo a la reivindicación 1 que tiene un espectro infrarrojo sin un pico significante en 1739 cm^{-1}.

10. Una forma de acuerdo a la reivindicación 1 que tiene un espectro infrarrojo sin un pico significante en 1399 cm^{-1}.

11. Una forma de acuerdo a la reivindicación 1 que tiene un espectro infrarrojo substancialmente como se muestra en la figura 3-B.

12. Una forma de acuerdo a la reivindicación 1 que tiene un espectro ^{13}C NMR con resonancias de carbono en 67.1 ppm.

13. Una forma de acuerdo a la reivindicación 1 que tiene un espectro ^{13}C NMR con resonancias de carbono de 16 ppm y hasta 67.1 ppm.

14. Una forma de acuerdo a la reivindicación 1 que no tiene un espectro ^{13}C NMR sin resonancias de carbono en 24.7 ppm.

15. Una forma de acuerdo a la reivindicación 1 que tiene un espectro ^{13}C NMR substancialmente como se muestra en la figura 5.

16. Una forma de acuerdo a la reivindicación 1 que tiene un de fusión de 223°C hasta 234°C, y un espectro infrarrojo con un pico de 1724 cm^{-1}.

17. Una forma de acuerdo a la reivindicación 1 que tiene un rango de fusión de 223°C hasta 234°C, y un espectro infrarrojo con un pico de 1724 cm^{-1} y sin picos significantes en 1399 cm^{-1} y en 1739 cm^{-1}.

18. Una forma de acuerdo a la reivindicación 2 que tiene un rango de fusión de 223°C hasta 234°C, y un espectro infrarrojo con un pico de 1724 cm^{-1} y sin picos significantes en 1399 cm^{-1} y en 1739 cm^{-1}; y un espectro ^{13}C NMR con una resonancia de carbono en 67.1 ppm.

19. Una forma de acuerdo a la reivindicación 1 que tiene un rango de fusión de 237°C hasta 242°C, y un espectro infrarrojo con un pico en 1724 cm^{-1}.

20. Una forma de acuerdo a la reivindicación 1 que tiene un rango de fusión de 237°C hasta 242°C, y un espectro infrarrojo con un pico en 1722 cm^{-1} y sin un pico significante en 1739 cm^{-1}.

21. Una forma de acuerdo a la reivindicación 2 que tiene un rango de fusión de 237°C hasta 242°C, y un espectro infrarrojo con un pico en 1724 cm^{-1} y sin un pico significante en 1739 cm^{-1}; y un espectro ^{13}C NMR con una resonancia de carbono en 67.1 ppm.

22. Una forma de acuerdo a la reivindicación 1 que tiene un sistema de cristal monocíclico.

23. Una forma de acuerdo a la reivindicación que tiene un grupo espacial $P2_1$.

24. Una forma de acuerdo a la reivindicación 1 que tiene valores de celda unitaria a, b y c de 8.78 Å, en 11.14 Å y en 11.06 Å, respectivamente.

25. Una forma de acuerdo a la reivindicación 1 que tiene valores de celda unitaria α, β y γ de 90°, de 90.52°, y de 90°, respectivamente.

26. Partículas de forma de la reivindicación 1 donde al menos 90% de dichas partículas tiene un tamaño de partícula menor a 400 micras.

27. Las partículas de la reivindicación 26 donde al menos 90% de dichas partículas tienen un tamaño de partícula de 25 hasta 400 micras.

28. Las partículas de la reivindicación 26 donde al menos 90% de dichas partículas tiene un tamaño de partícula de 50 hasta 150 micras.

29. Las partículas de la reivindicación 26 donde al menos 90% de dichas partículas tiene un tamaño de partícula de 75 hasta 125 micras.

30. Las partículas de la reivindicación 26 donde al menos 90% de dichas partículas tiene un tamaño de partícula de 40 hasta 100 micras.

31. Las partículas de la reivindicación 26 donde al menos 90% de dichas partículas tiene un tamaño de partícula de 30 hasta 50 micras.

32. Eplererona que comprende la forma cristalina de acuerdo a la reivindicación 1, donde dicha eplerenona tiene una fase de pureza de al menos 50%.

33. La eplerenona de la reivindicación 32 donde dicha eplerenona tiene una fase de pureza de al menos 90%.

34. La eplerenona de la reivindicación 32 donde dicha eplerenona es una fase substancialmente pura de la reivindicación 1.

35. Una composición farmacéutica que comprende una cantidad terapéuticamente efectiva de la forma de cualquiera de las reivindicaciones 1 a 35 en asociación con al menos un portador, adyuvante o diluyente farmacéuticamente aceptable.

36. La composición de la reivindicación 35 donde la única forma cristalina de eplerenona presente en la composición es una forma de acuerdo a la reivindicación 1.

37. Un método para tratar o prevenir una condición mediada de aldosterona o un desorden en un sujeto, dicho método comprende administrar a un sujeto que tiene o es susceptible a dicha

condición o desorden una cantidad terapéuticamente efectiva con una forma de acuerdo a la reivindicación 1.

38. El método de la reivindicación 37 para ser usado en el tratamiento de hiperaldosteronismo.

39. El método de acuerdo a la reivindicación 37 para ser usado en el tratamiento de hipertensión.

40. El método de la reivindicación 37 para ser usado en el tratamiento de fallas de corazón.

41. El método de acuerdo a la reivindicación 37 para ser usado en el tratamiento de cirrosis en el hígado.

42. El método de la reivindicación 37 para ser usado en el tratamiento de una condición seleccionada de un grupo que consiste de exceso de colágeno/fibrosis, hipertrofia benigna prostática y depresión.

43. Un método para la preparación eplerenona que comprende la forma de la reivindicación 1, el método comprende la cristalización de la eplerenona a partir de un solvente farmacéuticamente aceptable o una mezcla de solventes para producir directamente dicha forma.

44. El método de la reivindicación 43 donde el solvente o la mezcla de solventes comprende un solvente seleccionado de un grupo que

consiste de metanol, acetato de etilo, acetato de isopropilo, acetonitrilo, nitrobenceno, agua y etil benceno.

45. El método de la reivindicación 43 donde la eplerenona usada en la cristalización tiene una pureza química de al menos 99%.

46. El método de acuerdo a la reivindicación 43 donde la cristalización se lleva a cabo en presencia de un promotor de crecimiento de Forma II o un inhibidor de crecimiento de Forma I.

47. El método de acuerdo a la reivindicación 43 donde al solvente o las mezclas de los solventes se le adiciona un cristal pequeño que se utiliza para iniciar el proceso de cristalización con la forma de la reivindicación 1 antes de cristalizar la eplerenona.

48. El método de la reivindicación 43 donde al menos 50 por ciento en peso de la eplerenona cristalizada esta en la forma de la reivindicación 1.

49. El método de la reivindicación 43 donde al menos 90 por ciento en peso de la eplerenona cristalizada esta en la forma de la reivindicación 1.

50. El método de la reivindicación 43 donde al menos 99 por ciento en peso de la eplerenona cristalizada esta en la forma de la reivindicación 1.

51. Un método para la preparación de eplerenona que comprende la forma cristalina de la reivindicación 1, donde el método comprende:

La cristalización de eplerenona de un o unos solventes farmacéuticamente aceptables para formar un solvato;

y eliminar el solvente del solvato para producir la forma de acuerdo a la reivindicación 1.

52. El método de la reivindicación 51 donde el solvente o mezcla de solventes comprende un solvente seleccionado de un grupo que consiste de metiletil cetona, 2-pentanona, ácido acético, acetona, acetato de butilo, cloroformao, etanol, isobutanol, acetato de isobutilo, acetato de metilo, propionato de etilo, n-butanol, , n-octanol, n-propanol, isopropanol, acetato de propilo, propilen glicol, t-butanol, tetrahidrofurano, tolueno y acetato de t-butilo.

53. El método de la reivindicación 51 donde el solvente o la mezcla de solventes comprenden un solvente seleccionado de un grupo que consiste de 2-pentanona, ácido acético, acetona,acetato de butilo, cloroformo, isobutanol, acetato de isobutilo, acetato de metilo, propionato de etilo, , n-butanol, n-octanol, n-propanol, acetato de propilo, propilen glicol, t-butanol, tetrahidrofurano, tolueno y t-butil acetato.

54. El método de la reivindicación 51 donde el solvente o mezclas de solventes comprende un solvente seleccionado de un grupo que consiste de metiletil cetona y etanol.

55. El método de la reivindicación 51 donde la eplerenona usada en la cristalización tiene una pureza química de al menos de 99%.

56. El método de la reivindicación 51 donde la cristalización se lleva a cabo en presencia de un promotor de crecimiento de Forma II o un inhibidor de crecimiento de Forma I.

57. El método de acuerdo a la reivindicación 51 donde el solvente o mezcla de los solventes se le adiciona un cristal pequeño que se utiliza para iniciar el proceso de cristalización con la forma de la reivindicación 1 antes de cristalizar la eplerenona.

58. El método de acuerdo a la reivindicación 51 donde al menos 50 por ciento en peso de la eplerenona cristalizada esta en la forma de la reivindicación 1.

59. El método de acuerdo a la reivindicación 51 donde el menos 90 por ciento en peso de la eplerenona cristalizada está en la forma de la reivindicación 1.

60. El método de acuerdo a la reivindicación 51 donde al menos 99 por ciento en peso de la eplerenona cristalizada está en la forma de la reivindicación 1.

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61. Un método para preparar eplerenona que comprende una forma cristalina de acuerdo a la reivindicación 1, el método comprende digerir la eplerenona en un solvente o una mezcla de solventes farmacéuticamente aceptables.

62. El método de la reivindicación 61 donde el solvente comprende etanol o metiletil cetona.

63. El método de acuerdo a la reivindicación 61 donde al menos 50 por ciento en peso de la eplerenona preparada esta en la forma de la reivindicación 1.

64. El método de acuerdo a la reivindicación 61 donde al menos 90 por ciento en peso de la eplerenona preparada esta en la forma de la reivindicación 1.

65. El método de acuerdo a la reivindicación 61 donde al menos 99 por ciento en peso de la eplerenona preparada esta en la forma de la reivindicación 1.

IAA 105

PTO 2400010

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

January 02, 2001

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE UNDER 35 USC 111.

APPLICATION NUMBER: ***60/169,707***
FILING DATE: ***December 08, 1999***

By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

E. Bornett

E. BORNETT
Certifying Officer

12-09-99

Express Mail Label Number EL523767063US
Attorney Docket Number SRL 6171 (C-3256)

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

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[x] Additional inventors are being named on the 3rd & 4th separately numbered sheet(s) attached hereto.

Title of the Invention: NANOPARTICLES COMPRISING FORM II CRYSTALLINE EPLERENONE

Correspondence Address: Customer Number 000321

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The application includes:

[x] Specification - Number of Pages 99

[x] Drawings - Number of Sheets 92

[ ] Small Entity Statement

[x] Return Receipt Postcard

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Method of Payment of Filing Fee:

[X] A check in the amount of $ 150.00 is enclosed to cover the Provisional Filing Fee.

[ ] The Commissioner is hereby authorized to charge filing fees and credit Deposit Account No. 19-1345.

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

[X] No
[ ] Yes, the name of the U.S. Government agency and the Government contract number are: _____

Respectfully submitted,

Scott A. Williams, Reg. No. 39,876

Date: 12/8/99

SAWskg

660021 "2026 TO9

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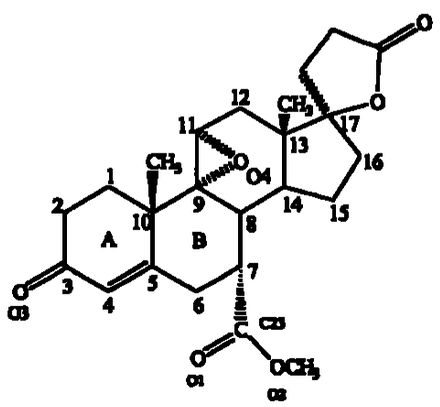
NANOPARTICLES COMPRISING FORM II CRYSTALLINE EPLERENONE

Field of the Invention

This invention is in the field of aldosterone receptor antagonist pharmaceutical agents and specifically relates to novel nanoparticles comprising the Form II crystalline form of eplerenone, methods of preparing these eplerenone nanoparticles, pharmaceutical compositions comprising these eplerenone nanoparticles, and methods for the treatment and/or prophylaxis of aldosterone-mediated conditions and/or disorders, including conditions and disorders associated with hyperaldosteronism such as hypertension.

Background of the Invention

The compound methyl hydrogen 9,11-epoxy-17-hydroxy-3-oxopregn-4-ene-7,21-dicarboxylate, γ-lactone 1 (also referred to herein as "eplerenone") was first reported in Grob et al., U.S. Patent 4,559,332 which discloses a class of 9,11-epoxy steroid compounds and their salts. Eplerenone is an aldosterone receptor antagonist that can be administered in a therapeutically effective amount where use of an aldosterone receptor antagonist is indicated, such as in the treatment of pathological conditions associated with hyperaldosteronism including hypertension, heart failure including cardiac insufficiency, and cirrhosis of the liver.



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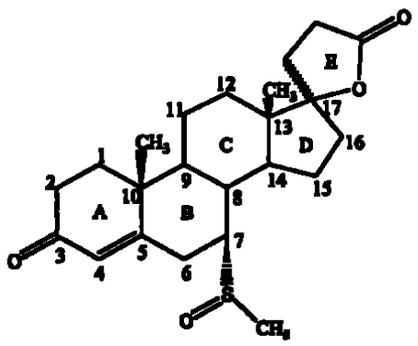
U.S. Patent 4,559,332 generally discloses the preparation of eplerenone and the preparation of pharmaceutical formulations comprising eplerenone.

Additional processes for the preparation of 9,11-epoxy steroid compounds and their salts, including eplerenone, are disclosed in Ng et al., WO97/21720 and Ng et al., WO98/25948.

Grob et al., "Steroidal Aldosterone Antagonists: Increased Selectivity of 9α,11-Epoxy Derivatives", Helvetica Chimica Acta, Vol. 80, pp. 566-585 (1997), discloses an X-ray crystal structure analysis of a solvate of eplerenone prepared by crystallizing eplerenone from a methylene chloride/diethyl ether solvent system.

De Gasparo et al., "Antialdosterones: Incidence and Prevention of Sexual Side Effects", J. Steroid Biochem., Vol. 32, No. 13, pp. 223-227 (1989), discloses the use of non-formulated eplerenone having a 20 micron particle size in a single dose study of eplerenone.

Spironolactone 2, a 20-spiroxane-steroid having activity as an aldosterone receptor antagonist, is commercially available for the treatment of hypertension. Spironolactone, however, has antiandrogenic activity that can result in gynecomastia and impotence in men. It also has weak progestational activity that can produce menstrual irregularities in women. Accordingly, there is interest in the development of additional active aldosterone receptor antagonists such as eplerenone that do not interact with other steroid receptor systems such as glucocorticoid, progestin and androgen steroid receptor systems and/or that provide for a broader range of treatment.



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Agafonov et al., "Polymorphism of Spironolactone", J. Pharm. Sciences, Vol. 80, No. 2, pp 181-185 (1991), discloses an acetonitrile solvate, an ethanol solvate, an ethyl acetate solvate, a methanol solvate and two non-solvated polymorphic crystalline forms of spironolactone. Brittan, Polymorphism in Pharmaceutical Solids, pp. 114-116, 207, 235, and 261 (Marcel Dekker, Inc. 1999), likewise discloses these solid state forms of spironolactone.

Summary of the Invention

A first aspect of the invention comprises novel particles comprising the Form II crystalline form of eplerenone wherein at least 90% of the particles have a particle size less than about 15 microns. These eplerenone particles possess activity as an aldosterone receptor antagonist and have unique properties relative to other solid state particulate forms of eplerenone.

A second aspect of the invention comprises substantially phase pure particles comprising Form II eplerenone wherein at least 90% of the particles have a particle size less than about 15 microns.

A third aspect of the invention comprises pharmaceutical compositions comprising particles comprising the Form II crystalline form of eplerenone wherein at least 90% of the particles have a particle size less than about 15 microns.

A fourth aspect of the invention comprises a method for the prophylaxis and/or treatment of an aldosterone-mediated condition and/or disorder comprising administering to a subject a therapeutically-effective amount of particles comprising the Form II crystalline form of eplerenone wherein at least 90% of the particles have a particle size less than about 15 microns.

A fifth aspect of the invention comprises methods for the preparation of particles comprising the Form II crystalline form of eplerenone wherein at least 90% of the particles have a particle size less than about 15 microns.

Additional aspects of the invention are discussed throughout the specification of this application.

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Brief Description of the Drawings

Fig. 1-A shows X-ray powder diffraction patterns of Form I eplerenone.

Fig. 1-B shows X-ray powder diffraction patterns of Form II eplerenone.

Fig. 1-C shows X-ray powder diffraction patterns of the methyl ethyl ketone solvate of eplerenone.

Figs. 1-D through 1-O show X-ray powder diffraction patterns for the following eplerenone solvates: n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, acetone solvate, toluene solvate, isopropanol solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, methyl acetate

solvate, and propyl acetate solvate, respectively.

Fig. 2-A shows a differential scanning calorimetry (DSC) thermogram of non-milled Form II directly crystallized from methyl ethyl ketone.

Fig. 2-B shows a differential scanning calorimetry (DSC) thermogram of non-milled Form II prepared by desolvation of a solvate obtained by crystallization of a high purity eplerenone from methyl ethyl ketone.

Fig. 2-C shows a differential scanning calorimetry (DSC) thermogram of Form II prepared by crystallizing a solvate from a solution of high purity eplerenone in methyl ethyl ketone, desolvating the solvate to yield Form II, and milling the resulting Form II.

Fig. 2-D shows a differential scanning calorimetry (DSC) thermogram of non-milled Form I prepared by desolvation of a solvate obtained by digestion of low purity eplerenone from appropriate solvents.

Figs. 2-E through 2-T show DSC thermograms for the following eplerenone solvates: n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, chloroform solvate, acetone solvate, toluene solvate, isopropanol solvate, t-butyl acetate solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, methyl acetate solvate, propyl acetate solvate, n-butanol solvate, and n-octanol solvate, respectively.

Fig. 3-A shows the infrared spectra (diffuse reflectance, DRIFTS) of Form I eplerenone.

Fig. 3-B shows the infrared spectra (diffuse reflectance, DRIFTS) of Form II eplerenone.

Fig. 3-C shows the infrared spectra (diffuse reflectance, DRIFTS) of the methyl ethyl ketone solvate of

eplerenone.

Fig. 3-D shows the infrared spectra (diffuse reflectance, DRIFTS) of eplerenone in chloroform solution.

Figs. 3-E through 3-Q show infrared absorption spectra for the following eplerenone solvates: n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, acetone solvate, toluene solvate, isopropanol solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, propyl acetate solvate, methyl acetate solvate, propylene glycol solvate, and t-butyl acetate solvate respectively.

Fig. 4 shows ^{13}C NMR spectra for Form I of eplerenone.

Fig. 5 shows ^{13}C NMR spectra for Form II of eplerenone.

Figs. 6-A through 6-R show thermogravimetry analysis profiles for the following eplerenone solvates: methyl ethyl ketone solvate, n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, chloroform solvate, acetone solvate, toluene solvate, isopropanol solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, methyl acetate solvate, propyl acetate solvate, propylene glycol solvate, n-butanol solvate, n-octanol solvate, and t-butyl acetate solvate, respectively.

Fig. 7 is a scanning electron micrograph of Form II eplerenone prepared by desolvation of the methyl ethyl ketone solvate.

Fig. 8 is a scanning electron micrograph of Form II eplerenone prepared by direct crystallization from ethyl acetate.

Fig. 9 shows an X-ray powder diffraction pattern of a crystalline form of 7-methyl hydrogen 4α,5α:9α,11α-diepoxy-

17-hydroxy-3-oxo-17α-pregnane-7α,21-dicarboxylate, γ-lactone isolated from methyl ethyl ketone.

Fig. 10 shows an X-ray powder diffraction pattern of the crystalline form of 7-methyl hydrogen 11α,12α-epoxy-17-hydroxy-3-oxo-17α-pregn-4-ene-7α,21-dicarboxylate, γ-lactone isolated from isopropanol.

Fig. 11 shows an X-ray powder diffraction pattern of the crystalline form of 7-methyl hydrogen 17-hydroxy-3-oxo-17α-pregna-4,9(11)-diene-7α,21-dicarboxylate, γ-lactone isolated from n-butanol.

Fig. 12 illustrates the Gibbs free energy-temperature relationship for enantiotropically related polymorphs.

Fig. 13 shows the X-ray powder diffraction patterns for the wet cake (methyl ethyl ketone solvate) obtained from (a) 0%, (b) 1%, (c) 3%, and (d) 5% diepoxide-doped methyl ethyl ketone crystallizations.

Fig. 14 shows the X-ray powder diffraction patterns for the dried solids obtained from (a) 0%, (b) 1%, (c) 3%, and (d) 5% diepoxide-doped methyl ethyl ketone crystallizations.

Fig. 15 shows the X-ray powder diffraction patterns for the dried solids from the methyl ethyl ketone crystallization with 3% doping of diepoxide (a) without grinding of the solvate prior to drying, and (b) with grinding of the solvate prior to drying.

Fig. 16 shows the X-ray powder diffraction patterns for the wet cake (methyl ethyl ketone solvate) obtained from (a) 0%, (b) 1%, (c) 5%, and (d) 10% 11,12-epoxide-doped methyl ethyl ketone crystallizations.

Fig. 17 shows the X-ray powder diffraction patterns for the dried solids obtained from (a) 0%, (b) 1%, (c) 5%, and

(d) 10% 11,12-epoxide-doped methyl ethyl ketone crystallizations.

Fig. 18 shows a cube plot of product purity, starting material purity, cooling rate and endpoint temperature based on the data reported in Table X-7A.

Fig. 19 shows a half normal plot prepared using the cube plot of Fig. 18 to determine those variables having a statistically significant effect on the purity of the final material.

Fig. 20 is an interaction graph based on the results reported in Table X-7A showing the interaction between starting material purity and cooling rate on final material purity.

Fig. 21 shows a cube plot of Form I weight fraction, starting material purity, cooling rate and endpoint temperature based on the data reported in Table X-7A.

Fig. 22 shows a half normal plot prepared using the cube plot of Fig. 21 to determine those variables having a statistically significant effect on the purity of the final material.

Fig. 23 is an interaction graph based on the results reported in Table X-7A showing the interaction between starting material purity and endpoint temperature on final material purity.

Fig. 24 shows an X-ray diffraction pattern of amorphous eplerenone.

Fig. 25 shows a DSC thermogram of amorphous eplerenone.

Fig. 26 shows the dissolution rates measured for four polymorph samples.

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Detailed Description of the Preferred Embodiments

As with all pharmaceutical compounds and compositions, the chemical and physical properties of eplerenone are important in its commercial development. These properties include, but are not limited to: (1) packing properties such as molar volume, density and hygroscopicity, (2) thermodynamic properties such as melting temperature, vapor pressure and solubility, (3) kinetic properties such as dissolution rate and stability (including stability at ambient conditions, especially to moisture and under storage conditions), (4) surface properties such as surface area, wettability, interfacial tension and shape, (5) mechanical properties such as hardness, tensile strength, compactibility, handling, flow and blend; and (6) filtration properties. These properties can affect, for example, the processing and storage of pharmaceutical compositions comprising eplerenone. Solid state forms of eplerenone that provide an improvement in one or more of these properties relative to other solid state forms of eplerenone are desirable.

The present invention comprises such novel solid state forms of eplerenone. Specifically, these novel solid state forms include solvated crystalline forms, non-solvated and non-hydrated crystalline forms (designated "Form I" and "Form II"), and an amorphous form of eplerenone. Each solid state form of eplerenone described in the present application possesses one or more of the above-described advantageous chemical and/or physical properties relative to the other solid state forms described herein or otherwise disclosed in the literature.

In one embodiment of the invention, the solid state form comprises Form I eplerenone. Form I exhibits a faster dissolution rate (approximately 30% higher) at lower temperatures (i.e., temperatures below the enantiotropic transition temperature as later discussed) than, for example, Form II. Where dissolution of eplerenone in the gastrointestinal tract is the rate-controlling step for delivery of the eplerenone to the target cells, faster dissolution generally results in improved bioavailability. Form I, therefore, can provide an improved bioavailability profile relative to Form II. In addition, selection of a solid state form of eplerenone having a faster dissolution rate likewise provides greater flexibility in the selection of excipients for, and in the formulation of, immediate release pharmaceutical compositions relative to other solid state forms having a slower dissolution rate.

In another embodiment of the invention, the solid state form comprises Form II eplerenone. Form II possesses greater physical stability at lower temperatures (i.e., at temperatures below the enantiotropic transition temperature as later discussed) than, for example, Form I. Solid state forms of eplerenone such as Form II that do not require the use of special processing or storage conditions, and that avoid the need for frequent inventory replacement, are desirable. For example, selection of a solid state form of eplerenone that is physically stable during the manufacturing process (such as during milling of eplerenone to obtain a material with reduced particle size and increased surface area) can avoid the need for special processing conditions and the increased costs generally associated with such special processing conditions. Similarly, selection of a

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solid state form of eplerenone that is physically stable at different conditions of storage (especially considering the different possible storage conditions during the lifetime of the eplerenone product) can help avoid polymorphic or other degradative changes in the eplerenone that can lead to product loss or deterioration of product efficacy. Therefore, the selection of a solid state form of eplerenone such as Form II having greater physical stability provides a meaningful benefit over less stable eplerenone forms.

In another embodiment of the invention, the solid state form of eplerenone comprises a solvated crystalline form of eplerenone. Such solvated crystalline forms are useful as intermediates in the preparation of Form I and Form II eplerenone. An additional benefit from the use of a solvated crystalline form as an intermediate is the "intrinsic micronizing" of the crystal that results upon desolvation as discussed later in this application. Such "intrinsic micronizing" can reduce or eliminate milling requirements. Further, where additional milling is still required, it is easier to mill certain solvates before the desolvation step than to mill Form I or Form II after desolvation of the solvate.

Pharmaceutically acceptable solvated crystalline forms also can be used directly in pharmaceutical compositions. These solvated crystalline forms preferably do not comprise methylene chloride, isopropanol or ethyl ether; and, in yet another embodiment, do not comprise methylene chloride, isopropanol, ethyl ether, methyl ethyl ketone or ethanol; and, in yet another embodiment, do not comprise methylene chloride, isopropanol, ethyl ether, methyl ethyl ketone, ethanol, ethyl acetate or acetone. More preferably, the solvated crystalline

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forms are substantially exclusive of solvents that are not pharmaceutically-acceptable solvents. Because Form I and Form II typically are more physically stable than the crystalline solvates at room temperature and under atmospheric pressure, the solvated crystalline forms used in such compositions generally comprise a pharmaceutically acceptable higher boiling and/or hydrogen bonding solvent such as, but not limited to, butanol. It is believed that the solvated crystalline forms collectively can offer a range of different dissolution rates and, where dissolution of eplerenone in the gastrointestinal tract is the rate-controlling step for delivery of the eplerenone to the target cells, bioavailabilities relative to Form I and Form II.

In another embodiment of the invention, the solid state form of eplerenone comprises an amorphous form of eplerenone. Amorphous eplerenone is useful as an intermediate in the preparation of Form I and Form II eplerenone. In addition, it is believed that amorphous eplerenone possesses a different dissolution rate and, where dissolution of eplerenone in the gastrointestinal tract is the rate-controlling step for delivery of the eplerenone to the target cells, bioavailability relative to Form I and Form II.

In another embodiment, the invention comprises combinations of solid state eplerenone forms selected from the group consisting of Form I eplerenone, Form II eplerenone, solvated crystalline eplerenone and amorphous eplerenone. Such combinations are useful, for example, in the preparation of pharmaceutical compositions having differing dissolution profiles, including controlled release compositions.

In addition, depending upon the intended use of the solid state form of eplerenone, processing considerations may favor the selection of a specific solid state form of eplerenone or a specific combination of such solid state forms. Pure phase Form II, for example, generally is more easily prepared than pure phase Form I. A mixture of Form I and Form II, however, generally is more easily prepared than phase pure Form II and allows for the use of a low purity eplerenone starting material. Use of a solvated crystalline form instead of Form I or Form II in a composition eliminates a processing step, desolvation, for those processes that otherwise would proceed by desolvation of the solvated crystalline form. Alternatively, the desolvation step can be eliminated, for example, if Form II is directly crystallized from an appropriate solvent without the intervening preparation and desolvation of the intermediate solvated crystalline form. These processes are described in greater detail later in the application.

It has been discovered that reduction of the particle size of the solid state form of eplerenone to a D_{90} particle size (later defined herein) between about 10 nm to about 15 microns can improve the bioavailability of unformulated eplerenone and pharmaceutical compositions comprising that solid state form of eplerenone. Accordingly, the D_{90} particle size of the unformulated eplerenone or the eplerenone used as a starting material in the pharmaceutical composition generally is less than about 15 microns, preferably less than about 1 micron, more preferably less than about 800 nm, still more preferably less than about 600 nm, and still more preferably less than about 400 nm. In one embodiment, the D_{90} particle size is between about 10 nm to about 1 micron. In

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another embodiment, the D_{90} particle size is between about 100 nm to about 800 nm. In another embodiment, the D_{90} particle size is between about 200 nm to about 600 nm. In another embodiment, the D_{90} particle size is between about 400 nm to about 800 nm.

Solid state forms of eplerenone having a particle size less than about 15 microns can be prepared in accordance with applicable particle size reduction techniques known in the art. Such techniques include, but are not limited to those described in U.S. Patents 5,145,684, 5,318,767, 5,384,124 and 5,747,001. U.S. Patents 5,145,684, 5,318,767, 5,384,124 and 5,747,001 are expressly incorporated by reference as if fully set forth at length. In accordance with the method of U.S. Patent 5,145,684, for example, particles of suitable size are prepared by dispersing the eplerenone in a liquid dispersion medium and wet-grinding the mixture in the presence of grinding media to reduce the particles to the desired size. If necessary or advantageous, the particles can be reduced in size in the presence of a surface modifier.

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Definitions

The term "amorphous" as applied to eplerenone refers to a solid state wherein the eplerenone molecules are present in a disordered arrangement and do not form a distinguishable crystal lattice or unit cell. When subjected to X-ray powder diffraction, amorphous eplerenone does not produce any characteristic crystalline peaks.

Where reference is made in this application to the "boiling point" of a substance or solution, the term "boiling point" means the boiling point of the substance or solution under the applicable process conditions.

The term "crystalline form" as applied to eplerenone refers to a solid state form wherein the eplerenone molecules are arranged to form a distinguishable crystal lattice (i) comprising distinguishable unit cells, and (ii) yielding diffraction peaks when subjected to X-ray radiation.

The term "crystallization" as used throughout this application can refer to crystallization and/or recrystallization depending upon the applicable circumstances relating to the preparation of the eplerenone starting material.

The term "digestion" means a process in which a slurry of solid eplerenone in a solvent or mixture of solvents is heated at the boiling point of the solvent or mixture of solvents under the applicable process conditions.

The term "direct crystallization" as used herein refers to the crystallization of eplerenone directly from a suitable solvent without the formation and desolvation of an intermediate solvated crystalline solid state form of eplerenone.

The term "particle size" as used herein refers to particle size as measured by conventional particle size measuring techniques well known in the art, such as laser light scattering, sedimentation field flow fractionation, photon correlation spectroscopy, or disk centrifugation. The term "D_{90} particle size" means the particle size of at least 90% of the particles as measured by such conventional particle size measuring techniques.

The term "DSC" means differential scanning calorimetry.

The term "HPLC" means high pressure liquid chromatography.

The term "IR" means infra red.

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The term "min" means minute.

The term "psi" means pounds per square inch absolute.

The term "purity" means the chemical purity of eplerenone according to conventional HPLC assay. As used herein, "low purity eplerenone" generally means eplerenone that contains an effective amount of a Form I growth promoter and/or a Form II growth inhibitor. As used herein, "high purity eplerenone" generally means eplerenone that does not contain, or contains less than an effective amount of, a Form I growth promoter and/or a Form II growth inhibitor.

The term "phase purity" means the solid state purity of eplerenone with regard to a particular crystalline or amorphous form of the eplerenone as determined by the infrared spectroscopy analytical methods described herein.

The term "XPRD" means X-ray powder diffraction.

The term "rpm" means revolutions per minute.

The term "TGA" means thermogravimetric analysis.

The term "T_m" means melting temperature.

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Characterization of Crystalline Form

1. Molecular Conformation

Single crystal X-ray analysis indicates that the eplerenone molecular conformation differs between Form I and Form II, particularly with respect to the orientation of the ester group at the 7-position of the steroid ring. The orientation of the ester group can be defined by the C8-C7-C23-O2 torsion angle.

In the Form I crystal lattice, the eplerenone molecule adopts a conformation in which the methoxy group of the ester is approximately aligned with the C-H bond at the 7-position

and the carbonyl group is approximately positioned over the center of the B-steroid ring. The C8-C7-C23-02 torsion angle is approximately -73.0° in this conformation. In this orientation, the carbonyl oxygen atom of the ester group (01) is in close contact with the oxygen atom of the 9,11-epoxide ring (04). The 01-04 distance is about 2.97 Å, which is just below the van der Waal's contact distance of 3.0 Å (assuming van der Waal's radii of 1.5Å for the oxygen).

In the Form II crystal lattice, the eplerenone molecule adopts a conformation in which the ester group is rotated approximately 150° relative to that of Form I and has a C8-C7-C23-02 torsion angle of approximately +76.9°. In this orientation, the methoxy group of the ester is directed toward the 4,5-alkene segment of the A-steroid ring. In this orientation, the distance between either oxygen atom of the ester group (01,02) and the oxygen atom of the 9,11-epoxide ring is increased relative to the distance determined for Form I. The 02-04 distance is approximately 3.04Å, falling just above the van der Waal's contact distance. The 01-04 distance is about 3.45Å.

The eplerenone molecule appears to adopt a conformation characteristic of Form II in the solvated crystalline forms analyzed by single crystal X-ray diffraction to date.

2. X-Ray Powder Diffraction

The various crystalline forms of eplerenone were analyzed with either a Siemens D5000 powder diffractometer or an Inel Multipurpose Diffractometer. For the Siemens D500 powder diffractometer, the raw data was measured for 2q values from 2 to 50, with steps of 0.020 and step periods of two seconds. For the Inel Multipurpose Diffractometer,

samples were placed in an aluminum sample holder and raw data was collected for 30 minutes at all two theta values simultaneously.

Tables 1A, 1B and 1C set out the significant parameters of the main peaks in terms of 2q values and intensities for the Form I (prepared by desolvation of the ethanol solvate obtained by digestion of low purity eplerenone), Form II (prepared by desolvation of the methyl ethyl ketone solvate obtained by recrystallization of high purity eplerenone), and methyl ethyl ketone solvate (prepared by room temperature slurry conversion of high purity eplerenone in methyl ethyl ketone) crystalline forms of eplerenone, respectively (X-ray radiation at a wavelength of 1.54056 Angstroms).

Minor shifts in peak positioning may be present in the diffraction patterns of Form I and Form II as a result of imperfections in the spacing of the crystal diffraction planes due to the route of manufacture of Form I and Form II (i.e. desolvation of a solvate). In addition, Form I is isolated from a solvate prepared by digestion of crude eplerenone. This method results in a lower overall chemical purity (approximately 90%) of the Form I. Finally, the solvated forms of eplerenone are expected to show some shifting in the positioning of the diffraction peaks due to the increased mobility of the solvent molecules within the solvent channels in the crystal lattice.

TABLE 1A: FORM I DATA

Angle 2-theta	d-spacing Angstrom	Intensity	Intensity %
6.994	12.628	1188	7.2
8.291	10.655	2137	13
10.012	8.827	577	3.5
11.264	7.849	1854	11.3
12.04	7.344	7707	46.8
14.115	6.269	3121	19
14.438	6.13	15935	96.8
15.524	5.703	637	3.9
16.169	5.477	1349	8.2
16.699	5.305	1663	10.1
16.94	5.23	1692	10.3
17.147	5.167	2139	13
17.66	5.018	6883	41.8
17.91	4.949	16455	100
18.379	4.823	3106	18.9
18.658	4.752	1216	7.4
19.799	4.48	1499	9.1
20.235	4.385	383	2.3
21.707	4.091	1267	7.7
21.8	4.073	1260	7.7
21.959	4.044	1279	7.8
22.461	3.955	4264	25.9
23.191	3.832	1026	6.2
23.879	3.723	1000	6.1
24.599	3.616	1688	10.3
25.837	3.445	931	5.7
26.034	3.42	686	4.2
26.868	3.316	912	5.5
27.093	3.288	1322	8
27.782	3.209	1236	7.5
28.34	3.147	1845	11.2
28.881	3.091	957	5.8
29.866	2.9892	745	4.5
30.627	2.9166	992	6
31.108	2.8728	1205	7.3
33.215	2.6951	1287	7.8
33.716	2.656	802	4.9
34.434	2.6024	914	5.6

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TABLE 1B: FORM II DATA

Angle 2-Theta	d-spacing Angstrom	Intensity Cps	Intensity %
7.992	11.054	11596	26.6
10.044	8.799	12048	27.6
11.206	7.889	4929	11.3
12.441	7.109	1747	4
12.752	6.936	4340	9.9
13.257	6.673	2444	5.6
14.705	6.019	43646	100
15.46	5.727	2670	6.1
15.727	5.63	7982	18.3
16.016	5.529	3519	8.1
17.671	5.015	8897	20.4
17.9	4.951	2873	6.6
18.352	4.83	612	1.4
18.703	4.74	669	1.8
19.524	4.543	1126	2.6
20.103	4.413	3753	8.6
20.63	4.302	1451	3.3
21.067	4.214	876	2
21.675	4.097	2760	6.3
22.232	3.995	1951	4.5
22.662	3.922	1657	3.8
23.624	3.763	827	1.9
24.279	3.663	1242	2.8
25.021	3.556	5144	11.8
25.486	3.492	1702	3.9
25.707	3.463	2483	5.7
26.251	3.392	1371	3.1
26.85	3.318	1970	4.5
27.319	3.262	1029	2.4
27.931	3.192	440	1
27.969	3.187	440	1
28.937	3.083	1128	2.6
29.703	3.005	1211	2.8
30.173	2.9594	1508	3.5
30.584	2.9206	1602	3.7
30.885	2.8928	1550	3.6
31.217	2.8628	1068	2.4
31.605	2.8285	1038	2.4
32.069	2.7895	1211	2.8
32.64	2.7412	684	1.6
32.747	2.7324	758	1.7
33.46	2.6759	508	1.2
34.194	2.6201	1085	2.5
34.545	2.5943	915	2.1

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TABLE 1C: METHYL ETHYL KETONE DATA

Angle 2-Theta	d-spacing Angstrom	Intensity Cps	Intensity %
7.584	11.648	5629	32.6
7.753	11.393	15929	92.3
10.151	8.707	2877	16.7
11.31	7.817	701	4.1
12.646	6.994	1027	5.9
13.193	6.705	15188	88
13.556	6.528	14225	82.4
14.074	6.287	1966	11.4
14.748	6.002	2759	16
15.165	5.837	801	4.6
15.548	5.694	1896	11
17.031	5.202	7980	46.2
17.28	5.127	17267	100
17.706	5.005	6873	39.8
18.555	4.778	545	3.2
18.871	4.699	1112	6.4
19.786	4.486	1704	9.9
20.158	4.401	1396	8.1
20.725	4.282	2644	15.3
21.787	4.076	1127	6.5
22.06	4.026	451	2.6
22.864	3.886	1542	8.9
23.412	3.796	14185	82.2
23.75	3.743	1154	6.7
24.288	3.662	3063	17.7
25.253	3.524	1318	7.6
25.603	3.49	1736	10.1
25.761	3.455	1225	7.1
26.176	3.402	1346	7.8
26.548	3.355	1098	6.4
27.357	3.257	1944	11.3
27.605	3.229	2116	12.3
27.9	3.195	858	5
28.378	3.142	583	3.4
28.749	3.103	763	4.4
29.3	3.046	1162	6.8
29.679	3.006	2606	15.1
30.402	2.9377	2184	12.6
30.739	2.9063	648	3.8

Graphical examples of the x-ray diffraction patterns for Form I, Form II, and the methyl ethyl ketone solvate

crystalline forms of eplerenone are shown in Figs. 1-A, 1-B, and 1-C, respectively. Form I shows distinguishing peaks at 7.0 ± 0.2, 8.3 ± 0.2, and 12.0 ± 0.2 degrees two theta. Form II shows distinguishing peaks at 8.0 ± 0.2, 12.4 ± 0.2, 12.8 ± 0.2, and 13.3 ± 0.2 degrees two theta. The methyl ethyl ketone solvated crystalline form shows distinguishing peaks at 7.6 ± 0.2, 7.8 ± 0.2, and 13.6 ± 0.2 degrees two theta.

Examples of the x-ray diffraction patterns are shown in Figs. 1-D through 1-O for the following solvate crystalline forms of eplerenone: n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, acetone solvate, toluene solvate, isopropanol solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, methyl acetate solvate, and propyl acetate solvate, respectively.

3. Melting/Decomposition Temperature

The temperatures of melting and/or decomposition of non-solvated eplerenone crystalline forms were determined using a TA Instruments 2920 differential scanning calorimeter. Each sample (1-2 mg) was placed in either a sealed or unsealed aluminum pan and heated at 10°C/minute. Melting/decomposition ranges were defined from the extrapolated onset to the maximum of the melting/decomposition endotherm.

The melting of the non-solvated eplerenone crystals forms (Form I and Form II) was associated with chemical decomposition and loss of trapped solvent from the crystal lattice. The melting/decomposition temperature also was affected by the manipulation of the solid prior to analysis. For example, non-milled Form II (approximate D_{90} particle size of about 180-450 microns) prepared by direct crystallization

from an appropriate solvent or from desolvation of a solvate obtained from crystallization of high purity eplerenone in an appropriate solvent or mixture of solvents generally had a melting range of about 237-242°C. Milled Form II (approximate D_{90} particle size of about 80-100 microns) (Form II prepared by crystallizing a solvate from a solution of high purity eplerenone in an appropriate solvent or mixture of solvents, desolvating the solvate to yield Form II, and milling the resulting Form II) generally had a lower and broader melting/decomposition range of about 223-234°C. Non-milled Form I (approximate D_{90} particle size of about 180-450 microns) prepared by desolvation of a solvate obtained by digestion of low purity eplerenone generally had a higher melting/decomposition range of about 247-251°C. Examples of the DSC thermograms of (a) non-milled Form II directly crystallized from methyl ethyl ketone, (b) non-milled Form II prepared by desolvation of a solvate obtained by crystallization of a high purity eplerenone from methyl ethyl ketone, (c) Form II prepared by milling a desolvated solvate obtained by crystallization of high purity eplerenone from methyl ethyl ketone, and (d) non-milled Form I prepared by desolvation of a solvate obtained by digestion of low purity eplerenone from methyl ethyl ketone are given in Figures 2-A, 2-B, 2-C and 2-D, respectively.

DSC thermograms of solvated forms of eplerenone were determined using a Perkin Elmer Pyris 1 differential scanning calorimeter. Each sample (1-10 mg) was placed in an unsealed aluminum pan and heated at 10°C/minute. One or more endothermal events at lower temperatures were associated with enthalpy changes that occurred as solvent was lost from the solvate crystal lattice. The highest temperature endotherm

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or endotherms were associated with the melting/decomposition of Form II or Form I eplerenone. Examples of the DSC thermograms are shown in Figs. 2-S through 2-T for the following solvated crystalline forms of eplerenone: n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, chloroform solvate, acetone solvate, toluene solvate, isopropanol solvate, t-butyl acetate solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, methyl acetate solvate, propyl acetate solvate, n-butanol solvate, and n-octanol solvate, respectively.

4. Infrared Absorption Spectroscopy

Infrared absorption spectra of the non-solvated forms of eplerenone (Form I and Form II) were obtained with a Nicolet DRIFT (diffuse reflectance infrared fourier transform) Magna System 550 spectrophotometer. A Spectra-Tech Collector system and a microsample cup were used. Samples (5%) were analyzed in potassium bromide and scanned from 400-4000 cm^{-1}. Infrared absorption spectra of eplerenone in dilute chloroform solution (3%) or in the solvated crystal forms were obtained with a Bio-rad FTS-45 spectrophotometer. Chloroform solution samples were analyzed using a solution cell of 0.2 mm path length with sodium chloride salt plates. Solvate FTIR spectra were collected using an IBM micro-MIR (multiple internal reflectance) accessory. Samples were scanned from 400-4000 cm^{-1}. Examples of the infrared absorption spectra of (a) Form I, (b) Form II, (c) the methyl ethyl ketone solvate, and (d) eplerenone in chloroform solution are shown in Figures 3-A, 3-B, 3-C and 3-D, respectively.

Table 2 discloses illustrative absorption bands for

eplerenone in the Form I, Form II, and methyl ethyl ketone solvate crystal forms. Illustrative absorption bands for eplerenone in chloroform solution are also disclosed for comparison. Differences between Form I and either Form II or the methyl ethyl ketone solvate were observed, for example, in the carbonyl region of the spectrum. Form I has an ester carbonyl stretch of approximately 1739 cm^{-1} while both Form II and the methyl ethyl ketone solvate have the corresponding stretch at approximately 1724 and 1722 cm^{-1}, respectively. The ester carbonyl stretch occurs at approximately 1727 cm^{-1} in the eplerenone in chloroform solution. The change in stretching frequency of the ester carbonyl between Form I and Form II reflects the change in orientation of the ester group between the two crystal forms. In addition, the stretch of the ester of the conjugated ketone in the A-steroid ring shifts from approximately 1664-1667 cm^{-1} in either Form I or the methyl ethyl ketone solvate to approximately 1655 cm^{-1} in Form II. The corresponding carbonyl stretch occurs at approximately 1665 cm^{-1} in dilute solution.

Another difference between Form I and Form II was seen in the C-H bending region. Form I has an absorption at approximately 1399 cm^{-1} which is not observed in Form II, the methyl ethyl ketone solvate, or the eplerenone in chloroform solution. The 1399 cm^{-1} stretch occurs in the region of CH_2 scissoring for the C2 and C21 methylene groups adjacent to carbonyl groups.

Table 2

Absorption Region	Form I (cm^{-1})	Form II (cm^{-1})	Methyl Ethyl Ketone Solvate (cm^{-1})	Eplerenone in Chloroform (cm^{-1})
ν C=O(lactone)	1773	1775	1767	1768
ν C=O(ester)	1739	1724	1722	1727
ν C=O(3keto)	1664	1655	1667	1665
ν C=C (3,4-olefin)	1619	1619	1622	1623
δ_{as}CH3, δCH2, δCH2(α to carbonyl)	1460, 1444, 1426	1467, 1438, 1422, 1399	1467, 1438, 1422	1464, 1438, 1422
δ_{s}CH3	1380	1381	~1380	1378

Examples of infrared absorption spectra are shown in Figs. 3-E through 3-S for the following solvated crystalline forms of eplerenone: n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, acetone solvate, toluene solvate, isopropanol solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, propyl acetate solvate, methyl acetate solvate, propylene glycol solvate, and t-butyl acetate solvate respectively.

5. Nuclear Magnetic Resonance

^{13}C NMR spectra were obtained at a field of 31.94 MHz. Examples of the ^{13}C NMR spectra of Form I and Form II eplerenone are shown in Figs. 4 and 5, respectively. The Form I eplerenone analyzed to obtain the data reflected in Fig. 4 was not phase pure and included a small amount of Form II eplerenone. Form I is most clearly distinguished by the carbon resonances at around 64.8 ppm, 24.7 ppm and 19.2 ppm.

Form II is most clearly distinguished by the carbon resonances at around 67.1 ppm and 16.0 ppm.

6. Thermogravimetry

Thermogravimetric analysis was performed using a TA Instruments TGA 2950 thermogravimetric analyzer. Samples were placed in an unsealed aluminum pan under nitrogen purge. Starting temperature was 25°C with the temperature increased at a rate of about 10°C/minute.

Examples of thermogravimetry analysis profiles are shown in Figs. 6-A through 6-R for the following solvated crystalline forms of eplerenone: methyl ethyl ketone solvate, n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, chloroform solvate, acetone solvate, toluene solvate, isopropanol solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, methyl acetate solvate, propyl acetate solvate, propylene glycol solvate, n-butanol solvate, n-octanol solvate, and t-butyl acetate solvate, respectively.

7. Microscopy

Hot-stage microscopy was performed on single crystals of the methyl ethyl ketone solvate using a Linkam THMS 600 Hot Stage with Zeiss Universal Polarized Light Microscope. Under polarized light at room temperature the solvate crystal was birefringent and translucent indicating that the crystal lattice was highly ordered. As the temperature increased to about 60°C, noticeable defects began to emerge along the long crystal dimension. A scanning electron micrograph of Form II eplerenone obtained by desolvation of the methyl ethyl ketone solvate is shown in Fig. 7 and reveals surface defects,

pores, cracks and fractures within the crystal lattice. A scanning electron micrograph of Form II eplerenone obtained by direct crystallization from ethyl acetate is shown in Fig. 8 and does not exhibit similar surface defects, pores, cracks and fractures within the crystal lattice.

8. Unit Cell Parameters

Tables 3A, 3B and 3C below summarize the unit cell parameters determined for Form I, Form II, and several solvated crystalline forms.

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TABLE 3A

Parameter	Form I	Form II	Methyl ethyl ketone Solvate
Crystal system	Ortho-rhombic	Monoclinic	Orthorhombic
Space group	$P2_12_12_1$	$P2_1$	$P2_12_12_1$
a	21.22 Å	8.78 Å	23.53 Å
b	15.40 Å	11.14 Å	8.16 Å
c	6.34 Å	11.06 Å	13.08 Å
α	90°	90°	90°
β	90°	93.52°	90°
γ	90°	90°	90°
z	4	2	4
Volume (Å)	2071.3	1081.8	2511.4
ρ (calculated)	1.329 g/cm³	1.275 g/cm³	1.287 g/cm³
R	0.0667	0.062	0.088

TABLE 3B

Parameter	Acetone Solvate	Toluene Solvate	Butyl Acetate Solvate[1]
Crystal system	Ortho-rhombic	Ortho-rhombic	Ortho-rhombic
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
a	23.31 Å	23.64 Å	23.07 Å
b	13.13 Å	13.46 Å	13.10 Å
c	8.28 Å	8.16 Å	8.24 Å
α	90°	90°	90°
β	90°	90°	90°
γ	90°	90°	90°
z	4	4	4
Volume (Å)	2533.7	2596.6	2490.0
ρ (calculated)	1.239 g/cm³	1.296 g/cm³	1.334 g/cm³
R	0.058	0.089	0.093

[1]the solvate molecules were not completely refined due to disorder of the solvent molecules in the channels.

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TABLE 3C

Parameter	Isobutyl Acetate Solvate[1]	Isopropanol Solvate[1]	Ethanol Solvate[1]
Crystal system	Ortho-rhombic	Ortho-rhombic	Ortho-rhombic
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
a	23.19 Å	23.15 Å	23.51 Å
b	12.95 Å	12.73 Å	13.11 Å
c	8.25 Å	8.25 Å	8.27 Å
α	90°	90°	90°
β	90°	90°	90°
γ	90°	90°	90°
Z	4	4	4
Volume (Å)	2476.4	2433.2	2548.6
ρ (calculated)	1.337 g/cm³	1.296 g/cm³	1.234 g/cm³
R	0.098	0.152	0.067

[1]The solvate molecules were not refined completely due to disorder of the solvent molecules in the channels.

Additional information on selected solvated crystalline forms of eplerenone is reported in Table 4 below. The unit cell data reported in Table 3A above for the methyl ethyl ketone solvate also are representative of the unit cell parameters for many of these additional eplerenone crystalline solvates. Most of the eplerenone crystalline solvates tested are substantially isostructural to each other. While there may be some minor shifting in the X-ray powder diffraction peaks from one solvated crystalline form to the next due to the size of the incorporated solvent molecule, the overall diffraction patterns are substantially the same and the unit cell parameters and molecular positions are substantially identical for most of the solvates tested.

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TABLE 4

Solvent	Stoichiometry (Solvent: Eplerenone)	Isostructural to Methyl Ethyl ketone Solvate?	Desolvation Temperature[1] (°C)
Methyl Ethyl Ketone	1:1	N/A	89
2-Pentanone	---	---	---
Acetic Acid	1:2	Yes	203
Acetone	1:1	Yes	117
Butyl Acetate	1:2	Yes	108
Chloroform	---	Yes	125
Ethanol	1:1	Yes	166
Isobutanol	---	---	---
Isobutyl Acetate	1:2	Yes	112
Isopropanol	1:1	Yes	121
Methyl Acetate	1:1	Yes	103
Ethyl Propionate	1:1	Yes	122
n-Butanol	1:1	Yes	103
n-Octanol	---	Yes	116
n-Propanol	1:1	Yes	129
Propyl Acetate	1:1	Yes	130
Propylene Glycol	---	Yes	188
t-Butanol	---	---	---
Tetrahydrofuran	1:1	Yes	136
Toluene	1:1	Yes	83
t-Butyl Acetate	---	Yes	109

[1]Defined as the extrapolated desolvation temperature from the final solvent weight loss step as determined by thermogravimetric analysis at a heating rate of 10°C/minute under nitrogen purge. Desolvation temperatures, however, can be affected by the method of manufacture of the solvate. Different methods can produce different numbers of nucleation sites capable of initiating desolvation in the solvate at lower temperatures.

The unit cell of the solvate is composed of four eplerenone molecules. The stoichiometry of the eplerenone molecules and solvent molecules in the unit cell is also reported in Table 4 above for a number of solvates. The unit cell of Form I is composed of four eplerenone molecules. The unit cell of Form II is composed of two eplerenone molecules. The solvate unit cells are converted during desolvation into

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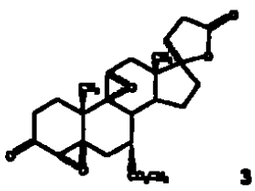
Form I and/or Form II unit cells when the eplerenone molecules undergo translation and rotation to fill the spaces left by the solvent molecules. Table 4 also reports the desolvation temperatures for a number of different solvates.

9. Crystal Properties of Impurity Molecules

Selected impurities in eplerenone can induce the formation of Form I during the desolvation of the solvate. In particular, the effect of the following two impurity molecules was evaluated: 7-methyl hydrogen 4α,5α:9α,11α-diepoxy-17-hydroxy-3-oxo-17α-pregnane-7α,21-dicarboxylate, γ-lactone 3 (the "diepoxide"); and 7-methyl hydrogen 11α,12α-epoxy-17-hydroxy-3-oxo-17α-pregn-4-ene-7α,21-dicarboxylate, γ-lactone 4 (the "11,12-epoxide").



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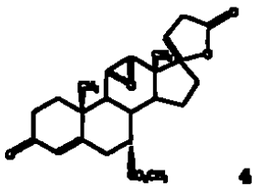

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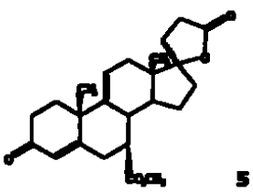
The effect of these impurity molecules on the eplerenone crystalline form resulting from desolvation is described in greater detail in the examples of this application.

Given the similarity in single crystal structure of 7-methyl hydrogen 17-hydroxy-3-oxo-17α-pregna-4,9(11)-diene-7α,21-dicarboxylate, γ-lactone 5 (the "9,11-olefin") and Form I, it is hypothesized that the 9,11-olefin also can induce the formation of Form I during the desolvation of the solvate.



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A single crystal form was isolated for each impurity compound. Representative X-ray powder diffraction patterns for the crystal forms isolated for the diepoxide, 11,12-epoxide and 9,11-olefin are given in Figs. 9, 10 and 11, respectively. The X-ray powder diffraction pattern of each impurity molecule is similar to the X-ray powder diffraction pattern of Form I, suggesting that Form I and the three impurity compounds have similar single crystal structures.

Single crystals of each impurity compound also were isolated and subjected to X-ray structure determination to verify that these three compounds adopt single crystal structures similar to that of Form I. Single crystals of the diepoxide were isolated from methyl ethyl ketone. Single crystals of the 11,12-epoxide were isolated from isopropanol. Single crystals of the 9,11-olefin were isolated from n-butanol. Crystal structure data determined for the crystalline form of each impurity compound are given in Table 5. The resulting crystal system and cell parameters were substantially the same for the Form I, diepoxide, 11,12-epoxide, and 9,11-olefin crystalline forms.

TABLE 5

Parameter	Form I	Diepoxide	11,12 Epoxide	9,11 olefin
Crystal system	Ortho-rhombic	Ortho-rhombic	Ortho-rhombic	Ortho-rhombic
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
a	21.22 Å	21.328 Å	20.90 Å	20.90 Å
b	15.40 Å	16.16 Å	15.55 Å	15.74 Å
c	6.34 Å	6.15 Å	6.38 Å	6.29 Å
α	90°	90°	90°	90°
β	90°	90°	90°	90°
γ	90°	90°	90°	90°
Z	4	4	4	4
Volume (Å)	2071.3	2119.0	2073.2	2069.3
ρ (calculated)	1.329 g/cm³	1.349 g/cm³	1.328 g/cm³	1.279 g/cm³
R	0.0667	0.0762	0.0865	0.0764

The four compounds reported in Table 5 crystallize into the same space group and have similar cell parameters (i.e., they are isostructural). It is hypothesized that the diepoxide, 11,12-epoxide and 9,11-olefin adopt a Form I conformation. The relative ease of isolation of a Form I packing (directly from solution) for each impurity compound, indicates that the Form I lattice is a stable packing mode for this series of structurally similar compounds.

Preparation of Eplerenone

The eplerenone starting material used to prepare the novel crystalline forms of the present invention can be prepared using the methods set forth in Ng et al., WO97/21720; and Ng et al., WO98/25948, particularly scheme 1 set forth in WO97/21720 and WO98/25948.

Preparation of Crystalline Forms

1. Preparation of Solvated Crystalline Form

The solvated crystalline forms of eplerenone can be prepared by crystallization of eplerenone from a suitable solvent or a mixture of suitable solvents. A suitable solvent or mixture of suitable solvents generally comprises an organic solvent or a mixture of organic solvents that solubilizes the eplerenone together with any impurities at an elevated temperature, but upon cooling, preferentially crystallizes the solvate. The solubility of eplerenone in such solvents or mixtures of solvents generally is about 5 to about 200 mg/mL at room temperature. The solvent or mixtures of solvents preferably are selected from those solvents previously used in the process to prepare the eplerenone starting material, particularly those solvents that would be pharmaceutically acceptable if contained in the final pharmaceutical composition comprising the eplerenone crystalline form. For example, a solvent system comprising methylene chloride that yields a solvate comprising methylene chloride generally is not desirable.

Each solvent used preferably is a pharmaceutically acceptable solvent, particularly a Class 2 or Class 3 solvent as defined in "Impurities: Guideline For Residual Solvents", International Conference On Harmonisation Of Technical Requirements For Registration Of Pharmaceuticals For Human Use (Recommended for Adoption at Step 4 of the ICH Process on July 17, 1997 by the ICH Steering Committee). Still more preferably, the solvent or mixture of solvents is selected from the group consisting of methyl ethyl ketone, 1-propanol,

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2-pentanone, acetic acid, acetone, butyl acetate, chloroform, ethanol, isobutanol, isobutyl acetate, methyl acetate, ethyl propionate, n-butanol, n-octanol, isopropanol, propyl acetate, propylene glycol, t-butanol, tetrahydrofuran, toluene, methanol and t-butyl acetate. Still more preferably, the solvent is selected from the group consisting of methyl ethyl ketone and ethanol.

In another embodiment of this process, the solvent or mixture of solvents is selected from the group consisting of 1-propanol, 2-pentanone, acetic acid, acetone, butyl acetate, chloroform, isobutanol, isobutyl acetate, methyl acetate, ethyl propionate, n-butanol, n-octanol, propyl acetate, propylene glycol, t-butanol, tetrahydrofuran, toluene, methanol and t-butyl acetate.

In another embodiment of this process, the solvent or mixture of solvents is selected from the group consisting of 1-propanol, 2-pentanone, acetic acid, acetone, butyl acetate, chloroform, isobutanol, isobutyl acetate, methyl acetate, ethyl propionate, n-butanol, n-octanol, n-propanol, propyl acetate, propylene glycol, t-butanol, tetrahydrofuran, toluene, methanol and t-butyl acetate.

To prepare the solvated crystalline form of eplerenone, an amount of the eplerenone starting material is solubilized in a volume of the solvent and cooled until crystals form. The solvent temperature at which the eplerenone is added to the solvent generally will be selected based upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, this solvent temperature typically is at least about 25°C, preferably from about 30°C to the boiling point of the solvent, and more preferably from about 25°C below the

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boiling point of the solvent to the boiling point of the solvent.

Alternatively, hot solvent may be added to the eplerenone and the mixture can be cooled until crystals form. The solvent temperature at the time it is added to the eplerenone generally will be selected based upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, the solvent temperature typically is at least 25°C, preferably from about 50°C to the boiling point of the solvent, and more preferably from about 15°C below the boiling point of the solvent to the boiling point of the solvent.

The amount of the eplerenone starting material mixed with a given volume of solvent likewise will depend upon the solubility curve of the solvent or mixture of solvents. Typically, the amount of eplerenone added to the solvent will not completely solubilize in that volume of solvent at room temperature. For most of the solvents described herein, for example, the amount of eplerenone starting material mixed with a given volume of solvent usually is at least about 1.5 to about 4.0 times, preferably about 2.0 to about 3.5 times, and more preferably about 2.5 times, the amount of eplerenone that will solubilize in that volume of solvent at room temperature.

After the eplerenone starting material has completely solubilized in the solvent, the solution typically is cooled slowly to crystallize the solvated crystalline form of eplerenone. For most of the solvents described herein, for example, the solution is cooled at a rate slower than about 20°C/minute, preferably at a rate of about 10°C/minute or slower, more preferably at a rate of about 5°C/minute or

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slower, and still more preferably at a rate of about 1°C/minute or slower.

The endpoint temperature at which the solvated crystalline form is harvested will depend upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, the endpoint temperature typically is less than about 25°C, preferably less than about 5°C, and more preferably less than about -5°C. Decreasing the endpoint temperature generally favors the formation of the solvated crystalline form.

Alternatively, other techniques may be used to prepare the solvate. Examples of such techniques include, but are not limited to, (i) dissolving the eplerenone starting material in one solvent and adding a co-solvent to aid in the crystallization of the solvate crystalline form, (ii) vapor diffusion growth of the solvate, (iii) isolation of the solvate by evaporation, such as rotary evaporation, and (iv) slurry converstion.

The crystals of the solvated crystalline form prepared as described above can be separated from the solvent by any suitable conventional means such as by filtration or centrifugation. Increased agitation of the solvent system during crystallization generally results in smaller crystal particle sizes.

2. Preparation of Form II From Solvate

Form II eplerenone can be prepared directly from the solvated crystalline form by desolvation. Desolvation can be accomplished by any suitable desolvation means such as, but not limited to, heating the solvate, reducing the ambient pressure surrounding the solvate, or combinations thereof.

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If the solvate is heated to remove the solvent, such as in an oven, the temperature of the solvate during this process typically does not exceed the enantiotropic transition temperature for Form I and Form II. This temperature preferably does not exceed about 150°C.

The desolvation pressure and time of desolvation are not narrowly critical. The desolvation pressure preferably is about one atmosphere or less. As the desolvation pressure is reduced, however, the temperature at which the desolvation can be carried out and/or the time of desolvation likewise is reduced. Particularly for solvates having higher desolvation temperatures, drying under vacuum will permit the use of lower drying temperatures. The time of desolvation need only be sufficient to allow for the desolvation, and thus the formation of Form II, to reach completion.

To ensure the preparation of a product that comprises substantially all Form II, the eplerenone starting material typically is a high purity eplerenone, preferably substantially pure eplerenone. The eplerenone starting material used to prepare Form II eplerenone generally is at least 90% pure, preferably at least 95% pure, and more preferably at least 99% pure. As discussed in greater detail elsewhere in this application, certain impurities in the eplerenone starting material can adversely affect the yield and Form II content of the product obtained from the process.

The crystallized eplerenone product prepared in this manner from a high purity eplerenone starting material generally comprises at least 10% Form II, preferably at least 50% Form II, more preferably at least 75% Form II, still more preferably at least 90% Form II, still more preferably at

least about 95% Form II, and still more preferably substantially phase pure Form II.

3. Preparation of Form I From Solvate

A product comprising Form I can be prepared in substantially the same manner as set forth above for the preparation of Form II by (i) using a low purity eplerenone starting material instead of a high purity eplerenone starting material, (ii) seeding the solvent system with phase pure Form I crystals, or (iii) a combination of (i) and (ii).

A. Use Of Impurities As Growth Promoters and Inhibitors

The presence and amount of selected impurities in the eplerenone starting material, rather than the total amount of all impurities in the eplerenone starting material, affect the potential for Form I crystal formation during the desolvation of the solvate. The selected impurity generally is a Form I growth promoter or Form II growth inhibitor. It may be contained in the eplerenone starting material, contained in the solvent or mixture of solvents before the eplerenone starting material is added, and/or added to the solvent or mixture of solvents after the eplerenone starting material is added. Bonafede et al., "Selective Nucleation and Growth of an Organic Polymorph by Ledge-Directed Epitaxy on a Molecular Crystal Substate", J. Amer. Chem. Soc., Vol. 117, No. 30 (August 2, 1995) discusses the use of growth promoters and growth inhibitors in polymorph systems and is incorporated by reference herein. For the present invention, the impurity generally comprises a compound having a single crystal structure substantially identical to the single crystal structure of Form I. The impurity preferably is a compound

having an X-ray powder diffraction pattern substantially identical to the X-ray powder diffraction pattern of Form I, and more preferably is selected from the group consisting of the diepoxide, the 11,12-epoxide, the 9,11-olefin and combinations thereof.

The amount of impurity needed to prepare Form I crystals typically can depend, in part, upon the solvent or mixture of solvents and the solubility of the impurity relative to eplerenone. In the crystallization of Form I from a methyl ethyl ketone solvent, for example, the weight ratio of diepoxide to low purity eplerenone starting material typically is at least about 1:100, preferably at least about 3:100, more preferably between about 3:100 and about 1:5, and still more preferably between about 3:100 and about 1:10. The 11,12-epoxide has a higher solubility in methyl ethyl ketone than the diepoxide and generally requires a larger amount of the 11,12-epoxide generally is necessary to prepare Form I crystals. Where the impurity comprises the 11,12-epoxide, the weight ratio of the diepoxide to the low purity eplerenone starting material typically is at least about 1:5, more preferably at least about 3:25, and still more preferably between about 3:25 and about 1:5. Where both the diexpoxide and the 11,12-epoxide impurities are used in the preparation of the Form I crystals, the weight ratio of each impurity to the eplerenone starting material may be lower than the corresponding ratio when only that impurity is used in the preparation of the Form I crystals.

A mixture of Form I and Form II is generally obtained when a solvate comprising the selected impurity is desolvated. The weight fraction of Form I in the product resulting from the initial desolvation of the solvate

typically is less than about 50%. Further treatment of this product by crystallization or digestion, as discussed below, generally will increase the weight fraction of Form II in the product.

B. Seeding

Form I crystals also can be prepared by seeding the solvent system with phase pure Form I crystals (or a Form I growth promoter and/or Form II growth inhibitor as previously discussed above) prior to crystallization of the eplerenone. The eplerenone starting material can be either a low purity eplerenone or a high purity eplerenone. When the resulting solvate prepared from either starting material is desolvated, the weight fraction of Form I in the product typically is at least about 70% and may be as great as about 100%.

The weight ratio of Form I seed crystals added to the solvent system to the eplerenone starting material added to the solvent system generally is at least about 0.75:100, preferably between about 0.75:100 to about 1:20, and more preferably between about 1:100 to about 1:50. The Form I seed crystals can be prepared by any of the methods discussed in this application for the preparation of Form I crystals, particularly the preparation of Form I crystals by digestion as discussed below.

The Form I seed crystals may be added at one time, in multiple additions or substantially continually over a period of time. The addition of the Form I seed crystals, however, generally is completed before the eplerenone begins to crystallize from solution, i.e., the seeding is completed before the cloud point (the lower end of the metastable zone) is reached. Seeding typically is performed when the solution

temperature ranges from about 0.5°C above the cloud point to about 10°C above the cloud point, preferably within about 2°C to about 3°C above the cloud point. As the temperature above the cloud point at which the seeds are added increases, the amount of seeding needed for crystallization of Form I crystals generally increases.

The seeding preferably occurs not only above the cloud point, but within the metastable zone. Both the cloud point and the metastable zone are dependent on the eplerenone solubility and concentration in the solvent or mixture of solvents. For a 12 volume dilution of methyl ethyl ketone, for example, the high end of the metastable zone generally is between about 70°C to about 73°C and the lower end of the metastable zone (i.e., the cloud point) is between about 57°C and 63°C. For a concentration of 8 volumes of methyl ethyl ketone, the metastable zone is even narrower because the solution is supersaturated. At this concentration, the cloud point of the solution occurs at about 75°C to about 76°C. Because the boiling point of methyl ethyl ketone is about 80° C under ambient conditions, seeding for this solution typically occurs between about 76.5°C and the boiling point.

An illustrative non-limiting example of seeding with Form I is set forth below in Example 7.

The crystallized eplerenone product obtained using a Form I growth promoter or Form II growth inhibitor, and/or Form I seeding generally comprises at least 2% Form I, preferably at least 5% Form I, more preferably at least 7% Form I, and still more preferably at least about 10% Form I.

The remaining crystallized eplerenone product generally is Form II.

C. Form I Prepared By Grinding Eplerenone

In yet another alternative, it has been discovered that a small amount of Form I can be prepared by suitable grinding eplerenone. Concentrations of Form I in ground eplerenone as high as about 3% have been observed.

4. Preparation of Form II From Solvate Prepared From Low Purity Eplerenone

As discussed above, crystallization of low purity eplerenone to form a solvate followed by desolvation of the solvate generally yields a product comprising both Form I and Form II. A product having a greater Form II content can be prepared from low purity eplerenone in substantially the same manner as set forth above for the preparation of Form I by seeding the solvent system with phase pure Form II crystals, or by using a Form II growth promoter and/or Form I growth inhibitor. The seeding protocol and the weight ratio of the amount of Form II seed crystals added to the solvent system to the amount of the eplerenone starting material added to the solvent system generally are similar to those ratios previously discussed above for the preparation of Form I eplerenone by seeding with phase pure Form I crystals.

The crystallized eplerenone product prepared in this manner generally comprises at least 10% Form II, preferably at least 50% Form II, more preferably at least 75% Form II, more preferably at least 90% Form II, still more preferably at least about 95% Form II, and still more preferably substantially phase pure Form II.

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The seeding protocols described in this section and in the prior section relating to the preparation of Form I eplerenone also may allow for improved control of the particle size of the crystallized eplerenone.

5. Crystallization of Form II Directly From Solution

Form II eplerenone also can be prepared by the direct crystallization of eplerenone from a suitable solvent or mixture of solvents without the formation of an intermediate solvate and the accompanying need for desolvation. Typically, (i) the solvent has a molecular size that is incompatible with the available channel space in the solvate crystal lattice, (ii) the eplerenone and any impurities are soluble in the solvent at elevated temperatures, and (iii) upon cooling, results in the crystallization of the non-solvated Form II eplerenone. The solubility of eplerenone in the solvent or mixture of solvents generally is about 5 to about 200 mg/mL at room temperature. The solvent or mixture of solvents preferably comprises one or more solvents selected from the group consisting of methanol, ethyl acetate, isopropyl acetate, acetonitrile, nitrobenzene, water and ethyl benzene.

To crystallize Form II eplerenone directly from solution, an amount of the eplerenone starting material is solubilized in a volume of the solvent and cooled until crystals form. The solvent temperature at which the eplerenone is added to the solvent generally will be selected based upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, this solvent temperature typically is at least about 25°C, preferably from about 30°C to the boiling point of the

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solvent, and more preferably from about 25°C below the boiling point of the solvent to the boiling point of the solvent.

Alternatively, hot solvent may be added to the eplerenone and the mixture can be cooled until crystals form. The solvent temperature at the time it is added to the eplerenone generally will be selected based upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, the solvent temperature typically is at least 25°C, preferably from about 50°C to the boiling point of the solvent, and more preferably from about 15°C below the boiling point of the solvent to the boiling point of the solvent.

The amount of the eplerenone starting material mixed with a given volume of solvent likewise will depend upon the solubility curve of the solvent or mixture of solvents. Typically, the amount of eplerenone added to the solvent will not completely solubilize in that volume of solvent at room temperature. For most of the solvents described herein, for example, the amount of eplerenone starting material mixed with a given volume of solvent usually is at least about 1.5 to about 4.0 times, preferably about 2.0 to about 3.5 times, and more preferably about 2.5 times, the amount of eplerenone that will solubilize in that volume of solvent at room temperature.

To ensure the preparation of a product that comprises substantially phase pure Form II, the eplerenone starting material generally is a high purity eplerenone. The eplerenone starting material preferably is at least 65% pure, more preferably at least 90% pure, still more preferably at least 98% pure, and still more preferably at least 99% pure.

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After the eplerenone starting material has completely solubilized in the solvent, the solution typically is cooled slowly to crystallize the solvated crystalline form of eplerenone. For most of the solvents described herein, for example, the solution is cooled at a rate slower than about 1.0°C/minute, preferably at a rate of about 0.2°C/minute or slower, and more preferably at a rate between about 5°C/minute and about 0.1°C/minute.

The endpoint temperature at which the Form II crystals are harvested will depend upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, the endpoint temperature typically is less than about 25°C, preferably less than about 5°C, and more preferably less than about -5°C.

Alternatively, other techniques may be used to prepare the Form II crystals. Examples of such techniques include, but are not limited to, (i) dissolving the eplerenone starting material in one solvent and adding a co-solvent to aid in the crystallization of Form II eplerenone, (ii) vapor diffusion growth of Form II eplerenone, (iii) isolation of Form II eplerenone by evaporation, such as rotary evaporation, and (iv) slurry conversion.

The crystals of the solvated crystalline form prepared as described above can be separated from the solvent by any suitable conventional means such as by filtration or centrifugation.

In addition, Form II eplerenone also can be prepared by digesting (as described below) a slurry of high purity eplerenone in methyl ethyl ketone and filtering the digested eplerenone at the boiling point of the slurry.

6. Preparation of Form I Directly From Solution

It is hypothesized that if the crystallization is performed above the enantiotropic transition temperature (T_t) for Form I and Form II, particularly if Form I growth promoters or Form II growth inhibitors are present or the solvent is seeded with phase pure Form I crystals, Form I should crystallize directly from solution since Form I is more stable at these higher temperatures. The solvent system used preferably comprises a high boiling solvent such as nitrobenzene. Suitable Form I growth promoters would include, but would not be limited to, the diepoxide and the 11,12-olefin.

7. Digestion of Eplerenone With A Solvent

The solvated crystalline forms, Form I and Form II of eplerenone also can be prepared by digestion of an eplerenone starting material in a suitable solvent or mixture of solvents. In the digestion process, a slurry of eplerenone is heated at the boiling point of the solvent or mixture of solvents. For example, an amount of eplerenone starting material is combined with a volume of solvent or mixture of solvents, heated to reflux, and the distillate is removed while an additional amount of the solvent is added simultaneously with the removal of the distillate. Alternatively, the distillate can be condensed and recycled without the addition of more solvent during the digestion process. Typically, once the original volume of solvent has been removed or condensed and recycled, the slurry is cooled and solvated crystals form. The solvated crystals can be separated from the solvent by any suitable conventional means such as by filtration or centrifugation. Desolvation of the

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solvate as previously described yields either Form I or Form II eplerenone depending upon the presence or absence of the selected impurities in the solvated crystals.

A suitable solvent or mixture of solvents generally comprises one or more of the solvents previously disclosed herein. The solvent may be selected, for example, from the group consisting of methyl ethyl ketone and ethanol.

The amount of eplerenone starting material added to the solvent used in the digestion process generally is sufficient to maintain a slurry (i.e., the eplerenone in the solvent or mixture of solvents is not completely solubilized) at the boiling point of the solvent or mixture of solvents. Illustrative values include, but are not limited to, about one gram of eplerenone per four mL methyl ethyl ketone and about one gram of eplerenone per eight mL ethanol.

The solution generally is cooled slowly once solvent turnover is complete to crystallize the solvated crystalline form of eplerenone. For the solvents tested, for example, the solution is cooled at a rate slower than about 20°C/minute, preferably about 10°C/minute or slower, more preferably about 5°C/minute or slower, and still more preferably about 1°C/minute or slower.

The endpoint temperature at which the solvated crystalline form is harvested will depend upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, the endpoint temperature typically is less than about 25°C, preferably less than about 5°C, and more preferably less than about -5°C.

If a product comprising primarily or exclusively Form II is desired, a high purity eplerenone starting material

typically is digested. The high purity eplerenone starting material preferably is at least 98% pure, more preferably at least 99% pure, and still more preferably at least 99.5% pure. The digested eplerenone product prepared in this manner generally comprises at least 10% Form II, preferably at least 50% Form II, more preferably at least 75% Form II, more preferably at least 90% Form II, still more preferably at least about 95% Form II, and still more preferably substantially phase pure Form II.

If a product comprising primarily or exclusively Form I is desired, a low purity eplerenone starting material typically is digested. The low purity eplerenone starting material generally contains only as much Form I growth promoter and/or Form II growth inhibitor as is needed to yield Form I. Preferably, the low purity eplerenone starting material is at least 65% pure, more preferably at least 75% pure, and still more preferably at least 80% pure. The digested eplerenone product prepared in this manner generally comprises at least 10% Form I, preferably at least 50% Form I, more preferably at least 75% Form I, more preferably at least 90% Form I, still more preferably at least about 95% Form I, and still more preferably substantially phase pure Form I.

8. Preparation of Amorphous Eplerenone

Amorphous eplerenone can be prepared in small quantities by suitable comminution of solid eplerenone, such as by crushing, grinding and/or micronizing. Phase pure amorphous eplerenone can be prepared, for example, by lyophilizing a solution of eplerenone, particularly an aqueous solution of

eplerenone. These processes are illustrated in Examples 13 and 14 below.

Additional Processing Considerations

1. Thermodynamic Stability Considerations

Form II is more thermodynamically stable than Form I at ambient temperatures. As described in Example 5 below, when an organic slurry containing equivalent amounts of Form I and Form II was allowed to stand overnight at room temperature and the residual solids were then collected and analyzed by X-ray powder diffraction, the analytical results indicated that the eplerenone had completely converted to Form II. The differential scanning calorimetry ("DSC") data (discussed previously) indicate that Form I is more thermodynamically stable than Form II at higher temperatures since it has the higher melting/decomposition temperature. Taken together, the slurry conversion and DSC data indicate that Form I and Form II are related enantiotropically, i.e., a change in the stability relationship between the two polymorphs occurs around a transition temperature (T_t), with Form II being the more stable at lower temperatures. Fig. 12 shows the Gibbs free energy-temperature relationship generally observed for enantiotropically related polymorphs such as Form I and Form II, wherein I and II refer to the crystal forms, T_t refers to the transition temperature, T_m refers to the melting points of Form I and Form II, and L represents the liquid or melt state.

Accordingly, processing temperatures preferably are maintained below the transition temperature during the preparation of compositions comprising Form II. For example, drying temperatures employed for desolvation typically are

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less than about 150°C, preferably less than about 125°C, more preferably less than about 115°C, more preferably less than about 110°C, and still more preferably between about 80°C to about 110°C. In addition, cooling (such as by use of liquid nitrogen) may be necessary during particle size reduction process steps to maintain the temperature of the Form II crystals below the transition temperature.

2. Intrinsic Micronizing Considerations

The method used to prepare crystalline eplerenone may affect the properties of the resulting crystal. For example, Form II prepared by desolvation of a solvate exhibits a higher incidence of surface defects, pores, cracks and fractures within the crystal lattice than Form II prepared by direct crystallization from solution. This "intrinsic micronizing" of the desolvated crystal results in an increase in both the available surface area of the crystal and the dissolution rate of the crystal. Dissolution times, therefore, can be shortened by selection of Form II crystals prepared by desolvation, lengthened by selection of Form II crystals prepared by direct crystallization, or otherwise adjusted by selection of an appropriate combination of Form II crystals prepared by desolvation and Form II crystals prepared by direct crystallization.

This "intrinsic micronizing" also may effectively reduce or eliminate the need for crystal particle size reduction during processing steps where Form II crystals prepared by desolvation are used in the preparation of the pharmaceutical composition. One disadvantage to using such Form II crystals, however, is the need for a desolvation step that is

not required for Form II crystals prepared by direct crystallization.

Product-By-Process Solid State Forms

The present invention also comprises each of the solid state eplerenone forms prepared in accordance with the processes disclosed in this application. In particular, the invention comprises solvated eplerenone, Form I eplerenone, Form II eplerenone, and combinations thereof, prepared as set forth in this application.

Combinations of Solid State Forms

Also embraced within this invention are combinations comprising a first solid state form of eplerenone and a second solid state form of eplerenone, wherein the first and second solid state forms of eplerenone are selected from Form I, Form II, solvated eplerenone and amorphous eplerenone. In general, the weight ratio of said first solid state form to said second solid state form preferably is at least about 1:9, more preferably is at least about 1:1, more preferably at least about 2:1, more preferably at least about 5:1, and still more preferably at least about 9:1.

In one embodiment, for example, the first solid state form is Form I and the second solid state form is Form II.

In another embodiment, for example, the first solid state form is Form II and the second solid state form is Form I.

Pharmaceutical Compositions

Also embraced within this invention is a class of pharmaceutical compositions comprising a first solid state

form of eplerenone and a second solid state form of eplerenone in association with one or more pharmaceutically-acceptable carriers and/or diluents and/or adjuvants (collectively referred to herein as "carrier" materials) and, if desired, other active ingredients. The first solid state form of eplerenone and the second solid state form of eplerenone are selected from Form I, Form II, solvated eplerenone and amorphous eplerenone. In general, the weight ratio of said first solid state form to said second solid state form preferably is at least about 1:9, more preferably is at least about 1:1, more preferably at least about 2:1, more preferably at least about 5:1, and still more preferably at least about 9:1.

In one embodiment of the invention, the entire amount of eplerenone contained in the composition is present as phase pure Form II.

In another embodiment of the invention, the entire amount of eplerenone contained in the composition is present as phase pure Form I.

In still another embodiment of the invention, the entire amount of eplerenone contained in the composition is present as phase pure solvated crystalline eplerenone.

In still another embodiment of the invention, the entire amount of eplerenone contained in the composition is present as amorphous eplerenone.

In still another embodiment of the invention, the composition comprises both Form I and Form II. The ratio of the amount of Form II to Form I in the composition generally is between about 1:20 to about 20:1. In other embodiments, for example, this ratio is between about 10:1 to about 1:10;

about 5:1 to about 1:5; about 2:1 to about 1:2; or is about 1:1.

In still another embodiment of the invention, the composition comprises a first solid state form of eplerenone and a second solid state form of eplerenone, wherein the first and second solid state forms of eplerenone are selected from Form I, Form II, solvated eplerenone and amorphous eplerenone. In general, the weight ratio of said first solid state form to said second solid state form preferably is at least about 1:9, preferably about 1:1, more preferably at least about 2:1, more preferably at least about 5:1, and still more preferably at least about 9:1.

In each of the above combination and pharmaceutical composition embodiments, the D_{90} particle size of the eplerenone used as a starting material in the pharmaceutical composition generally is between about 10 nm to about 15 microns, preferably less than about 1 micron, more preferably less than about 800 nm, still more preferably less than about 600 nm, and still more preferably less than about 400 nm. In one embodiment, the D_{90} particle size is between about 10 nm to about 1 micron. In another embodiment, the D_{90} particle size is between about 100 nm to about 800 nm. In another embodiment, the D_{90} particle size is between about 200 nm to about 600 nm. In another embodiment, the D_{90} particle size is between about 400 nm to about 800 nm.

These solid state forms of eplerenone of the present invention may be administered by any suitable route, preferably in the form of a pharmaceutical composition adapted to such a route, and in a dose effective for the treatment intended. The solid state forms of eplerenone and compositions thereof may, for example, be administered

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orally, intravascularly, intraperitoneally, subcutaneously, intramuscularly or topically.

The amount of eplerenone that is administered and the dosage regimen for treating a condition or disorder with the solid state forms of eplerenone and/or compositions of this invention depends on a variety of factors, including the age, weight, sex and medical condition of the subject, the severity of the disease, the route and frequency of administration, and the particular compound employed, and thus may vary widely. The pharmaceutical compositions may contain eplerenone in the range of about 0.1 to 2000 mg, preferably in the range of about 0.5 to 500 mg and most preferably between about 1 and 200 mg. A daily dose of about 0.01 to 100 mg/kg body weight, preferably between about 0.5 and about 20 mg/kg body weight and most preferably between about 0.1 to 10 mg/kg body weight, may be appropriate. The daily dose can be administered in one to four doses per day.

For oral administration, the pharmaceutical composition may be in the form of, for example, a tablet, capsule, or suspension. The pharmaceutical composition is preferably made in the form of a dosage unit containing a particular amount of eplerenone. Examples of such dosage units are tablets or capsules. The eplerenone may also be administered by injection of a suspension wherein, for example, saline, dextrose or water may be used as a suitable carrier.

Other formulations may be in the form of a topical ointment or cream, or a suppository, containing eplerenone in a total amount of, for example, 0.075 to 30% w/w, preferably 0.2 to 20% w/w and most preferably 0.4 to 15% w/w. When formulated in an ointment, the eplerenone may be employed with either paraffinic or a water-miscible ointment base.

Alternatively, the eplerenone may be formulated in a cream with an oil-in-water cream base. If desired, the aqueous phase of the cream base may include, for example at least 30% w/w of a polyhydric alcohol such as propylene glycol, butane-1,3-diol, mannitol, sorbitol, glycerol, polyethylene glycol and mixtures thereof. The topical formulation may desirably include a compound which enhances absorption or penetration of the eplerenone through the skin or other affected areas. Examples of such dermal penetration enhancers include dimethylsulfoxide and related analogs.

The novel solid state forms of eplerenone also can be administered by a transdermal device. Preferably, topical administration will be accomplished using a patch either of the reservoir and porous membrane type or of a solid matrix variety. In either case, the eplerenone is delivered continuously from the reservoir or microcapsules through a membrane into the eplerenone permeable adhesive, which is in contact with the skin or mucosa of the recipient. If the eplerenone is absorbed through the skin, a controlled and predetermined flow of the eplerenone is administered to the recipient. In the case of microcapsules, the encapsulating agent may also function as the membrane.

The oily phase of the emulsions of this invention may be constituted from known ingredients in a known manner. While the phase may comprise merely an emulsifier, it may comprise a mixture of at least one emulsifier with a fat or an oil or with both a fat and an oil. Preferably, a hydrophilic emulsifier is included together with a lipophilic emulsifier which acts as a stabilizer. It is also preferred to include both an oil and a fat. Together, the emulsifier(s) with or without stabilizer(s) make-up the so-called emulsifying wax,

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and the wax together with the oil and fat make up the so-called emulsifying ointment base which forms the oily dispersed phase of the cream formulations. Emulsifiers and emulsion stabilizers suitable for use in the formulation of the present invention include Tween 60, Span 80, cetostearyl alcohol, myristyl alcohol, glyceryl monostearate, and sodium lauryl sulfate, among others.

The choice of suitable oils or fats for the formulation is based on achieving the desired cosmetic properties, since the solubility of the eplerenone in most oils likely to be used in pharmaceutical emulsion formulations is very low. Thus, the cream should preferably be a non-greasy, non-staining and washable product with suitable consistency to avoid leakage from tubes or other containers. Straight or branched chain, mono- or dibasic alkyl esters such as di-isoadipate, isocetyl stearate, propylene glycol diester of coconut fatty acids, isopropyl myristate, decyl oleate, isopropyl palmitate, butyl stearate, 2-ethylhexyl palmitate or a blend of branched chain esters may be used. These may be used alone or in combination depending on the properties required. Alternatively, high melting point lipids such as white soft paraffin and/or liquid paraffin or other mineral oils can be used.

Formulations suitable for topical administration to the eye also include eye drops wherein the eplerenone is suspended in suitable carrier, especially an aqueous carrier for the eplerenone. The eplerenone is preferably present in such formulations in a concentration of 0.5 to 20%, advantageously 0.5 to 10% and particularly about 1.5% w/w.

For therapeutic purposes, the novel solid state forms of eplerenone ordinarily are combined with one or more adjuvants

appropriate to the indicated route of administration. If administered per os, the compound may be admixed with lactose, sucrose, starch powder, cellulose esters of alkanoic acids, cellulose alkyl esters, talc, stearic acid, magnesium stearate, magnesium oxide, sodium and calcium salts of phosphoric and sulfuric acids, gelatin, acacia gum, sodium alginate, polyvinylpyrrolidone, and/or polyvinyl alcohol, and then tableted or encapsulated for convenient administration. Such capsules or tablets may contain an immediate release formulation, or may contain a controlled-release formulation such as a dispersion of the eplerenone in hydroxypropylmethyl cellulose. Formulations for parenteral administration may be in the form of aqueous or non-aqueous isotonic sterile injection suspensions. These suspensions may be prepared from sterile powders or granules having one or more of the carriers or diluents mentioned for use in the formulations for oral administration. Other adjuvants and modes of administration are well and widely known in the pharmaceutical art.

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Methods of Treatment or Prophylaxis

The present invention also comprises methods for the treatment and/or prophylaxis of an aldosterone-mediated condition and/or disorder such as hypertension in a subject, the methods comprising treating the subject having or susceptible to such condition or disorder with a therapeutically-effective amount of Form I, Form II, solvated crystalline form, or an amorphous form of eplerenone, or combinations thereof. These solid state forms of eplerenone would be useful for the treatment and/or prophylaxis of a condition and/or disorder in a subject where administration

of an aldosterone-antagonist is indicated including, but not limited to, the treatment of hyperaldosteronism such as hypertension, heart failure including cardiac insufficiency, cirrhosis of the liver, excess collagen/fibrosis, benign prostate hypertrophy and depression.

Besides being useful for human treatment, these solid state forms of eplerenone are also useful for veterinary treatment of companion animals, exotic animals and farm animals, including mammals, rodents, and the like. More preferred animals include horses, dogs, and cats.

These solid state forms of eplerenone also may be used (i) in combination therapies partially or completely in place of other conventional aldosterone receptor antagonists, or (ii) in combination therapies with other active drugs. The phrase "combination therapy" is intended to embrace the administration of each agent in a sequential manner in a regimen that will provide beneficial effects of the drug combination, and is intended as well to embrace co-administration of these agents in a substantially simultaneous manner, such as in a single capsule or injection having a fixed ratio of these active agents or in multiple, separate capsules or injections for each agent. Non-limiting examples of combination therapy include the treatment of cardiovascular diseases using a combination of an aldosterone receptor antagonist and an angiotensin II receptor antagonist as described in WO96/24373; the treatment of congestive heart failure using a combination of an aldosterone receptor antagonist and an angiotensin II antagonist as described in WO96/40257; and the treatment of heart failure using a combination of an aldosterone receptor antagonist, an ACE inhibitor and a diuretic as described in WO96/24372.

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Working Examples

The following examples contain detailed descriptions of the methods of preparation of the various solid state forms of eplerenone described in this application. These detailed descriptions fall within the scope, and serve to exemplify the invention. These detailed descriptions are presented for illustrative purposes only and are not intended as a restriction on the scope of the invention. All parts are by weight and temperatures are in degrees Centigrade unless otherwise indicated. The eplerenone starting material used in each of the following examples was prepared in accordance with scheme 1 set forth in Ng et al., WO98/25948.

Example 1: Preparation of (a) methyl ethyl ketone solvate from high purity eplerenone starting material and (b) Form II crystalline eplerenone from resulting solvate

A. Preparation of Methyl Ethyl Ketone Solvate:

High purity eplerenone (437 mg; greater than 99% purity with less than 0.2% diepoxide and 11,12 epoxide present) was dissolved in 10 mL of methyl ethyl ketone by heating to boiling on a hot plate with magnetic stirring at 900 rpm. The resulting solution was allowed to cool to room temperature with continuous magnetic stirring. Once at room temperature, the solution was transferred to a 1°C bath with maintenance of the stirring for one hour. After one hour, the solid methyl ethyl ketone solvate was collected by vacuum filtration.

B. Preparation of Form II crystalline eplerenone:

The solid methyl ethyl ketone solvate prepared in Step A above was dried in an oven at 100°C for four hours at ambient

pressure. The dried solid was determined to be pure Form II by DSC and XPRD analysis.

Example 2: Preparation of additional solvates from high purity eplerenone starting material

Additional solvated crystalline forms were prepared by replacing methyl ethyl ketone with one of the following solvents: n-propanol, 2-pentanone, acetic acid, acetone, butyl acetate, chloroform, ethanol, isobutanol, isobutyl acetate, isopropanol, methyl acetate, ethyl propionate, n-butanol, n-octanol, propyl acetate, propylene glycol, t-butanol, tetrahydrofuran, and toluene and carrying out the crystallization substantially as described above in Step A of Example 1.

Example 3: Preparation of Methyl Ethyl Ketone Solvate by Vapor Diffusion Growth

Eplerenone (400 mg; greater than 99.9% purity) was dissolved in 20 mL of methyl ethyl ketone by warming on a hot plate to form a stock solution. An 8 mL amount of the stock solution was transferred to a first 20 mL scintillation vial and diluted to 10 mL with methyl ethyl ketone (80%). A 10 mL amount of the stock solution was transferred to a second 20 mL scintillation vial and diluted to 10 mL with methyl ethyl ketone (40%). The final 2 mL of the stock solution was diluted to 10 mL with methyl ethyl ketone (20%). The four vials containing the dilutions were transferred to a dessicator jar containing a small amount of hexane as an anti-solvent. The dessicator jar was sealed and the hexane vapor allowed to diffuse into the methyl ethyl ketone

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solutions. Methyl ethyl ketone solvate crystals grew in the 80% dilution sample by the next day.

Example 4: Preparation of Methyl Ethyl Ketone Solvate by Rotary Evaporation

About 400 mg of eplerenone (greater than 99.9% purity) is weighed into a 250 mL round bottom flask. Solvent (150 mL) is added to the flask and, if necessary, the solution is heated gently until the solid is dissolved. The resulting clear solution is placed on a Buchi rotary evaporator with a bath temperature of about 85°C and the solvent is removed under vacuum. Solvent removal is stopped when approximately 10 mL of solvent remain in the round bottom flask. The resulting solids are analyzed by appropriate method (XPRD, DSC, TGA, microscopy, etc.) for determination of form.

Example 5: Slurry Conversion

Approximately 150 mg of Form II eplerenone and 150 mg of Form I eplerenone were added to 5 mL of ethyl acetate. The resulting slurry was allowed to stir at 300 rpm (magnetic stirring) overnight. The next day a sample of the solid was collected by filtration. Analysis of the sample by XPRD indicated that the sample was entirely composed of Form II eplerenone.

Example 6: Preparation of (a) solvate from low purity eplerenone starting material and (b) Form I crystalline eplerenone from resulting solvate

Samples containing varying amounts of the impurity 7-methyl hydrogen 4α,5α:9α,11α-diepoxy-17-hydroxy-3-oxo-17α-pregnane-7α,21-dicarboxylate, γ-lactone (the "diepoxide") or

the impurity 7-methyl hydrogen 11α,12α-epoxy-17-hydroxy-3-oxo-17α-pregn-4-ene-7α,21-dicarboxylate, γ-lactone (the "11,12-epoxide") were prepared by adding the desired amount of the impurity to a 7 mL scintillation vial together with an amount of eplerenone sufficient to provide a total sample mass of 100 mg. The weight percent of the diepoxide or 11,12-epoxide in each sample is given in Tables X-6A and X-6B, respectively. A micro-flea magnetic stirrer was added to each scintillation vial along with 1 mL of methyl ethyl ketone. The vials were loosely capped and the solid dissolved by heating to reflux on a hot plate with magnetic stirring. Once the solids were dissolved, the solutions were allowed to cool to room temperature on the hot plate. Magnetic stirring was maintained during the cooling period. After the solutions reached room temperature, the solids were collected by vacuum filtration and immediately analyzed by X-ray powder diffraction (XPRD). The solids were then placed in a 100°C oven and dried for one hour at ambient pressure. The dried solids were analyzed by XPRD for Form I content by monitoring the area of the Form I diffraction peak at about 12.1 degrees two theta. All XPRD diffraction patterns were recorded using an Inel Multipurpose Diffractometer.

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TABLE X-6A

Weight Percent Diepoxide	Weight Eplerenone (mg)	Weight Diepoxide (mg)
0%	100.44	--
1%	99.08	1.24
2%	98.09	2.24
3%	97.08	3.04
5%	95.09	5.04

TABLE X-6B

Weight Percent 11,12-Epoxide	Weight Eplerenone (mg)	Weight 11,12-Epoxide (mg)
0%	101.38	0
1%	99.23	1.10
5%	94.97	5.36
10%	90.13	10.86

A. Diepoxide Results

Fig. 13 shows the X-ray powder diffraction patterns for the wet cake (methyl ethyl ketone solvate) obtained from the (a) 0%, (b) 1%, (c) 3%, and (d) 5% diepoxide-doped methyl

ethyl ketone crystallizations. The peak intensities have been normalized for ease of comparison. No peaks characteristic of Form I or the diepoxide are present in the diffraction patterns. The patterns are characteristic of the methyl ethyl ketone solvate of eplerenone.

Fig. 14 shows the X-ray powder diffraction patterns for the dried solids obtained from the (a) 0%, (b) 1%, (c) 3%, and (d) 5% diepoxide-doped methyl ethyl ketone crystallizations. The peak intensities have been normalized for ease of comparison. No Form I was detected for the dried samples corresponding to the methyl ethyl ketone crystallizations performed at doping levels of 0 and 1%. Form I was detected in the dried samples corresponding to the methyl ethyl ketone crystallizations performed at doping levels of 3 and 5%. The area for the Form I diffraction peak at about 12.1 degrees two theta and the estimated Form I content for each sample are given in Table X-6C below.

TABLE X-6C

Weight Percent of Diepoxide in Starting Eplerenone Mixture	Weight Percent of Diepoxide in Resulting Crystals (HPLC)	Form I Peak Area 12° Two Theta Peak	Estimated Weight Percent of Form I
0%	---	None Detected	None Detected
1%	0.29%	None Detected	None Detected
3%	0.58%	1168	10%
5%	1.05%	4175	30%

The results reported in Table X-6C confirm that the presence of the diepoxide affects the formation of Form I during the desolvation. These results indicate that the

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diepoxide is effective in inducing the formation of Form I eplerenone when it is incorporated into and/or adsorbed onto the methyl ethyl ketone solvate crystals.

The 3% diepoxide doping experiment was repeated to analyze the impact of the route of preparation on the amount of Form I formed during the desolvation. In this experiment, the methyl ethyl ketone solvate obtained from the doped crystallization was divided into two portions. The first portion was left untreated while the second portion was lightly ground in a mortar and pestle to induce a higher level of crystal defects. The two portions were both dried at 100 °C for one hour at ambient pressure. The dried solids were analyzed by XPRD. The XPRD patterns are given in Fig. 15 for the dried solids from the methyl ethyl ketone crystallization with 3% doping of diepoxide (a) without grinding of the solvate prior to drying, and (b) with grinding of the solvate prior to drying. The XPRD patterns indicated a greater amount of Form I in the ground sample relative to the unground sample. These results suggest that the conditions under which the methyl ethyl ketone solvate is isolated and handled can affect the crystal form that results from the desolvation.

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B. 11,12-Epoxide Results

Fig. 16 shows the X-ray powder diffraction patterns for the wet cake (methyl ethyl ketone solvate) obtained from the (a) 0%, (b) 1%, (c) 5%, and (d) 10% 11,12-epoxide-doped methyl ethyl ketone crystallizations. The peak intensities have been normalized for ease of comparison. No peaks characteristic of Form I or the 11,12-epoxide are present in the diffraction

patterns. The patterns are characteristic of the methyl ethyl ketone solvate of eplerenone.

Fig. 17 shows the X-ray powder diffraction patterns for the dried solids obtained from the (a) 0%, (b) 1%, (c) 5%, and (d) 10% 11,12-epoxide-doped methyl ethyl ketone crystallizations. The peak intensities have been normalized for ease of comparison. No Form I was detected for the dried samples corresponding to the methyl ethyl ketone crystallizations performed at doping levels of 0, 1% and 5%. Form I was detected in the dried samples corresponding to the methyl ethyl ketone crystallization performed at a doping level of 10%. The area for the Form I diffraction peak at 12.1 degrees two theta and estimated Form I content for each sample are given in Table X-6D.

TABLE X-6D

Weight Percent 11,12-Epoxide in Starting Eplerenone Mixture	Weight Percent 11,12-Epoxide in Resulting Crystals (HPLC)	Form I Peak Area 12° Two Theta Peak	Estimated Weight Percent of Form I
0%	Not Available	None Detected	None Detected
1%	Not Available	None Detected	None Detected
5%	Not Available	None Detected	None Detected
10%	Not Available	1541	10-15%

The results reported in Table X-6D confirm that the presence of the 11,12-epoxide impacts the formation of Form I during the desolvation. The percentage of impurity in the methyl ethyl ketone crystallization required to induce the formation of Form I eplerenone appears to be greater for the 11,12-epoxide than for the diepoxide.

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Example 7: Effect of Crystallization and Drying on Final Crystal Form

The following four experiments analyzing the effect of crystallization and drying on the final crystal form were conducted: (i) methyl ethyl ketone crystallization of eplerenone (2^3+3 statistical design of experiment), (ii) crystallization of poor quality mother liquor residue, (iii) crystallization of high purity eplerenone with form I seeding, and (iv) crystallization of low purity eplerenone with form II seeding. Variables in the design of the experiments included cooling rate, starting material purity level, and end point temperature of crystallization. For purposes of this Example, high purity eplerenone was defined as ultra-pure milled epleranone (HPLC analysis showed this material to be 100.8% pure) and low purity eplerenone was defined as 89% pure eplerenone. To prepare the low purity eplerenone, stripped-down mother liquors from the process for the preparation of epleranone were analyzed and blended to yield a material that was 61.1% eplerenone, 12.8% diepoxide and 7.6% 11,12-epoxide. This material was then blended with a sufficient amount of high purity eplerenone to yield the 89% eplerenone.

A. Methyl Ethyl Ketone Crystallization

In the methyl ethyl ketone crystallization experiment, all runs were performed using 60 g of high purity eplerenone. High endpoint was defined as 45°C and low endpoint was defined as 5°C. High cooling rate was defined as 3°C/minute cooling and low cooling rate was defined as 0.1°C/minute cooling. Center points were 1.5°C/minute cooling, 94.5% pure eplerenone, and a 25°C endpoint.

After a background reading was taken with the FTIR, 250 mL of methyl ethyl ketone was charged to a 1L Mettler RC-1, MP10 reactor and stirred at 100 rpm. After several scans, eplerenone was charged to the reactor followed by an additional 470 mL of methyl ethyl ketone. Agitation was increased to 500 rpm to suspend solids and the batch temperature was increased to 80°C. The batch temperature was held at 80°C to ensure dissolution of the eplerenone. Black or white specks generally were visible in the resulting transparent solution. The batch temperature was then ramp cooled at the desired rate to the desired endpoint, where it was maintained for one hour before being pulled into a transfer flask and filtered. The vacuum was reactor, transfer flask and cake were then washed with 120 mL methyl ethyl ketone. Once the wash was pulled through the cake, the stopped. About 10 grams of each wet cake were dried in a vacuum oven under nominal conditions of 75°C with a light nitrogen bleed. For the "high, high, high" and "low, low, low" experiments described below, fluid bed drying was operated under high and low conditions. High fluid bed drying was defined as 100°C with a blower setting of "4" while low fluid bed drying was defined as 40°C with a blower setting of "1".

B. Crystallization of Poor Quality Mother Liquor Residue

In the crystallization of poor quality mother liquor residue experiment, 60 g of the 61.1% pure material and 720 mL methyl ethyl ketonewere charged directly to a 1L Mettler RC-1, MP10 reactor. The 61.1% pure material was not blended with high purity eplerenone prior to being charged to the reactor. The resulting mixture was heated to 80°C and was an opaque

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slurry at that temperature. The crystallization continued and the mixture was filtered at 45°C under fast cooling conditions.

C. Form I Seeding

In the Form I seeding experiment, 60 g of pure (100.8%) eplerenone and 720 mL of methyl ethyl ketone were charged to a 1L Mettler RC-1, MP10 reactor. The mixture was heated to 80°C and then cooled to 25°C at a rate of 1.5° C/minute. When the solution had cooled to 62°C, it was seeded with 3 g of phase pure Form I crystals to initiate crystallization. The Form I seed crystals were prepared by the digestion process described in Example 9 below.

D. Form II Seeding

In the Form II seeding experiment, 66.6 g of 89.3% eplerenone (prepared by mixing 48.3 g of 100% eplerenone with 18.3 g of 61.1% eplerenone) and 720 mL of methyl ethyl ketone were charged to a 1L Mettler RC-1, MP10 reactor. The mixture was heated to 80°C and then cooled to 25°C at a rate of 1.5° C/minute. When the solution had cooled to 63°C, it was seeded with 3 g of phase pure Form II crystals to initiate crystallization. The Form II seed crystals were prepared by the crystallization and desolvation process described in Example 1 above.

Results from the experiments are reported in Table X-7A. In the n+1 crystallization experiment, Form I was detected only in the experiments employing low purity eplerenone where the product contained the diepoxide. Elevated levels of the diepoxide in the final product were also observed with higher cooling rates.

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The crystallization of poor quality mother liquor residue experiment yielded poor quality material that appeared to be a mixture of the diepoxide and Form I when analyzed by X-ray powder diffraction.

The Form I seeding experiment (where high purity eplerenone was seeded with Form I) yielded a product that was 77% Form I based on X-ray powder diffraction analysis, but entirely Form I based on DSC. The X-ray powder diffraction model, however, had not been tested for linearity beyond about 15% Form I. This experiment was the only one of the four experiments of this Example where Form I was created in the absence of the diepoxide.

The Form II seeding experiment (where low purity eplerenone was seeded with Form II) yielded a product that was entirely Form II.

The data obtained for the high fluid bed drying of eplerenone appeared to correspond to the data obtained for the vacuum oven drying. The low fluid bed dryings yielded results that differed from those of the vacuum oven dryings.

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TABLE X 7A

Cooling Rate[1]	Cooling Endpoint[2]	Impurity Level	Nucleation Temperature (°C)	Weight Percent 11 12 Epoxide	Weight Percent Diepoxide[4]	Assay For Desolvated Crystal	Percent Yield	Weight Percent Form I (XPRD)
+	+		57 0	ND	ND	100 3	66 1	ND
+			54 9	ND	ND	100 3	98 1	ND
	+		60 9	ND	ND	100 3		ND
			63 4	ND	ND	100 5	79 3	ND
+	+	++	N/A	4 8	36 6	43 3	27	100[5]
+	+	+	52 2	0 49	0 88	98 3	62	29
+		+	53 3	0 56	1 0	98 1	87	9
0	0	0	59 0	0 18	0 36	99 4	75	5
	+	+	63 3	0 20	0 44	99 4	36	31
		+	61 4	0 18	0 40	99 5	87	ND
0	0	0	60 6	0 18	0 36	99 5	79 2	ND
0	0	0	55 9	0 38	0 80	98 6	80 5	<3%
0	0	100 8% eplerenone seeded with Form I		0 03	ND	100 4	82 2	77/100[5]
0	0	89 3% eplerenone seeded with Form II		0 33	0 50	97 5	80 2	ND

[1]Cooling Rate (+) = 3°C/min (0) = 1 5 C/min ; and ( ) = 0 1 °C/min

[2]Cooling Endpoint (+) = 45°C; (0) = 25 °C; and ( ) = 5°C

Impurity Level (+) = 89 3% purity eplerenone starting material (++) = 61 1% purity eplerenone starting material (0) = 100 8% purity eplerenone starting material; and ( ) = 94 5% purity eplerenone starting material

[4]Weight percent after drying solvate in a vacuum oven at 75 C

Appears to be mixture of Form I and diepoxide when analysed by XPRD

[5]Appears to be 77% Form I when analysed by XPRD and 100% Form I when analysed by DSC

A. Material Purity

A cube plot of product purity, starting material purity, cooling rate and endpoint temperature based on the data reported in Table X-7A is shown in Fig. 18. The cube plot suggests that the use of a higher purity material at the start of crystallization will yield a higher purity product. The endpoint temperature of crystallization does not appear to greatly affect the product purity. The cooling rate, however, appears to have an effect with slightly less pure product resulting from a faster cooling rate. In fact, the level of diepoxide generally was higher with faster cooling rates.

Fig. 19 shows a half normal plot that was prepared using the results of cube plot to determine which variables, if any, had a statistically significant effect on the product purity. Starting material purity had the greatest statistically significant effect on product purity, although cooling rate and the interaction between cooling rate and starting material purity were also seen as statistically significant effects.

Fig. 20 is an interaction graph based on these results and showing the interaction between starting material purity and cooling rate on product purity. With the high purity eplerenone (100.8% eplerenone starting material) the cooling rate appears to have little or no effect on final purity. With the low purity eplerenone (89.3% eplerenone starting material), however, the product purity decreases as cooling rate increases. This result suggests that more impurities crystallize out in eplerenone crystallizations conducted at higher cooling rates.

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B. Form I Content

A cube plot of Form I weight fraction, starting material product purity, cooling rate and endpoint temperature based on the data reported in Table X-7A is shown in Fig. 21. The cube plot suggests that the use of a higher purity eplerenone at the start of crystallization will yield a lower amount of Form I. The endpoint temperature of crystallization also appears to have an effect on the form of the final product. The cooling rate does not appear to greatly affect the formation of Form I although some Form I may result from faster cooling at the low endpoint temperature in the presence of impurities.

Fig. 22 shows a half normal plot that was prepared using the results of the cube plot to determine which variables, if any, had a statistically significant effect on the amount of Form I in the final material. Starting material purity, endpoint temperature of the crystallization and the interaction between the two variables were seen as statistically significant effects.

Fig. 23 is an interaction graph based on these results and showing the interaction between starting material purity and endpoint temperature on final Form I content. With the high purity eplerenone (100.8% eplerenone starting material), endpoint temperature appears to have little effect on Form I content. No Form I resulted in either case with pure eplerenone. With low purity eplerenone (89.3% eplerenone starting material), however, Form I was present in both cases, with significantly more Form I at higher endpoint temperatures.

Table X-7B reports the weight fraction of Form I measured in materials dried using either a fluid bed (LAB-LINE/P.R.L.

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Hi-Speed Fluid Bed Dryer, Lab-Line Instruments, Inc.) or a vacuum oven (Baxter Scientific Products Vacuum Drying Oven, Model DP-32). Similar Form I content was observed for comparable materials dried in either the high fluid bed or the vacuum oven. A difference was observed, however, for comparable materials dried in the low fluid bed relative to the vacuum oven.

TABLE X-7B

Cooling Rate	End Point	Impurity Level	Drying Type	Weight Percent Form I
High	High	High	Vacuum Oven	29%
High	High	High	High Fluid Bed	25%
High	High	High	Low Fluid Bed	4.7%
Low	Low	Low	Vacuum Oven	ND
Low	Low	Low	High Fluid Bed	ND
Low	Low	Low	Low Fluid Bed	5.5%

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Example 8: Crystallization of a Mixture of Form I and Form II From Methyl Ethyl Ketone To Prepare a Solvate, and (b) Desolvation of the Solvate to Prepare Form II

Form I eplerenone (10 g) was combined with 80 mL of methyl ethyl ketone. The mixture was heated to reflux (79°C) and stirred at this temperature for about 30 minutes. The resulting slurry was then cooled with a stepwise, holdpoint protocol by maintaining the slurry at 65°C, 50°C, 35°C and 25°C for about 90 minutes at each temperature. The slurry was filtered and rinsed with about 20 mL methyl ethyl ketone. The isolated solid was initially dried on the filter and then in a vacuum oven at 40-50°C. The drying was completed in the vacuum oven

at 90-100°C. The desolvated solid was obtained with an 82% recovery. XPRD, MIR and DSC confirmed that the solid had a Form II crystalline structure.

Example 9: Digestion of Low Purity Eplerenone Starting Material With a Solvent to Prepare Form I

A. Digestion With Ethanol Solvent:

Low purity eplerenone (24.6 g; 64% by weight assay via HPLC) was combined with 126 mL of ethanol 3A. The slurry was heated to reflux and the distillate removed. An additional 126 mL of ethanol 3A was simultaneously added as 126 ml of solvent was removed via atmospheric distillation. Upon completion of the solvent turnover, the mixture was cooled to 25 °C and stirred for one hour. The solid was filtered and rinsed with ethanol 3A. The solid was air-dried to give the ethanol solvate. The solvate was further dried in a vacuum oven at 90-100°C for six hours to obtain 14.9 g of Form I eplerenone.

B. Digestion With Methyl Ethyl Ketone Solvent

In an alternative digestion process, 1 gram of low purity eplerenone (about 65% pure) was digested in 4 mL of methyl ethyl ketone for two hours. After the two hours, the mixture was allowed to cool to room temperature. Once cooled, the solid was collected by vacuum filtration and determined to be the methyl ethyl ketone solvate by XPRD analysis. The solid was dried at 100°C for 30 to 60 minutes. The dried solids were determined to be pure Form I by XPRD.

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Example 10: Digestion of High Purity Eplerenone Starting Material With a Solvent to Prepare Form II

A. Digestion With Ethanol Solvent:

High purity eplerenone (1 gram) was digested in 8 mL of ethanol for approximately two hours. The solution was then allowed to cool to room temperature and the solids were collected by vacuum filtration. Analysis of the solids by XPRD immediately after filtration indicated that the solids were a solvate (presumably the ethanol solvate). The solids were subsequently dried at 100°C at atmospheric pressure for 30 minutes. The dried solid was analyzed by XPRD and determined to be predominately Form II (no Form I detected).

B. Digestion with Methyl Ethyl Ketone Solvent:

High purity eplerenone (1 gram) was digested in 4 mL of methyl ethyl ketone for two hours. After the two hours, the solution was allowed to cool to room temperature and the solids collected by vacuum filtration. The solid was immediately analyzed by XPRD and determined to be a solvate of eplerenone (presumably the methyl ethyl ketone solvate). The solvate was subsequently dried at 100°C at ambient pressure for 30 to 60 minutes. The dried solids were analyzed by XPRD and determined to be primarily Form II with no diffraction peaks for Form I present.

Example 11: Crystallization of Form II Directly From Solution

Procedure A: Eplerenone (2.5 g) was dissolved in ethyl acetate by heating to 75°C. Once the eplerenone dissolved,

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the solution was held at 75°C for 30 minutes to ensure complete dissolution. The solution was then cooled at 1 °C/min to 13°C. Once at 13°C, the slurry was allowed to stir for two hours at 750 rpm with an overhead stirrer. The crystals were collected by vacuum filtration and dried in a vacuum oven at 40°C for one hour. The XPRD pattern and DSC thermogram of the solid were characteristic of Form II eplerenone. Thermal gravimetric analysis (TGA) of the solid indicated no weight loss from the solid up to 200°C.

Procedure B: In an alternative procedure, 2 g of eplerenone was dissolved in 350 mL of 15/85% acetonitrile/water by heating on a hot plate with magnetic stirring. Once the eplerenone was dissolved, the solution was allowed to cool to room temperature overnight with magnetic stirring. The resulting solid was collected by vacuum filtration. The crystals were birefringent and had a triangular, plate-like crystal habit. The solid had an XPRD and DSC characteristic of Form II eplerenone. TGA indicated no weight loss up to 200°C.

Procedure C: In an alternative procedure, 640 mg of eplerenone was placed in a 50 mL flask with 20 mL of ethyl benzene. The resulting slurry was heated to 116°C and became a clear solution. The clear solution was cooled to 25°C over 30 minutes. Nucleation began at 84°C during the cooling period. The resulting solids were filtered from the solution and air-dried to give 530 mg of solids (83% recovery). Hot-stage microscopy and XPRD confirmed that the solids were Form II crystals.

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Procedure D: In an alternative procedure, 1.55 g of eplerenone was added to 2.0 mL of nitrobenzene and heated to 200°C. The resulting slurry was stirred overnight at 200°C. The solution was allowed to cool to room temperature (natural air convection) the following day and the solid was isolated. The solid was determined to be Form II eplerenone by XPRD and polarized light microscopy.

Procedure E: In an alternative procedure, 5.0 g of eplerenone (purity greater than 99%) was added to 82 g of methanol (104 mL). Under stirring action (210 rpm), the solution was heated to 60°C and held at that temperature for 20 minutes to ensure complete dissolution. The solution was then cooled to -5°C at a rate of 0.16°C/minute under stirring. The crystals were collected by filtration and dried in a vacuum oven at 40°C for 20 hours. The dried solids were determined to be pure Form II eplerenone by DSC and XPRD analysis.

Procedure F: In an alternative procedure, 6.0 g of eplerenone (ethanol solvate containing 9% ethanol and having a corrected purity of 95.2%) was added to 82 g of methanol (104 mL). Under stirring action (210 rpm), the solution was heated to 60°C and held at that temperature for 20 minutes to ensure complete dissolution. The solution was then cooled to 50°C at a rate of 0.14°C/minute and then held at that temperature for about 2.5 hours. The solution was then cooled to -5°C at a rate of 0.13°C/minute under stirring. The crystals were collected by filtration and dried in a vacuum

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oven at 40°C for 16 hours. The dried solids were determined to be pure Form II eplerenone by DSC and XPRD analysis.

Example 12: Crystallization of Form I Directly From Solution

150.5 mg of the diepoxide and 2.85 g of eplerenone were added to 1.5 mL of nitrobenzene. The mixture was magnetically stirred at 200°C for several hours. The slurry was then allowed to cool to room temperature by natural air convection. The sample was dried and analyzed by polarized light microscopy and XPRD. The XPRD indicated that the sample was a mixture of Form I and Form II. The crystals were translucent by microscopy, indicating that desolvation (and conversion to either Form I or Form II) did not occur.

Example 13: Preparation of Amorphous Eplerenone By Comminution

Approximately one-half of a steel Wig-L-Bug container was filled with about 60 g of eplerenone (greater than 99.9% purity). A steel ball and cap were placed on the sample container and agitated for 30 seconds by the Wig-L-Bug apparatus. The eplerenone was scraped off the surface of the Wig-L-Bug container and the container agitated for an additional 30 seconds. The resulting solid was analyzed by XPRD and DSC and determined to be a mixture of amorphous eplerenone and Form II crystalline eplerenone.

Example 14: Preparation of Amorphous By Lyophilization

Approximately 100 mg of crude eplerenone was weighed into a beaker containing 400 mL of water. The solution was heated slightly for five minutes, and then sonicated and heated with stirring for an additional five minutes.

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Approximately 350 mL of the eplerenone solution was filtered into a 1000 mL round bottom flask containing 50 mL of HPLC water. The solution was flashed frozen in a dry ice/acetone bath over a time period of one to two minutes. The flask was attached to a Labconco Freezone 4.5 freeze dryer and dried overnight. The solids in the flask were transferred to a small brown bottle. A small aliquot was observed under polarized light microscopy at 10X, 1.25X optivar in cargille oil (1.404) and observed to be at least 95% amorphous eplerenone. Figures 24 and 25 show the XPRD pattern and DSC thermogram obtained for the amorphous eplerenone. The peak observed at 39 degrees two theta in Figure 24 is attributable to the aluminum sample container.

Example 15: Measurement of Form II Solubility

The aqueous solubility of Form II eplerenone was measured at pH 7 (100 mM phosphate buffer) at 5, 25, and 40°C. Approximately 30 mg of Form II eplerenone was mixed with approximately 10 mL of buffer to form a slurry of eplerenone at both 5 and 25 °C. Approximately 40 mg of Form II eplerenone was mixed with approximately 10 mL of buffer to form a slurry of eplerenone at 40 °C. Samples were prepared in duplicate for each condition. The slurries were allowed to equilibrate in appropriate temperature water shaker baths and the solutions were analyzed for eplerenone content by ultraviolet visible analysis (245 nm) at the 1, 5, 12, 19, 27, and 36 day time intervals. Data from each condition was appropriately averaged to determine the solubility of eplerenone at each condition and is reported in Table X-15. The residual solids from each time point were analyzed by DSC and TGA at the end of the 36 day equilibration and were

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determined to be Form II.

TABLE X-15

Temperature (°C)	Form II Solubility
5	0.24 mg/mL
25	0.29 mg/mL
40	0.39 mg/mL

Example 16: Measurement of Intrinsic Dissolution Rates

Intrinsic dissolution rates were measured for the following four eplerenone polymorph samples: (i) Form II eplerenone prepared by direct crystallization from acetonitrile using water as an anti-solvent in the same manner as Example 12, Procedure B, (ii) Form I eplerenone prepared by digestion in ethanol in the same manner as Example 10, Procedure A, (iii) a mixture of Form I (5 weight percent) and Form II (95 weight percent), and (iv) Form II eplerenone that was micronized to provide the following particle size distribution: 10% of the particles under 9 microns, 50% of the particles under 22 microns, and 90% of the particles under 41 microns.

Eplerenone (150 mg) was weighed and placed into a VanKel intrinsic dissolution cavity. The powder was compressed at 1200 psi using a Carver press. The sample was then mounted onto the intrinsic dissolution apparatus. The dissolution media used was 1% SDS in HPLC grade water. All experiments were conducted at 37°C for two hours. Before the start of the experiment, 500 mL of the dissolution medium was equilibrated at 37°C for 30 minutes in the dissolution bathing chamber. An initial sample was taken from each dissolution vessel which served as time zero for the experiment. The eplerenone

tablets were then lowered into the dissolution medium. Samples were drawn at determined intervals for the determination of rate of dissolution. Care was taken to avoid air bubbles forming at the surface of the tablet. The samples were analyzed by UV absorbance detection at 243 nm. Intrinsic dissolution rates were calculated from the slope of the linear portion of the concentration versus time profiles corrected for volume and normalized for the surface area of the dissolution tablet (0.5 cm^2).

Fig. 26 reports the intrinsic dissolution rates measured for the four samples. These studies appear to indicate that Form I eplerenone has faster intrinsic dissolution rate than Form II eplerenone. X-Ray powder diffraction measurements comparing compressed against uncompressed eplerenone confirmed that the polymorphs did not interconvert upon compression or during the course of the dissolution studies.

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Example 17: Eplerenone Polymorph Composition

Tablets containing 25 mg, 50 mg, 100 mg and 200 mg doses of Form II eplerenone are prepared and have the following composition:

Ingredient	Weight % of Tablet
Form II Eplerenone	29.41
Form I Eplerenone	Not Detected
Lactose Monohydrate (#310, NF)	42.00
Microcrystalline Cellulose (NF, Avicel PH101)	18.09
Croscarmellose Sodium (NF, Ac-Di-Sol)	5.00
Hydroxypropyl Methylcellulose (#2910, USP, Pharmacoat 603)	3.00
Sodium Lauryl Sulfate (NF)	1.00
Talc (USP)	1.00
Magnesium Stearate (NF)	0.5
Total	100.00

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Example 18: Eplerenone Polymorph Composition

Capsules (hard gelatin capsule, #0) are prepared containing a 100 mg dose of eplerenone and have the following composition:

Ingredient	Amount (mg)
Form II Eplerenone	90.0
Form I Eplerenone	10.0
Lactose, Hydrous, NF	231.4
Microcrystalline Cellulose, NF	45.4
Talc, USP	10.0
Croscarmellose Sodium, NF	8.0
Sodium Lauryl Sulfate, NF	2.0
Colloidal Silicon Dioxide, NF	2.0
Magnesium Stearate, NF	1.2
Total Capsule Fill Weight	400.0

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Example 19: Eplerenone Polymorph Composition

Capsules (hard gelatin capsule, size #0) are prepared containing a 200 mg dose of eplerenone and have the following composition:

Ingredient	Amount (mg)
Form II Eplerenone	190.0
Form I Eplerenone	10.0
Lactose, Hydrous, NF	147.8
Microcrystalline Cellulose, NF	29.0
Talc, USP	10.0
Croscarmellose Sodium, NF	8.0
Sodium Lauryl Sulfate, NF	2.0
Colloidal Silicon Dioxide, NF	2.0
Magnesium Stearate, NF	1.2
Total Capsule Fill Weight	400.0

Example 20: Preparation of Milled Eplerenone

Dried methyl ethyl ketone solvate is first delumped by passing the solvate through a 20 mesh screen on a Fitzmill. The delumped solid is then pin milled using an Alpine Hosakawa stud disk pin mill operating under liquid nitrogen cooling at a feed rate of approximately 250 kilograms/hour. Pin milling produces milled eplerenone with a D_{90} size of approximately 65-100 microns.

Example 21: Effect of Eplerenone Particle Size (Dog Study)

The effect of the particle size of Form II eplerenone on eplerenone plasma concentrations and relative bioavailability was studied in a dog model. Four heathy female beagle dogs

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weighing between 8 to 12 kg were intragastrically administered one immediate release capsule containing the formulation described in Table X-21A below followed by about 10 mL of water.

Table X-21A

INGREDIENT	WEIGHT % OF TABLET	Amount (mg)
Eplerenone	50.00	200.00
Lactose, Fast-Flo, Hydrous	36.95	147.80
Microcrystalline Cellulose (Avicel PH102)	7.25	29.00
Sodium Lauryl Sulfate	0.50	2.00
Croscarmellose Sodium	2.00	8.00
Talc	2.50	10.00
Colloidal Silicon Dioxide	0.50	2.00
Magnesium Stearate	0.30	1.20
Total	100.00	400.00
Capsules, Size #0, White Opaque	1 Capsule	

The dogs were fasted for 15 to 20 hours prior to administration of the capsule and were not fed again until at least 4 hours after dose administration. Blood samples (approximately 3 mL) were collected by venipuncture in chilled tubes containing heparin at 0, 0.5, 1, 2, 3, 4, 6, 8 and 24 hours after dose administration. The blood samples were immediately placed on ice. Separation of plasma from the blood samples was complete after about 15 minutes of centrifugation. The resulting plasma samples were frozen at about -20°C and stored until analyzed. Analysis was performed using an LC/MS/MS procedure.

This experiment was conducted using the same four dogs

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for three formulations that were identical except for the particle size of the eplerenone starting material. The three formulations tested used eplerenone starting materials having a D_{90} particle size (that is, at least 90% of the particles were) less than about 212 microns, less than about 86 microns, and less than about 36 microns, respectively. A minimum of a five day wash-out period was allowed between administration of each of the formulations. Reducing the D_{90} particle size of the eplerenone starting material from about 212 microns to above 86 microns increased relative bioavailability by almost 100%. The mean results are reported in Tables X-21B and X-21C below.

Table X-21B

Time (hours)	Blood Serum Eplerenone Concentration (μg/mL)		
	D_{90} = 212 microns	D_{90} = 86 microns	D_{90} = 36 microns
0	0	0	0
0.5	1.83	3.65	1.99
1.0	2.40	6.18	5.86
2.0	3.77	6.89	6.77
3.0	2.85	5.70	6.60
4.0	2.61	4.39	5.56
6.0	1.63	3.11	3.31
8.0	1.10	1.90	2.09
24.0	0.0252	0.032	0.0706

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Table X-21C

Pharmacokinetic Parameter	Pharmacokinetic Parameter Value		
	D_{90} = 212 microns	D_{90} = 86 microns	D_{90} = 36 microns
C_{max} (μg/mL)	3.98	7.02	7.39
T_{max} (hours)	1.50	1.75	2.25
AUC ((μg/mL)hr)	26.6	49.2	53.1
Relative Bioavailability (%)	53.25	100	107.9

Example 22: Effect of Eplerenone Particle Size (Human Study)

The effect of the particle size of a Form II eplerenone starting material used in a pharmaceutical composition on eplerenone plasma concentrations and relative bioavailability was studied in a human model. Subjects received single doses of Form II eplerenone medication on Days 1, 8, 15, 22 and 29 according to a randomization schedule. All medication was administered with 180 mL of water at 0800 hours. Blood samples for eplerenone pharmacokinetic analyses were collected at -0.5 (predose), 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 16, 24, 36 and 48 hours postdose. Urine samples were collected and pooled between the hours of 0-24 and 24-48 hour postdose.

Plasma and urinary concentrations of eplerenone were determined using a validated high performance liquid chromatography method with MS/MS Detection. Pharmacokinetic data obtained is reported in Table X-23A. The particle size distributions of the eplerenone used in the preparation of the medications was determined in the dry powder state using laser light scattering.

Table X-23A

Pharmacokinetic Parameter	Pharmacokinetic Parameter Value		
	100 mg Capsule A (D_{90}=40 microns)	100 mg Tablet (D_{90}=82 microns)	100 mg Capsule B (D_{90}=96 microns)
C_{max} (ng/mL)	1747	1704	1669
T_{max} (hours)	1.8	1.8	1.3
AUC ((ng/mL)hr)	11349	11945	11981

The specific composition of each medication is reported in Table X-23B below.

Table X-23B

INGREDIENT	WEIGHT % OF TABLET/CAPSULE		
	Capsule A	Tablet A	Capsule B
Form II Eplerenone	25	30	25
Lactose Monohydrate	–	42	57.86 (fast flo lactose)
Lactose, Hydrous NF	57.8	–	–
Microcrystalline Cellulose (Avicel PH101)	11.4	17.5 (7.5% intragranular plus 10% extragranular)	11.34 (Avicel PH 102)
Croscarmellose Sodium (Ac-Di-Sol)	2	5	2
Hydroxypropyl Methylcellulose (Pharmacoat 603)	–	3	–
Sodium Lauryl Sulfate	0.5	1	0.5
Talc	2.5	1	2.5
Magnesium Stearate	0.3	0.5	0.3
Colloidal Silicon Dioxide	0.5	–	0.5
Total	100	100	100

All mentioned references are expressly incorporated by reference herein as if fully set forth at length. In addition, U.S. Provisional Application Serial No. 60/111,646 filed December 9, 1998, and the corresponding nonprovisional application, U.S. Application Serial No. 09/_____ filed December 8, 1999, are expressly incorporated by reference herein as if fully set forth at length.

When introducing elements of the present invention or the preferred embodiment(s) thereof, the articles "a", "an", "the" and "said" are intended to mean that there are one or more of the elements. The terms "comprising", "including" and "having" are intended to be inclusive and mean that there may be additional elements other than the listed elements.

Although this invention has been described with respect to specific embodiments, the details of these embodiments are not to be construed as limitations.

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WHAT IS CLAIMED IS:

1. Particles comprising the Form II crystalline form of eplerenone, wherein at least 90% of said particles have a particle size less than about 15 microns.

2. The particles of Claim 1 wherein the crystalline form has an X-ray powder diffraction pattern with a peak at 8.0 ± 0.2 degrees two theta.

3. The particles of Claim 1 wherein the crystalline form has an X-ray powder diffraction pattern with peaks at 8.0 ± 0.2, 12.4 ± 0.2, 12.8 ± 0.2, and 13.3 ± 0.2 degrees two theta.

4. The particles of Claim 1 wherein the crystalline form has an X-ray powder diffraction pattern without a significant peak at 12.0 ± 0.2.

5. The particles of Claim 1 wherein the crystalline form has an X-ray powder diffraction pattern substantially as shown in Figure 1-B.

6. The particles of Claim 1 wherein the crystalline form has a melting range from about 223°C to about 234°C.

7. The particles of Claim 1 wherein the crystalline form has a melting range from about 237°C to about 242°C.

8. The particles of Claim 1 wherein the crystalline form has an IR spectrum with a peak at about 1724 cm^{-1}.

9. The particles of Claim 1 wherein the crystalline form has an IR spectrum without a significant peak at about 1739 cm^{-1}.

10. The particles of Claim 1 wherein the crystalline form has an IR spectrum without a significant peak at about 1399 cm^{-1}.

11. The particles of Claim 1 wherein the crystalline form has an IR spectrum substantially as shown in Figure 3-B.

12. The particles of Claim 1 wherein the crystalline form has a ^{13}C NMR spectrum with a carbon resonance at about 67.1 ppm.

13. The particles of Claim 1 wherein the crystalline form has a ^{13}C NMR spectrum with carbon resonances at about 16.0 ppm and about 67.1 ppm.

14. The particles of Claim 1 wherein the crystalline form has a ^{13}C NMR spectrum without a carbon resonance at about 24.7 ppm.

15. The particles of Claim 1 wherein the crystalline form has a ^{13}C NMR spectrum substantially as shown in Figure 5.

16. The particles of Claim 1 wherein the crystalline form has a melting range from about 223°C to about 234°C, and an IR spectrum with a peak at about 1724 cm^{-1}.

17. The particles of Claim 1 wherein the crystalline form has a melting range from about 223°C to about 234°C, and an IR spectrum with a peak at about 1724 cm^{-1} and without significant peaks at about 1399 cm^{-1} and about 1739 cm^{-1}..

18. The particles of Claim 1 wherein the crystalline form has a melting range from about 223°C to about 234°C; an IR spectrum with a peak at about 1724 cm^{-1} and without significant peaks at about 1399 cm^{-1} and about 1739 cm^{-1}; an X-ray powder diffraction pattern with a peak at 8.0 ± 0.2; and a ^{13}C NMR spectrum with a carbon resonance at about 67.1 ppm.

19. The particles of Claim 1 wherein the crystalline form has a melting range from about 237°C to about 242°C, and an IR spectrum with a peak at about 1724 cm^{-1}.

20. The particles of Claim 1 wherein the crystalline form has a melting range from about 237°C to about 242°C, and an IR spectrum with a peak at 1722 cm^{-1} and without a significant peak at 1739 cm^{-1}.

21. The particles of Claim 1 wherein the crystalline form has a melting range from about 237°C to about 242°C; an IR spectrum with a peak at about 1724 cm^{-1} and without significant peaks at about 1399 cm^{-1} and about 1739 cm^{-1}; an X-ray powder diffraction pattern with a peak at 8.0 ± 0.2; and a ^{13}C NMR spectrum with a carbon resonance at about 67.1 ppm.

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22. The particles of Claim 1 wherein the crystalline form has a monoclinic crystal system.

23. The particles of Claim 1 wherein the crystalline form has a $P2_1$ space group.

23. The particles of Claim 1 wherein the crystalline form has unit cell a, b and c values of about 8.78 Å, about 11.14 Å, and about 11.06 Å, respectively.

25. The particles of Claim 1 wherein the crystalline form has unit cell α, β and γ values of about 90°, about 90.52°, and about 90°, respectively.

26. Particles of Claim 1 wherein at least 90% of said particles have a particle size less than about 1 micron.

27. The particles of Claim 26 wherein at least 90% of said particles have a particle size less than about 800 nm.

28. The particles of Claim 26 wherein at least 90% of said particles have a particle size between about 10 nm to about 1 micron.

29. The particles of Claim 26 wherein at least 90% of said particles have a particle size between about 100 nm to about 800 nm.

30. The particles of Claim 26 wherein at least 90% of said particles have a particle size between about 200 nm to

about 800 nm.

31. The particles of Claim 26 wherein at least 90% of said particles have a particle size between about 400 nm to about 800 nm.

32. Eplerenone comprising the particles of Claim 1, wherein said eplerenone has a phase purity of at least about 50% Form II.

33. The eplerenone of Claim 32 wherein said eplerenone has a phase purity of at least about 90% Form II.

34. The eplerenone of Claim 32 wherein said eplerenone is substantially phase pure Form II eplerenone.

35. A pharmaceutical composition comprising a therapeutically-effective amount of the particles of any of Claims 1 to 35 in association with at least one pharmaceutically-acceptable carrier, adjuvant or diluent.

36. A composition of Claim 35 wherein the only eplerenone crystalline form present in the composition is Form II eplerenone.

37. A method of treating or preventing an aldosterone-mediated condition or disorder in a subject, said method comprising administering to a subject having or susceptible to said condition or disorder a therapeutically-effective amount of the particles of Claim 1.

1246
1207

38. The method of Claim 37 for use in treatment of hyperaldosteronism.

39. The method of Claim 37 for use in treatment of hypertension.

40. The method of Claim 37 for use in treatment of heart failure.

41. The method of Claim 37 for use in treatment of cirrhosis of the liver.

42. The method of Claim 37 for use in treatment of a condition selected from the group consisting of excess collagen/fibrosis, benign prostate hypertrophy and depression.

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ABSTRACT OF THE DISCLOSURE

Novel nanoparticles comprising the Form II crystalline form of eplerenone are described. These eplerenone nanoparticles have unique chemical and physical properties relative to other solid state forms of eplerenone and are characterized by their x-ray powder diffraction pattern, infrared spectrum, nuclear magnetic resonance spectrum, differential scanning calorimetric thermogram, and other physical characterizations.

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Figure 1-A

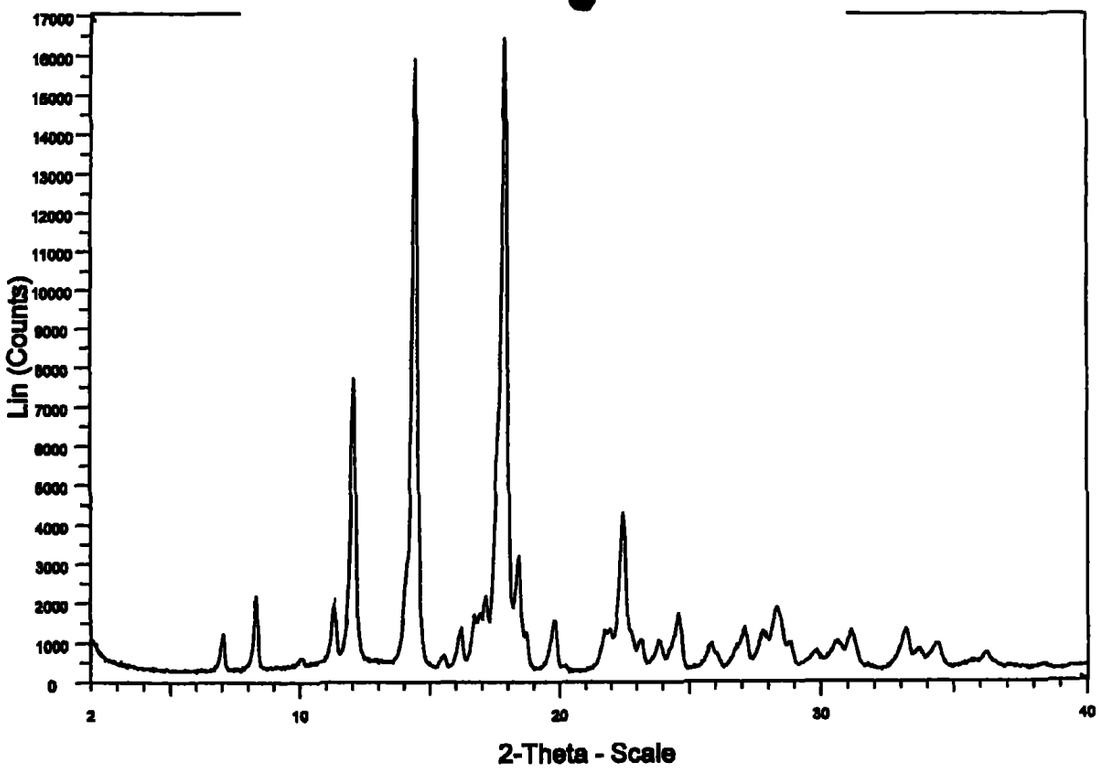
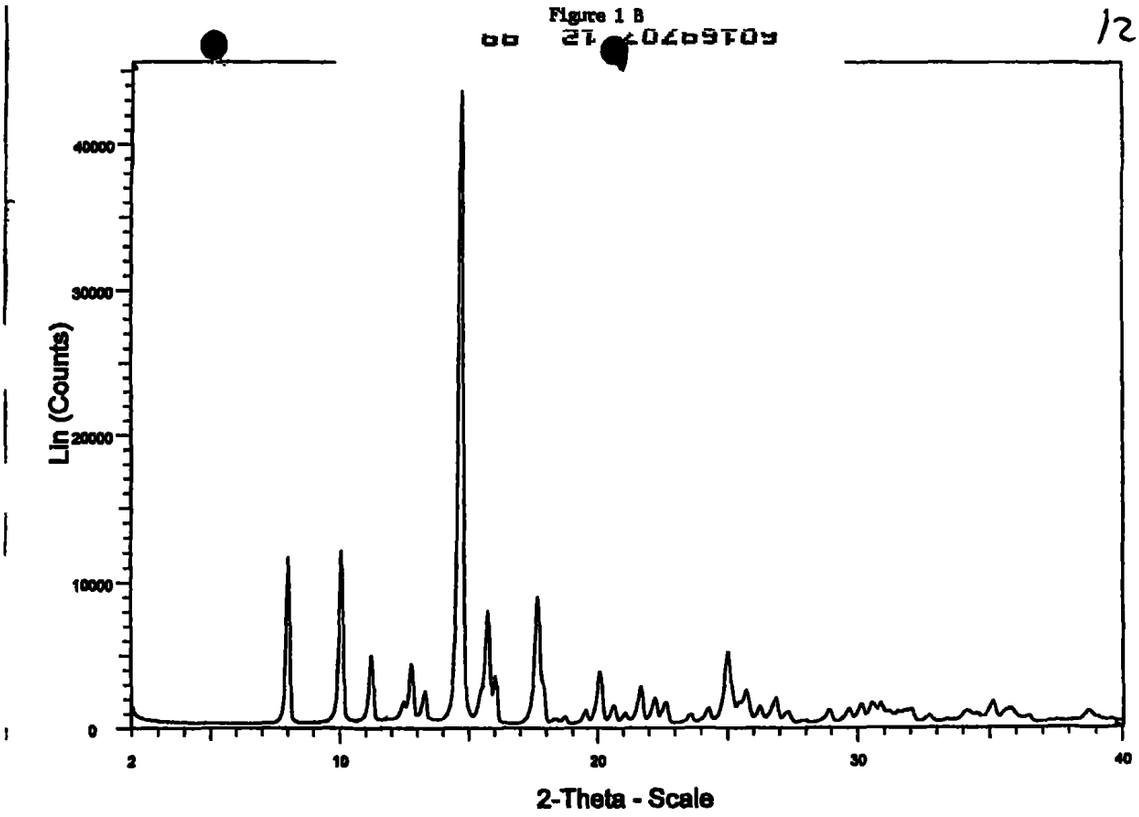

Lin (Counts)
17000
16000
15000
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
2
10
20
30
40
2-Theta - Scale

Figure 1 B


Lin (Counts)
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30000
20000
10000
0
2
10
20
30
40
2-Theta - Scale

Figure 1-C

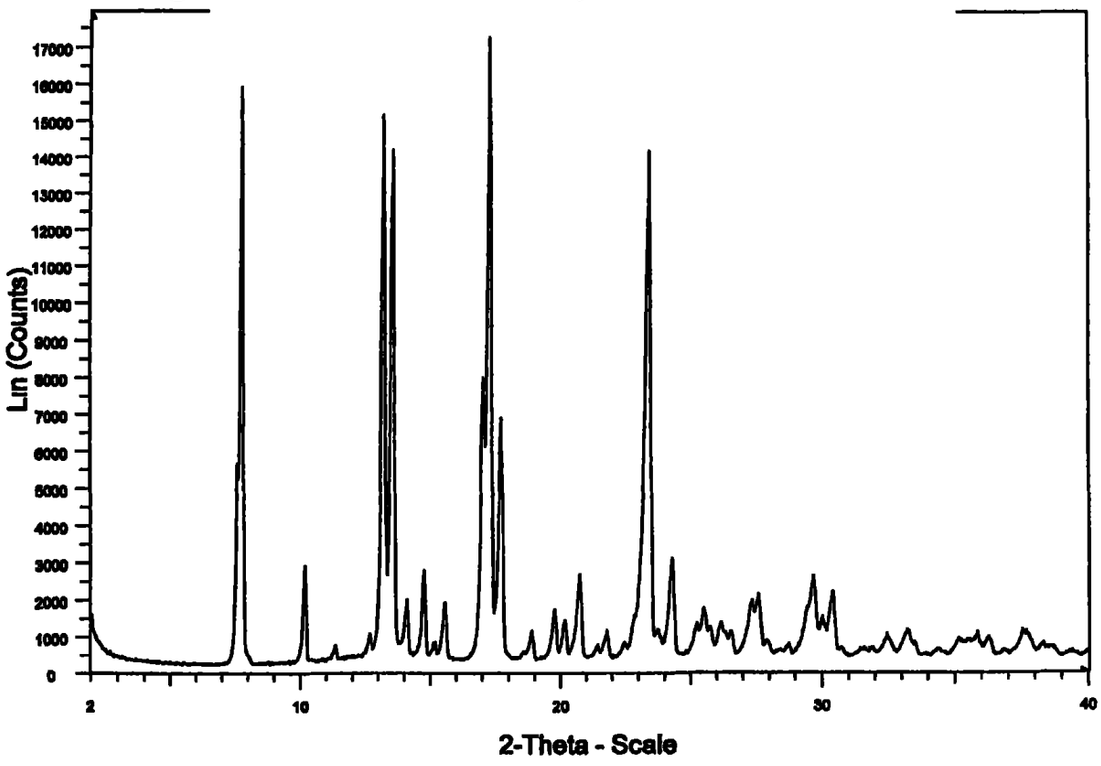

Lin (Counts)
17000
16000
15000
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
2
10
20
30
40
2-Theta - Scale

Figure 1-D

n-propyl alcohol solvate

INEL

1212

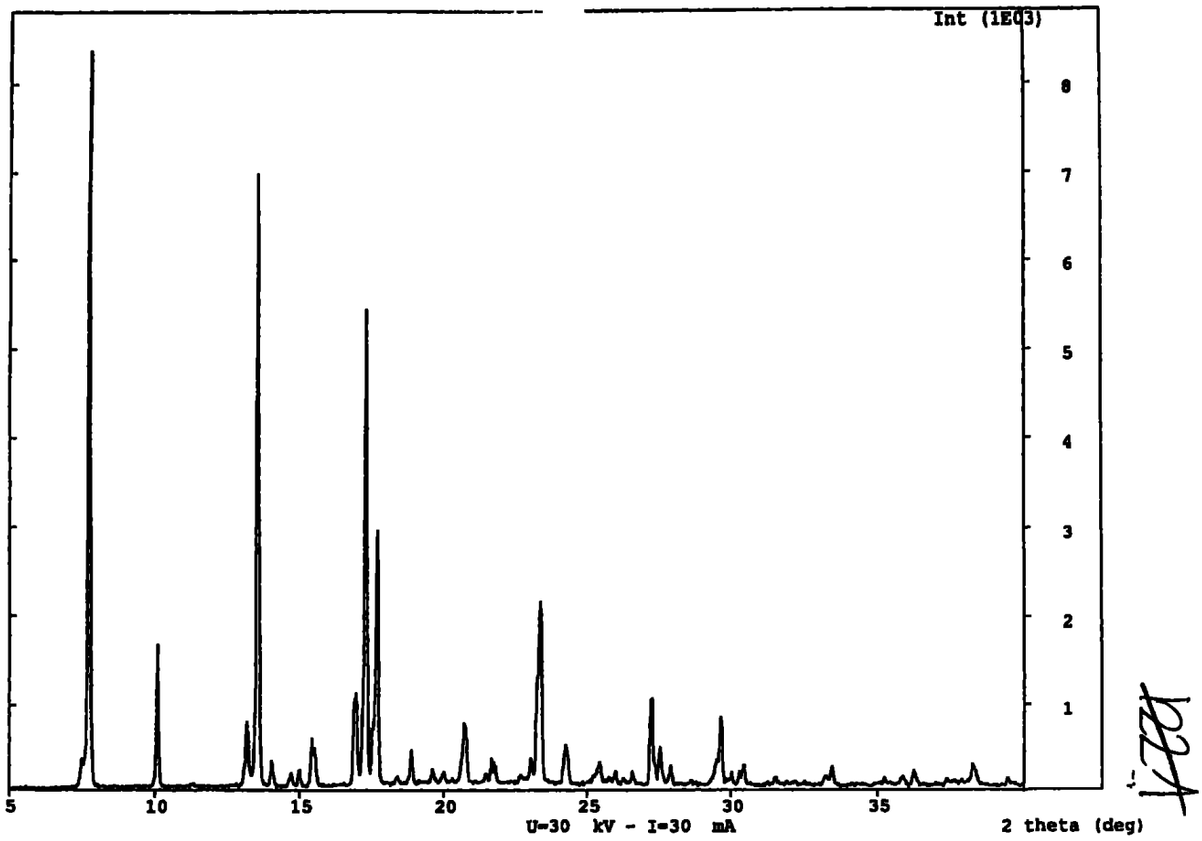


Figure 1-E

THF Solvate

INEL

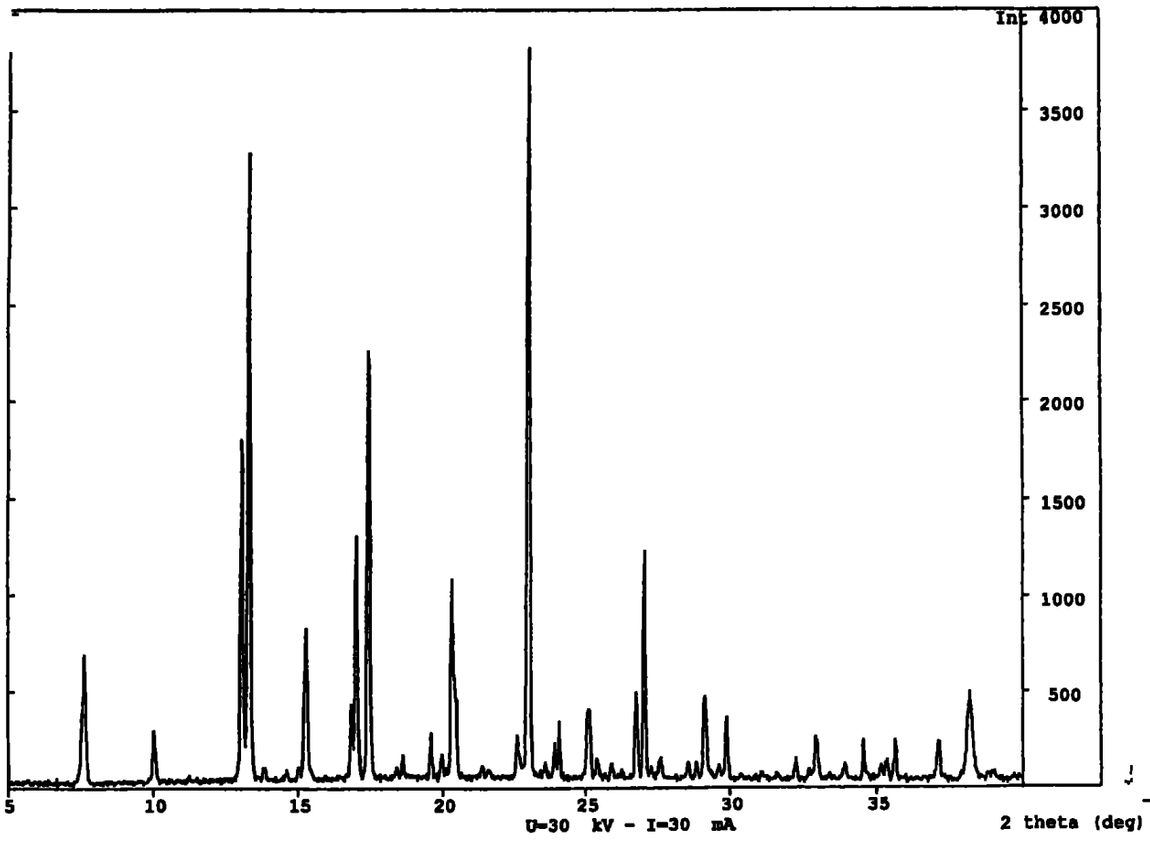


Figure 1 F

Ethyl Propionate solvate

INEL

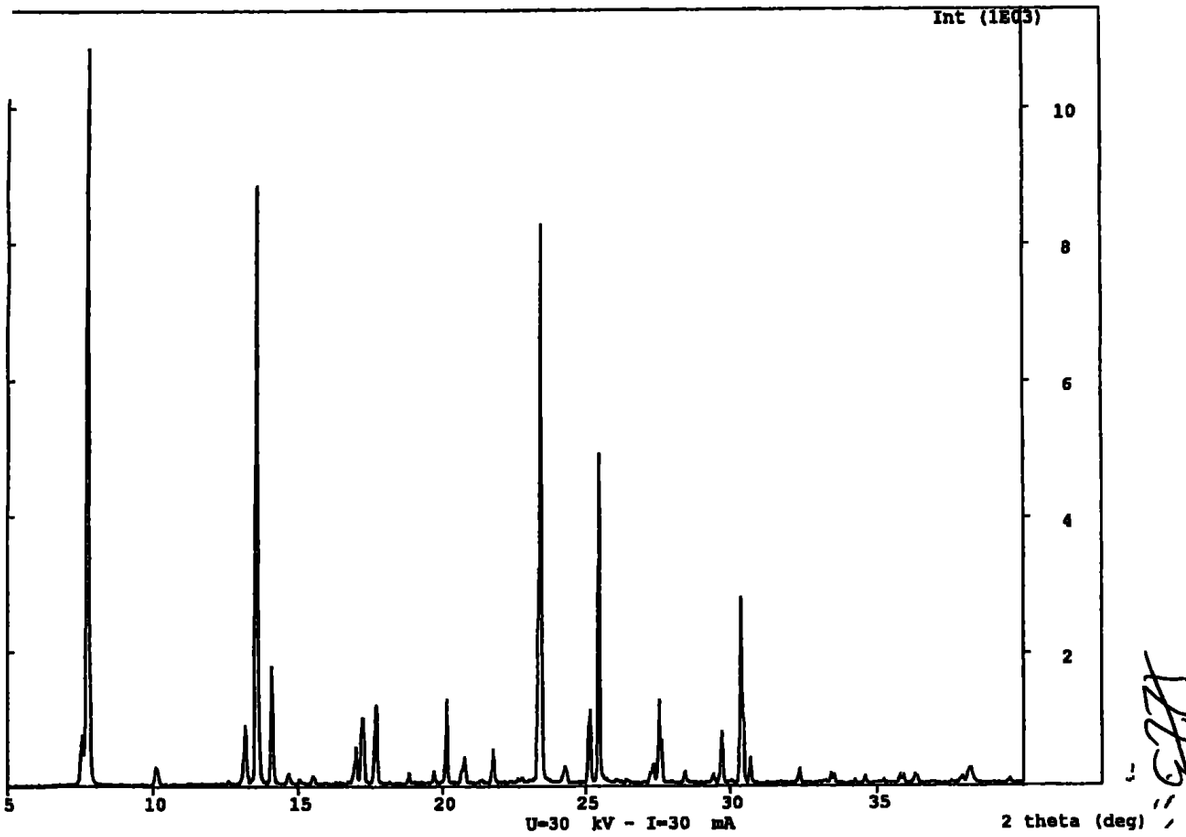


Figure 1-G

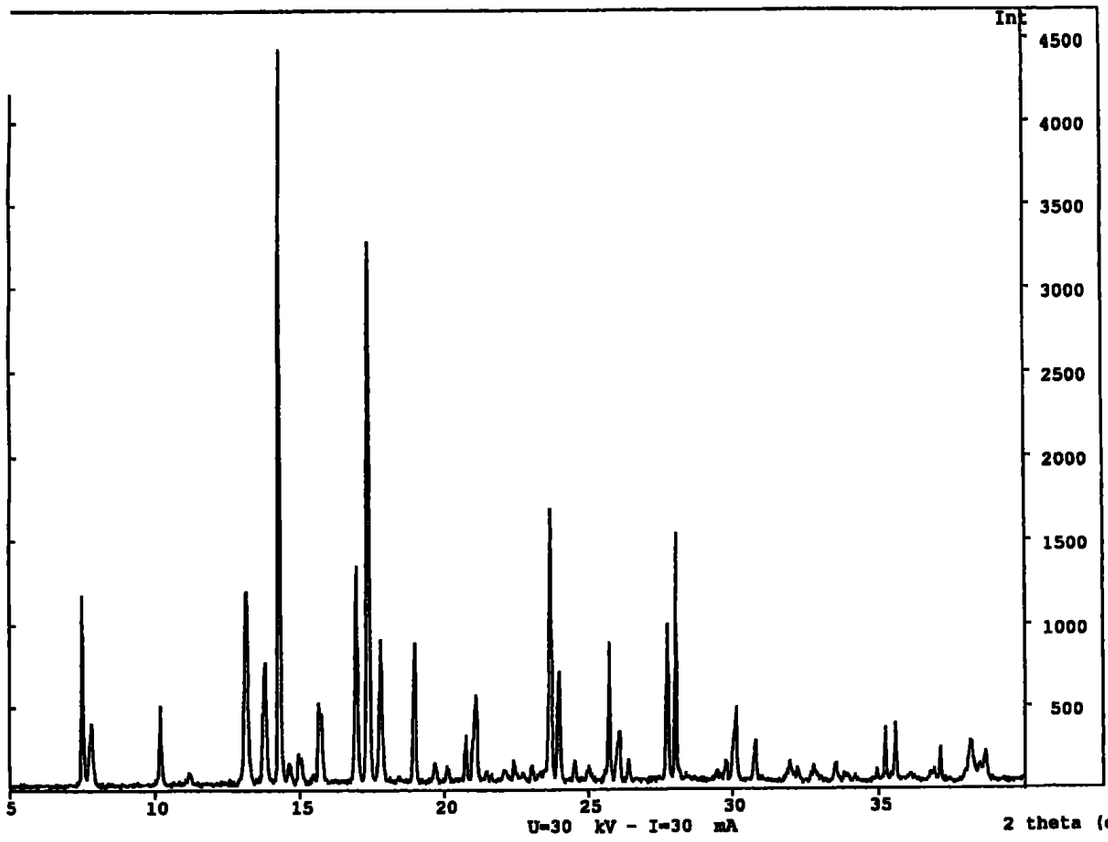

Int
4500
4000
3500
3000
2500
2000
1500
1000
500
5
10
15
20
25
30
35
U=30 kV - I=30 mA
2 theta (deg)

Figure 1-H

0880211-0268709

Acetone Solvate

INEL

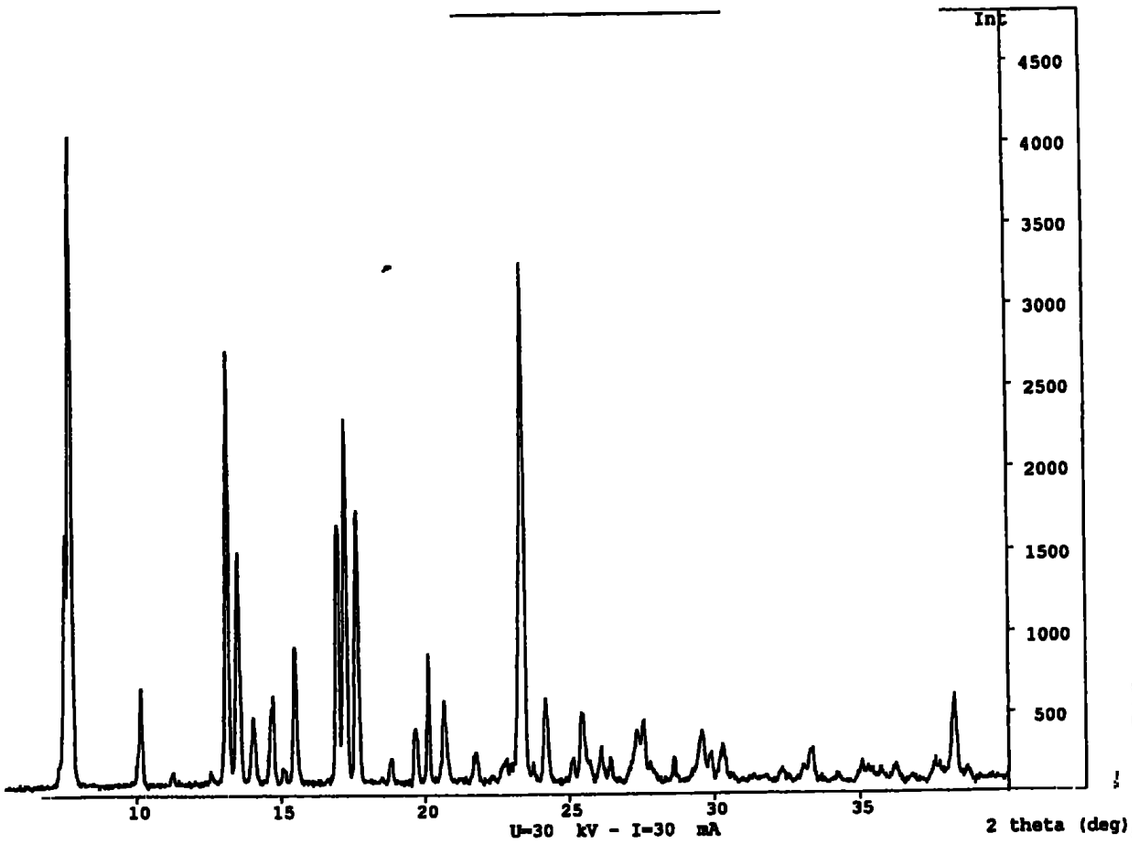


Figure 1-I

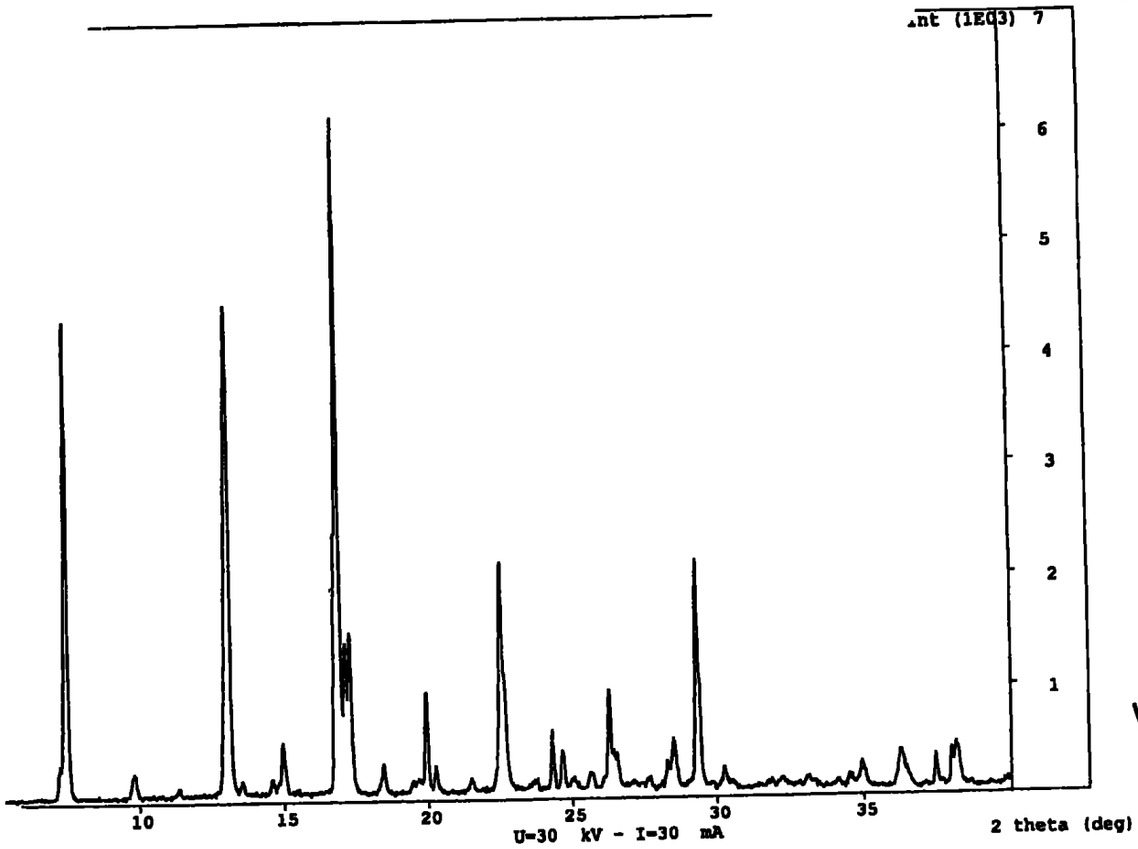


Figure 1-J

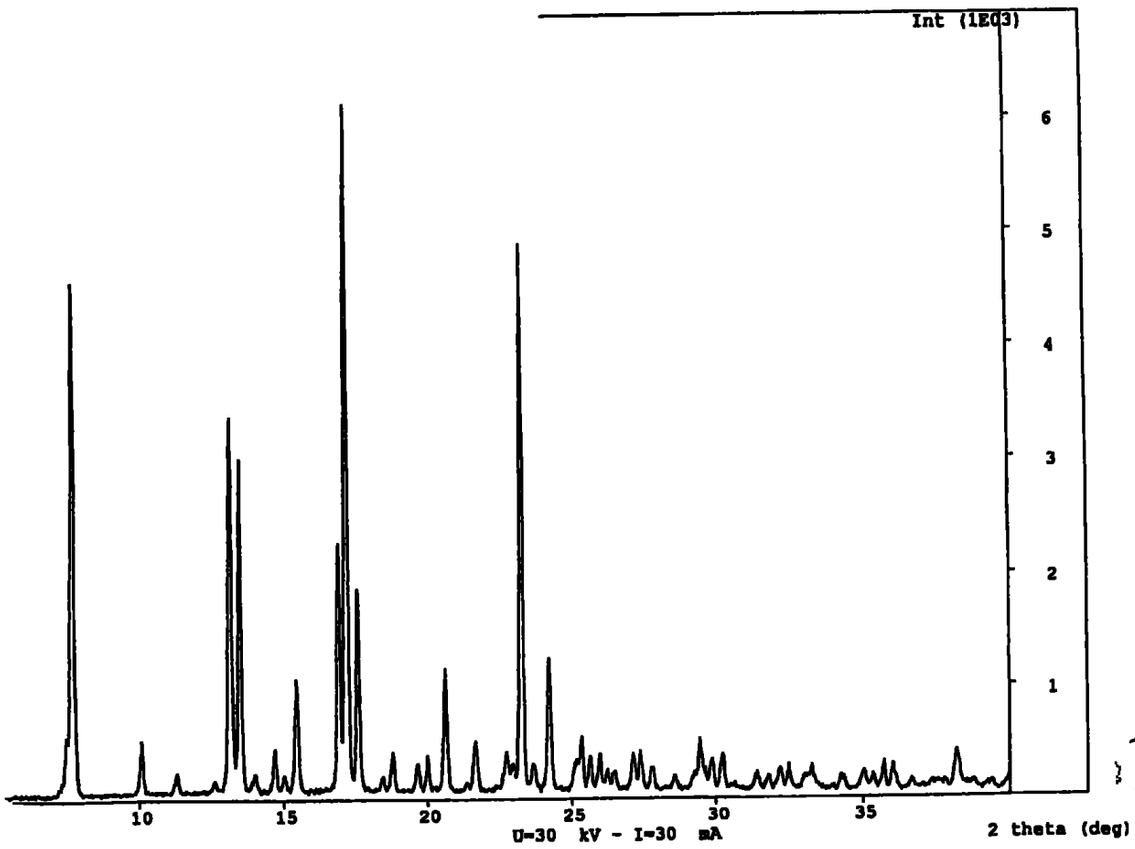
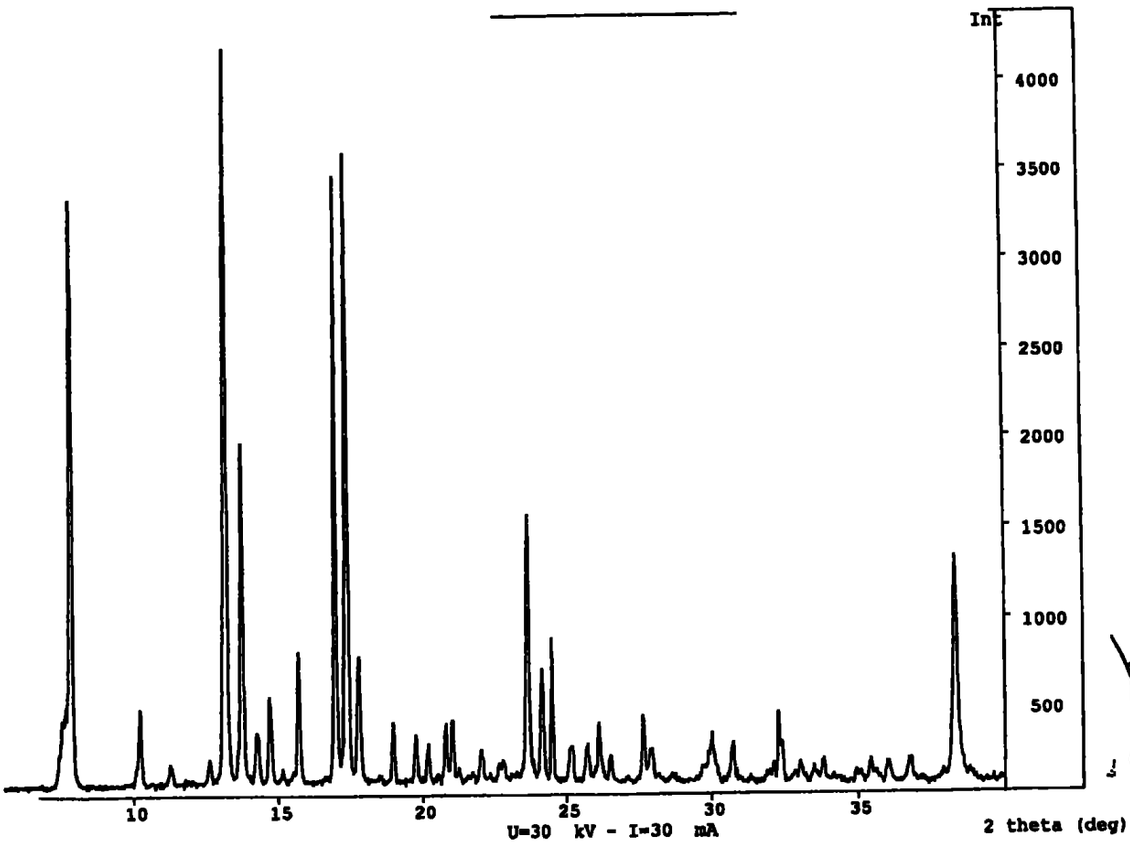


Figure 1-K

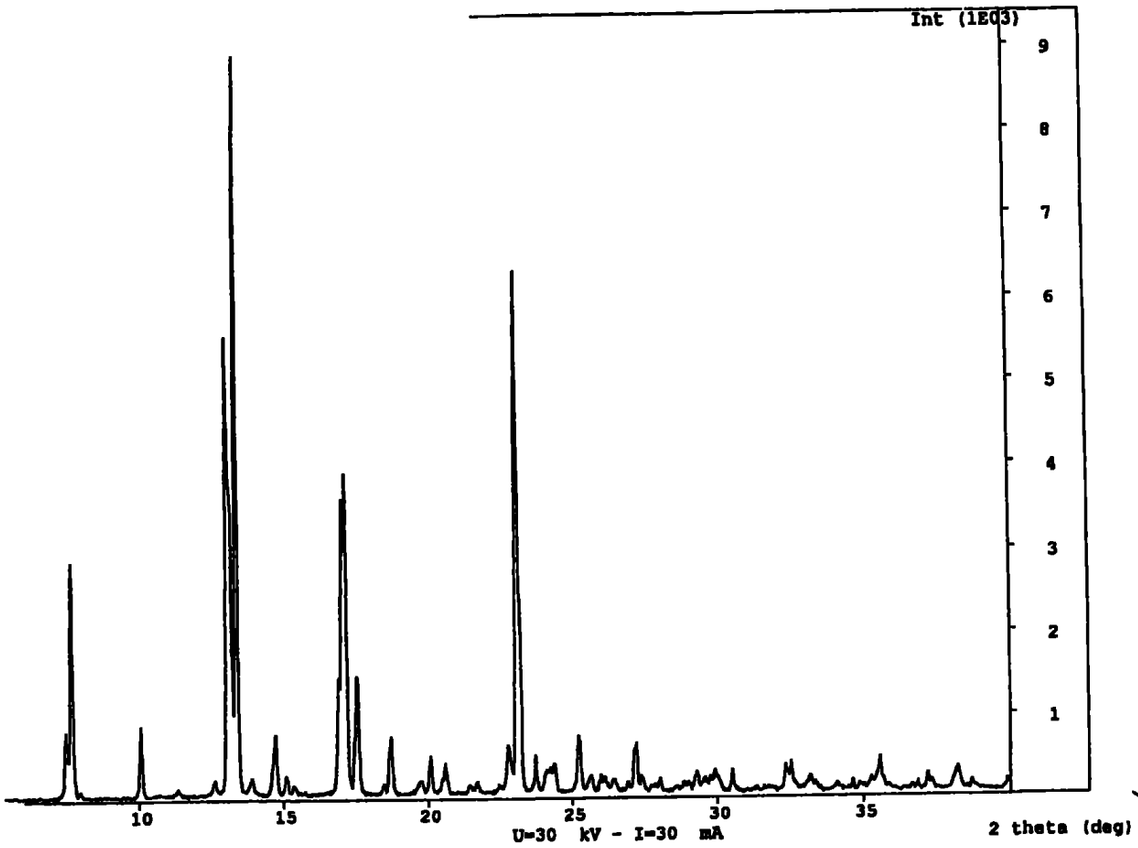
ethanol solvate

INEL 1219



1228

Figure 1-L



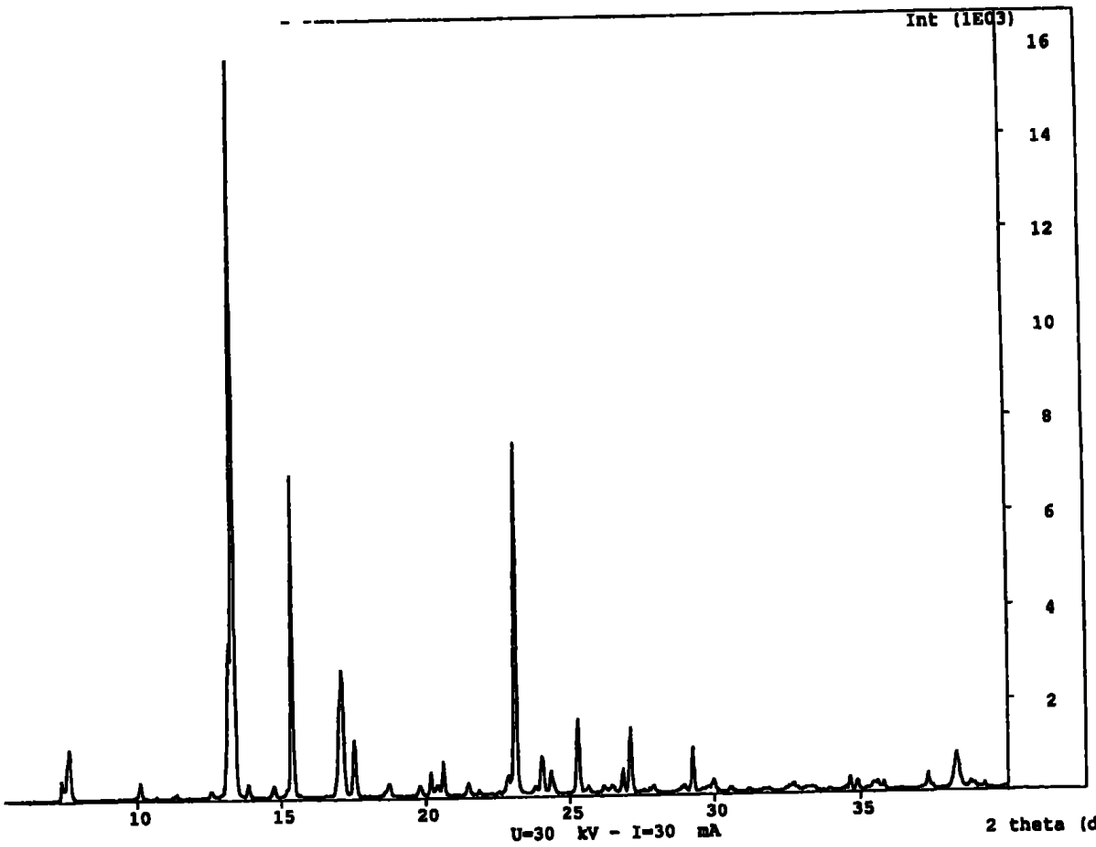
1229

Figure 1-M

6688021 20080109

bu[redacted] acetate solvate

INEL 1221

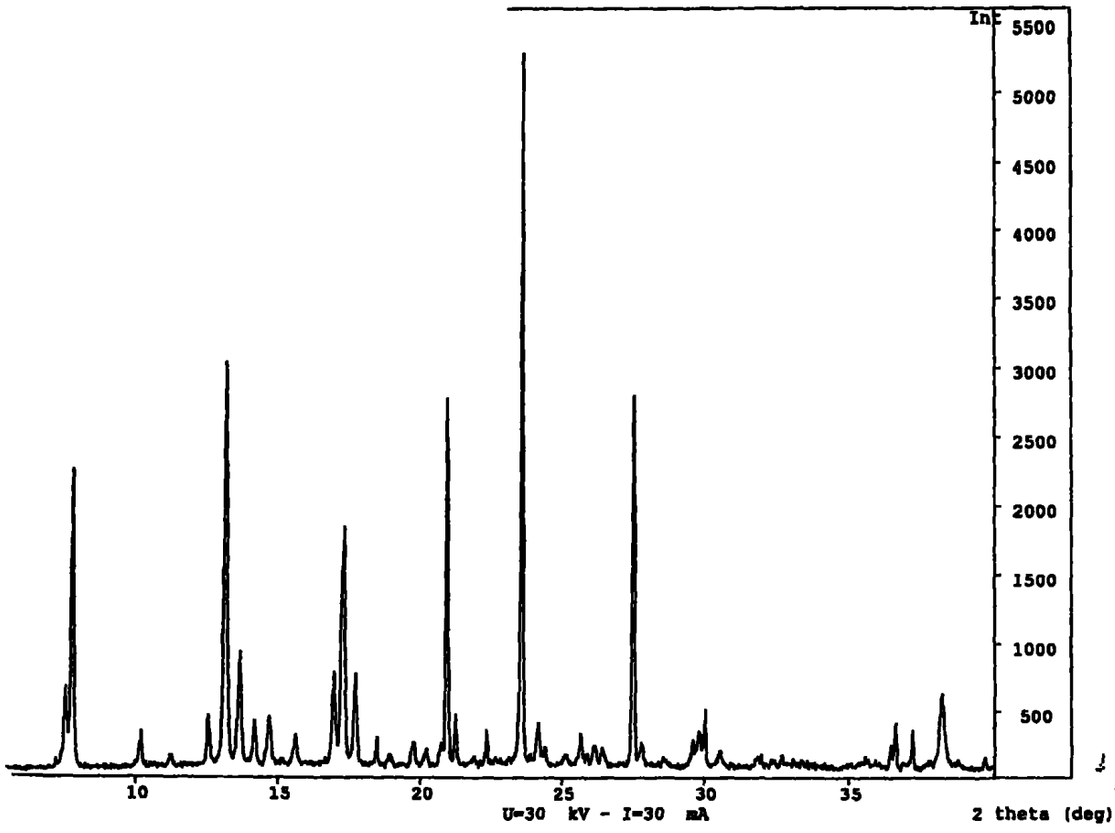


1230

Figure 1-N

[redacted] Methanesulfonate Solvate

INEL 1222

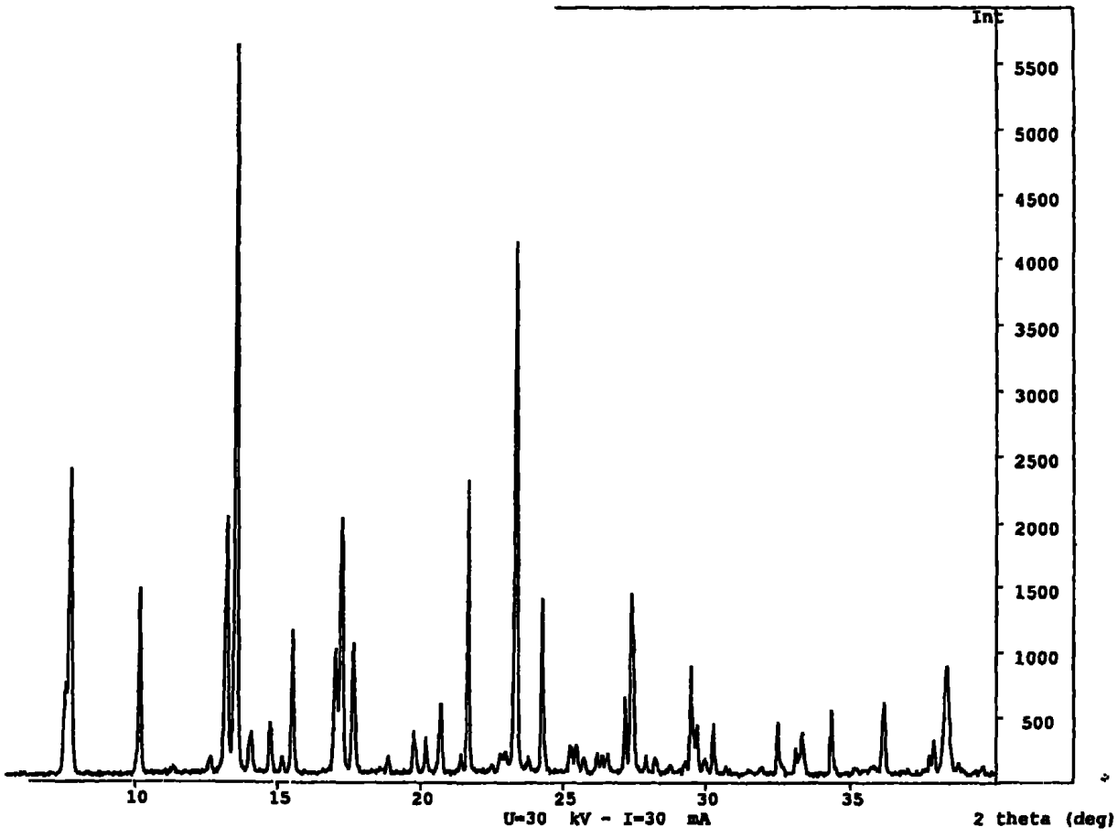


1234

Figure 1-0

[redacted] solvate

INEL



Size 0 6360 mg
Method 10 DEG C/MIN AMB TO 300
Comment: SEALED PAN

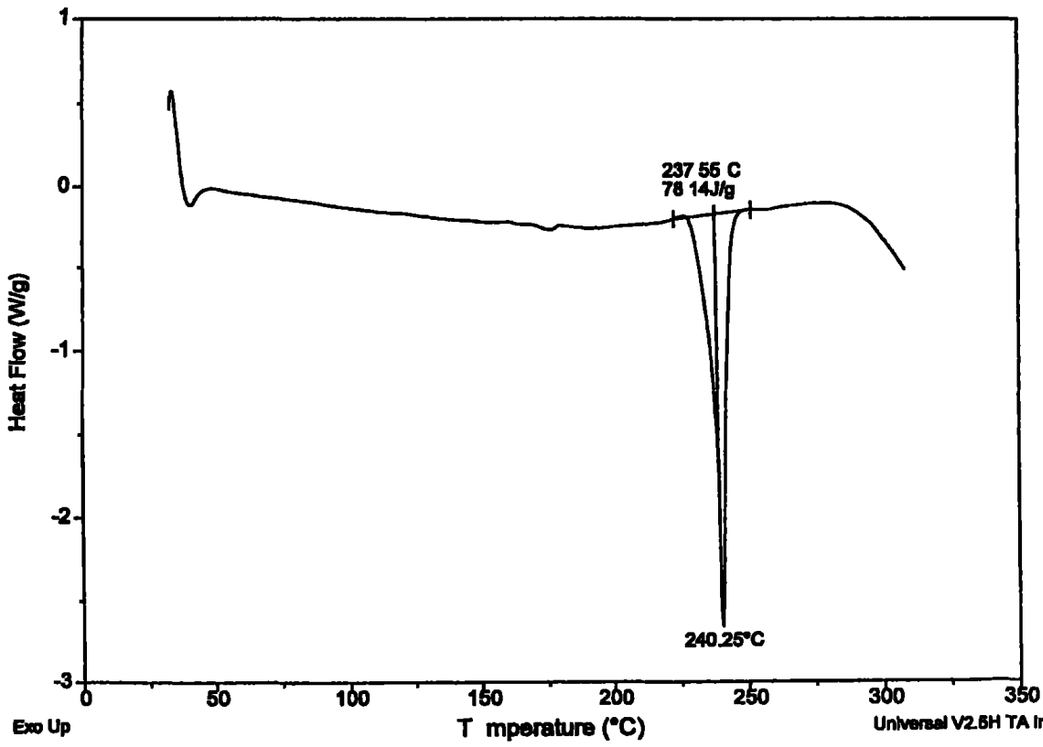
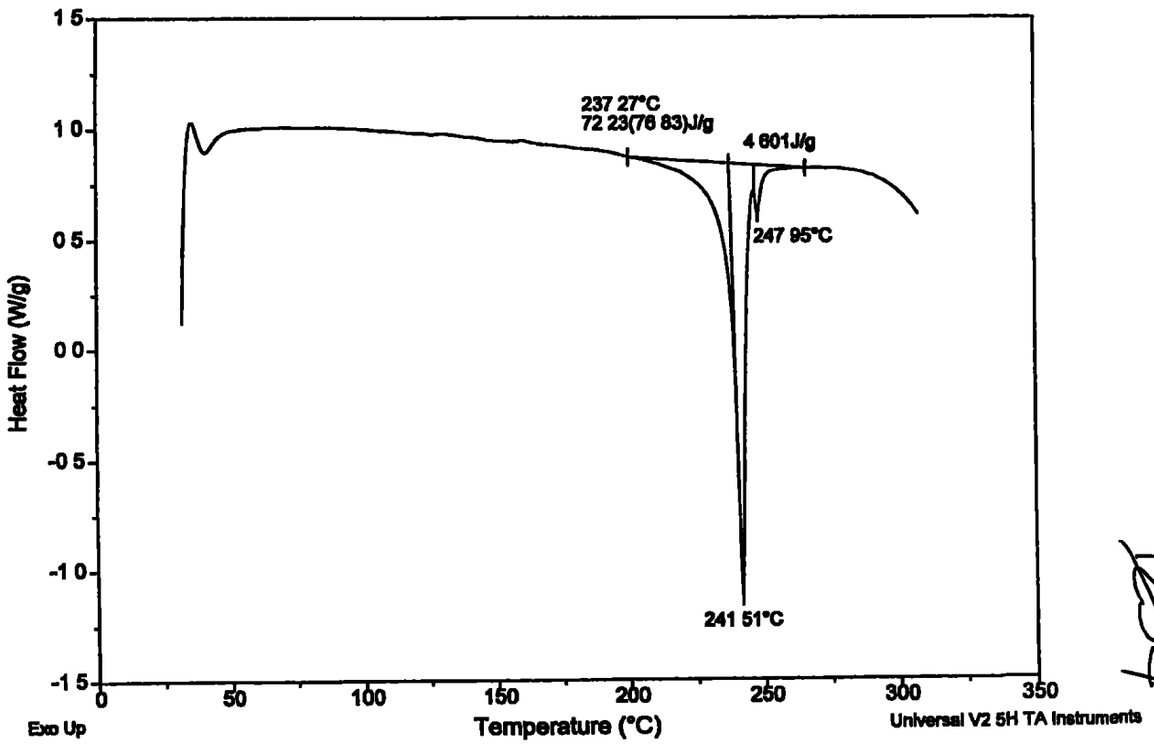
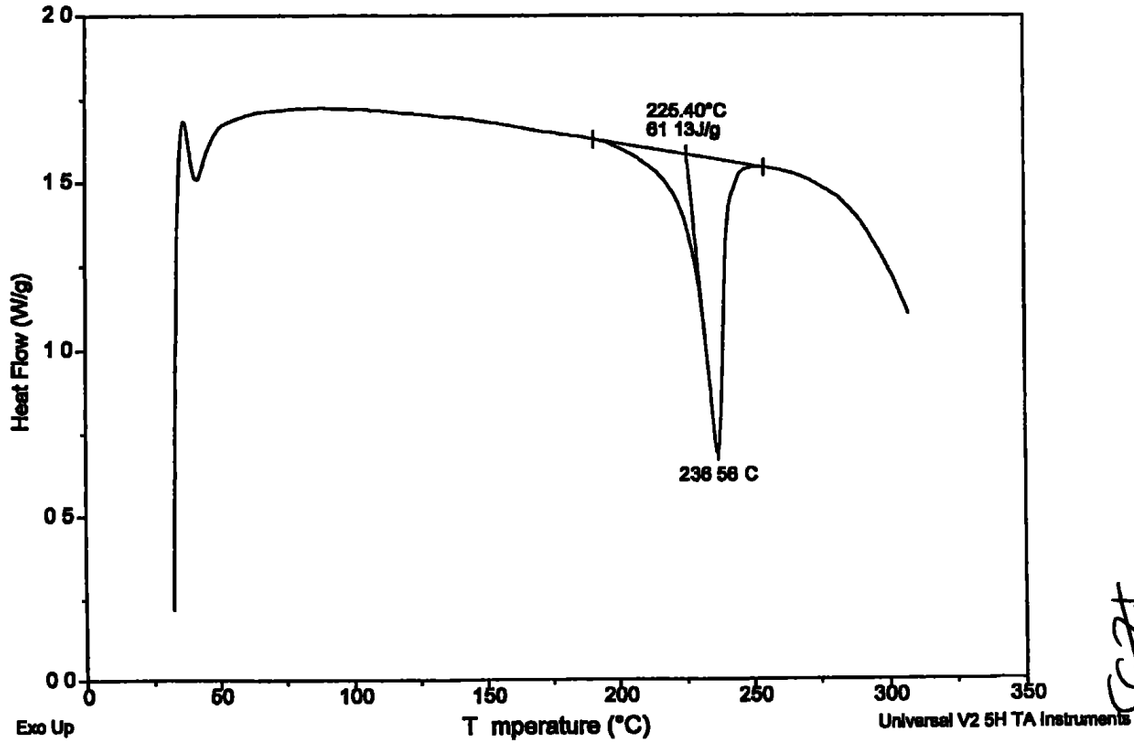


Figure 2-5

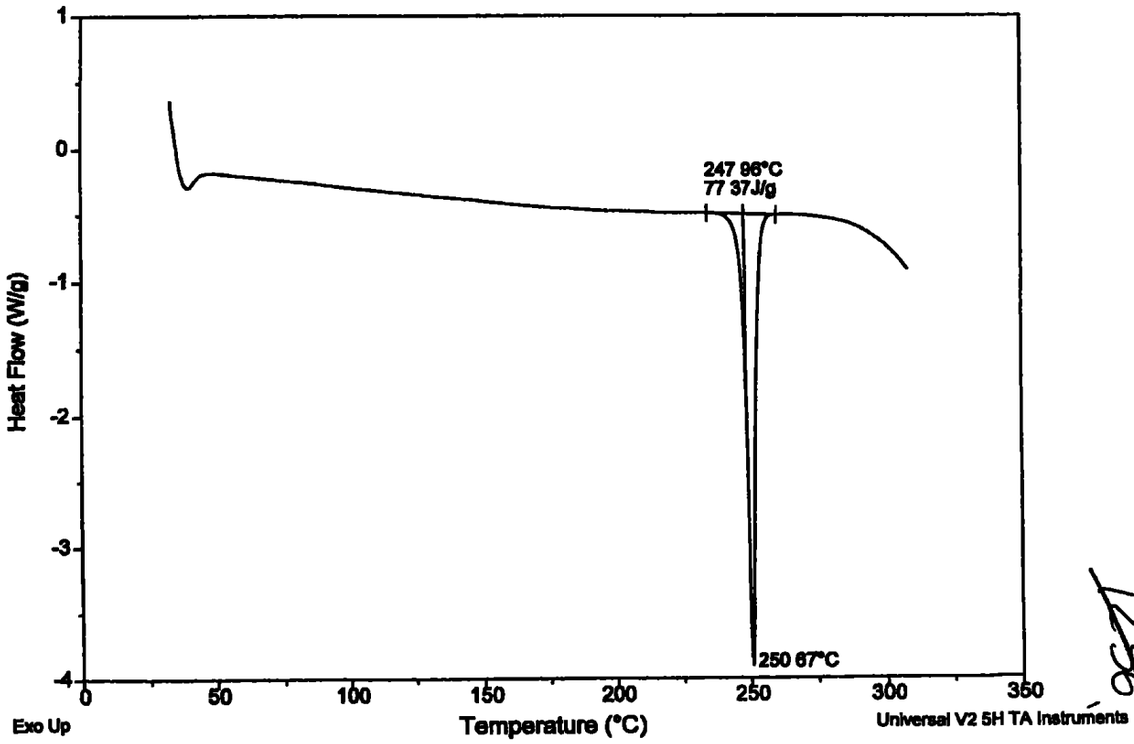
Size 1 7840 mg
Method 10 DEG C/MIN AMB TO 300
Comment: SEALED PAN



Size 1 4230 mg
Method 10 DEG C/MIN AMB TO 300
Comment: SEALED PAN



Size 1 0400 mg
Method 10 DEG C/MIN AMB TO 300
Comment: SEALED PAN



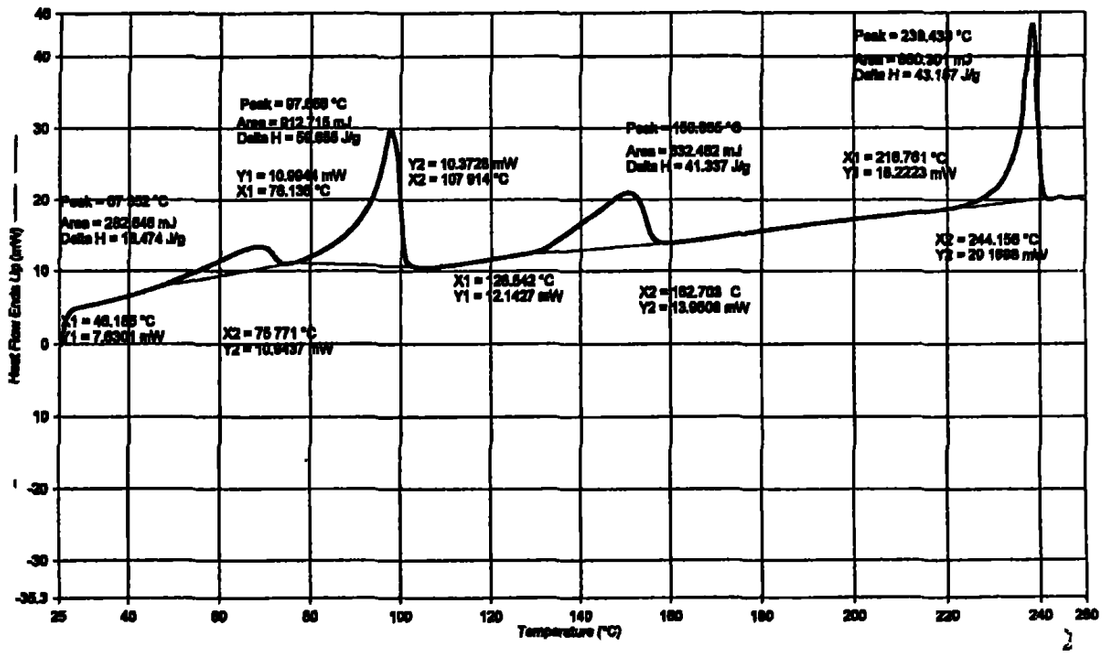
1236

Figure 2-E

[redacted]

1228

Sample Weight: 18.300 mg
Comment:
N-Propyl Alcohol solvate 1
unwashed sgn

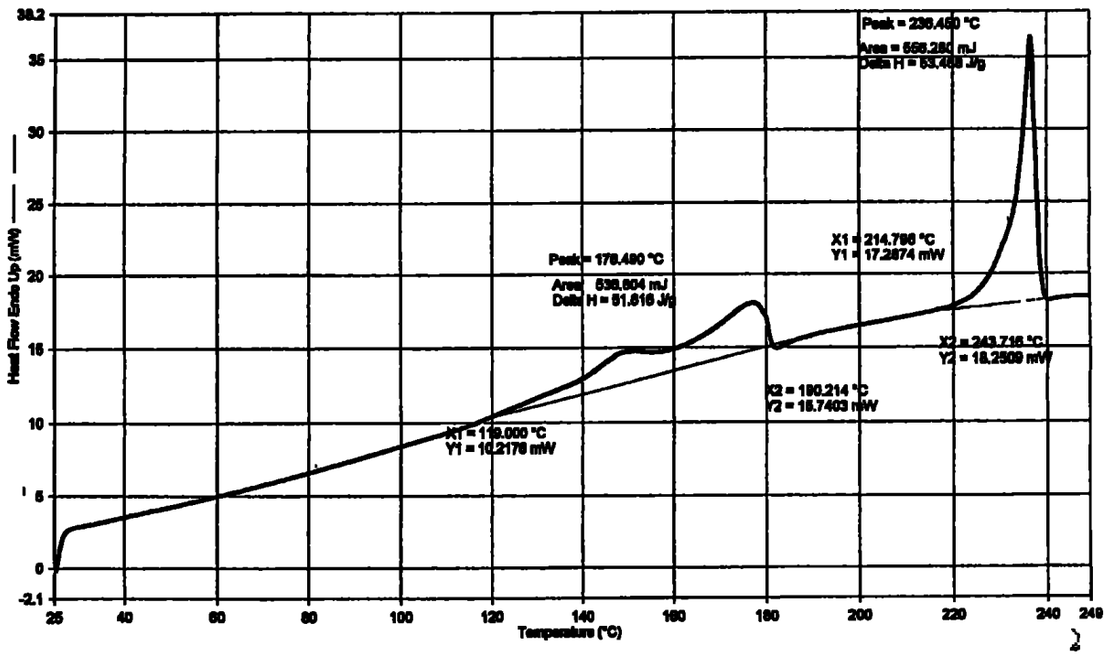


1227

Figure 2-F

1229

Sample Weight: 10.400 mg
Comment: THF activate 1
unsealed pan



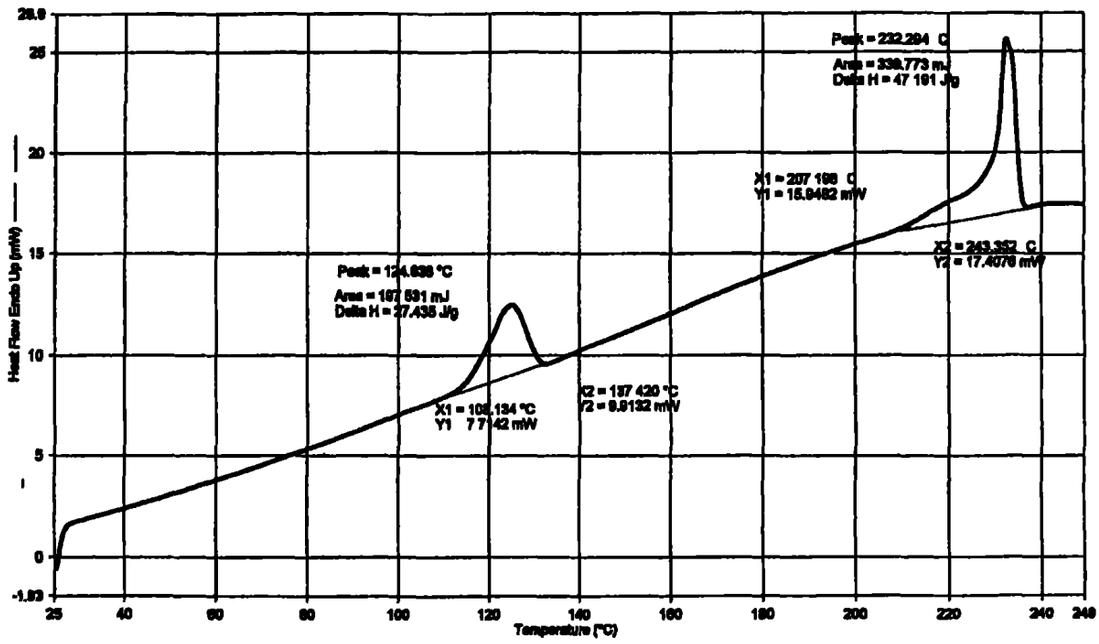
1228

Figure 2-G

[redacted]

Sample Weight: 7.200 mg
Comment: Lot 96K020-F3b
Ethyl propionate solvate
unsealed pan

1230

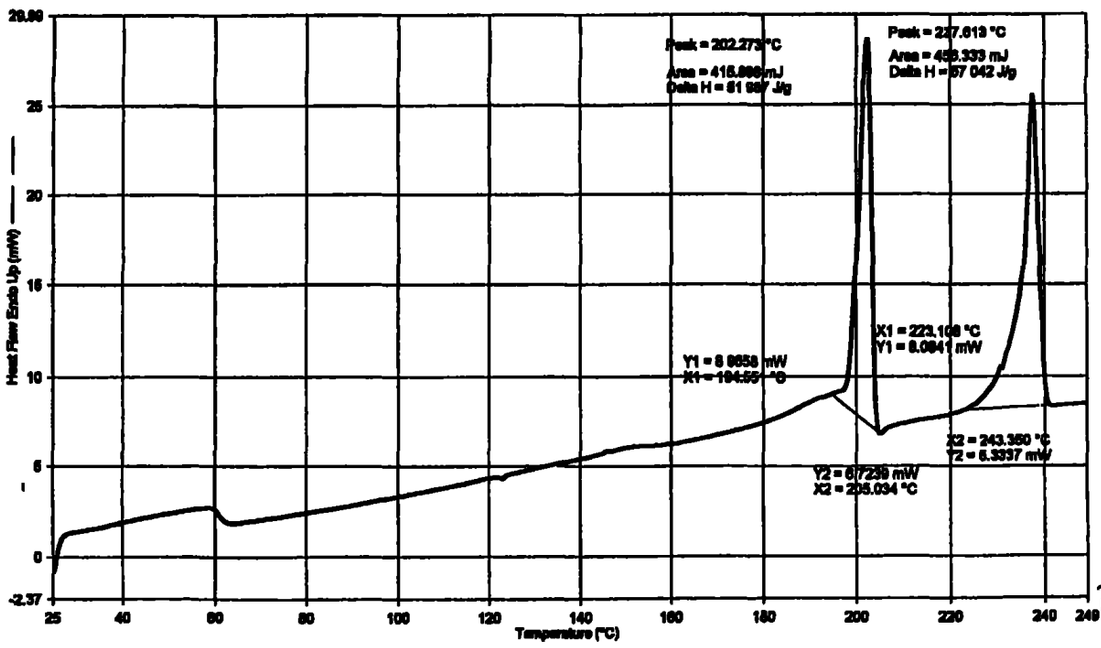


1239

Figure 2-H

R31

Sample Weight: 6.000 mg
Comment: Acetic ACid solvate 1
unsealed pan

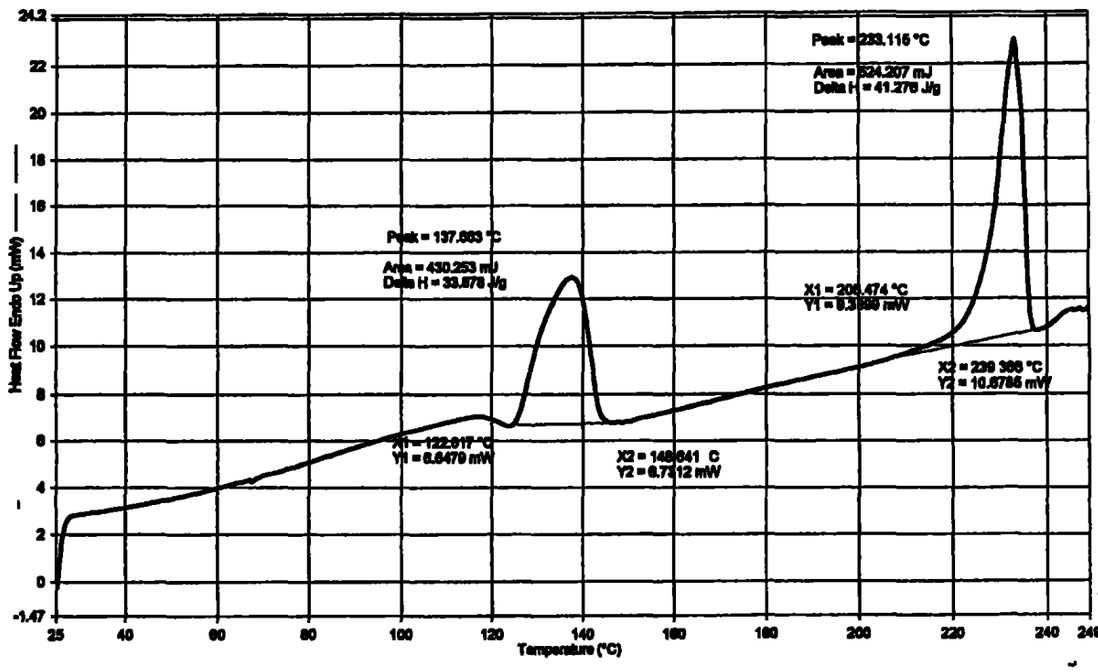


Q42

Figure 21

1232

Sample Weight: 12.700 mg
Comment: Chloroform solvate 1
unsealed pan



1234

Figure 2-J

1233

Sample Weight: 8.704 mg
Comment: Acetone Solvate

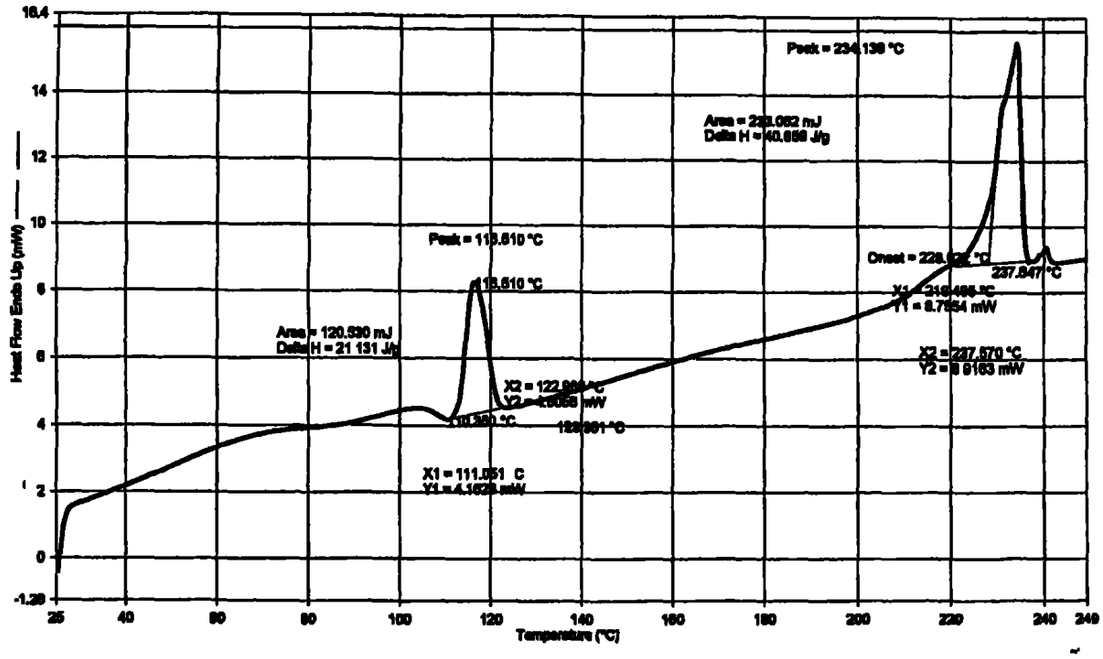
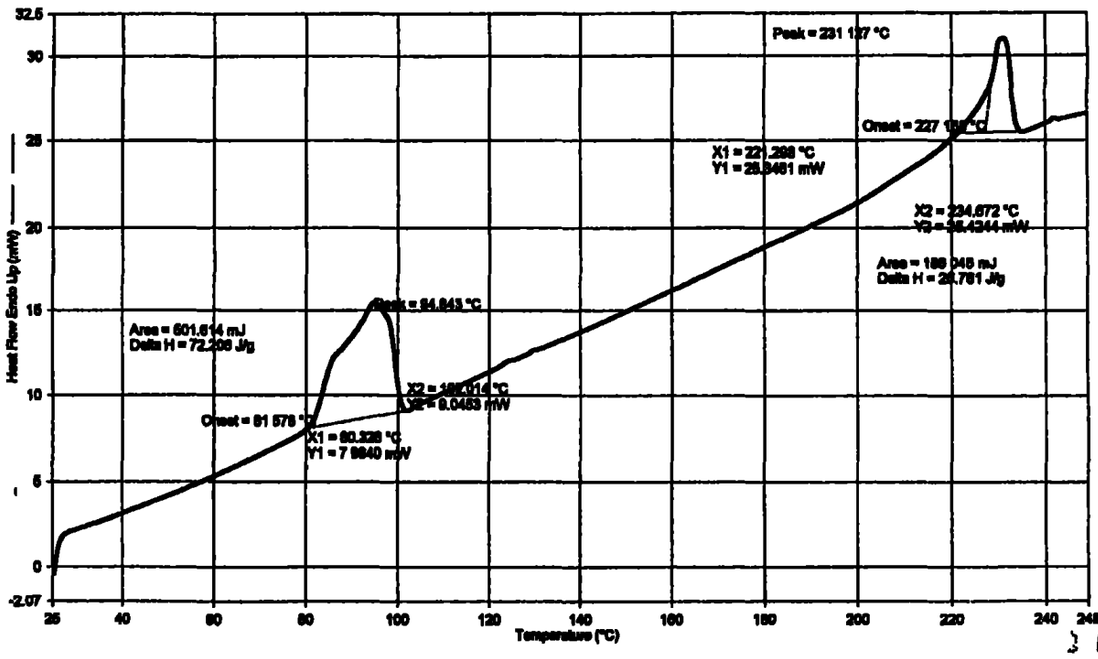


Figure 2-A

1234

Sample Weight: 6.947 mg
Comment: Toluene Solvate



1243

Figure 7-L

1235

Sample Weight: 8.174 mg
Comment: Isopropanol Solvate

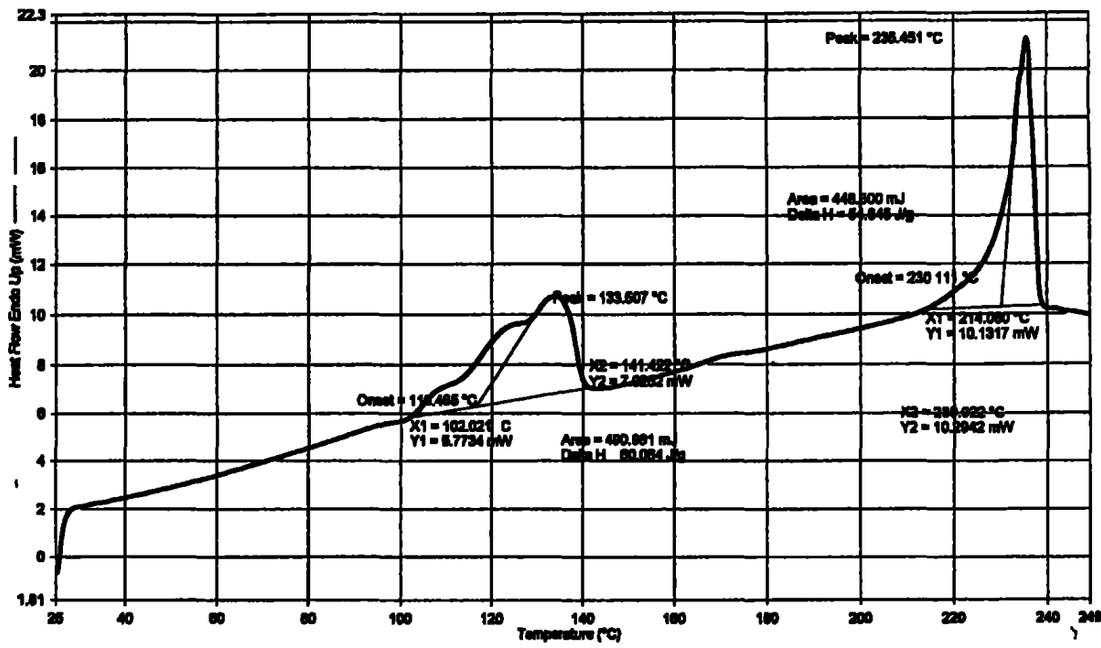
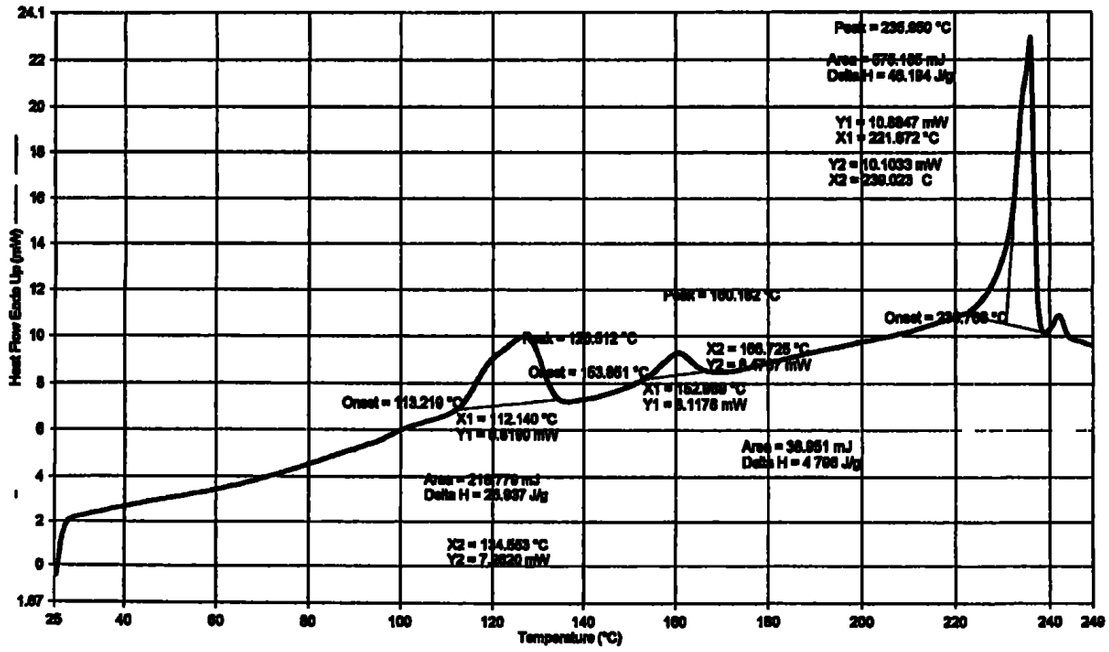


Figure 2

1236

Sample Weight: 8.122 mg
Comment:
ethanol solvate
10 °C/min sealed pan



1245

Figure 2 N

1237

Sample Weight: 8.840 mg
Comment:
i-butyl acetate extrate
10 °C/min sealed pan

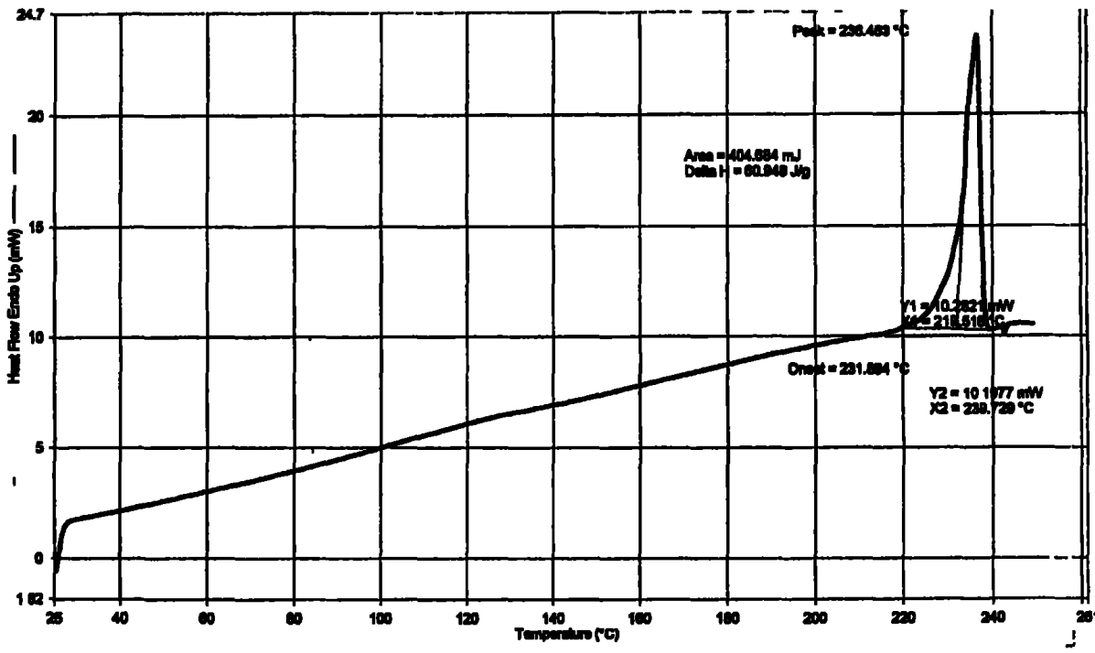


Figure 2-O

Sample Weight: 9.900 mg
Comment:
Isobutyl acetate solvate
10 °C/min sealed pan

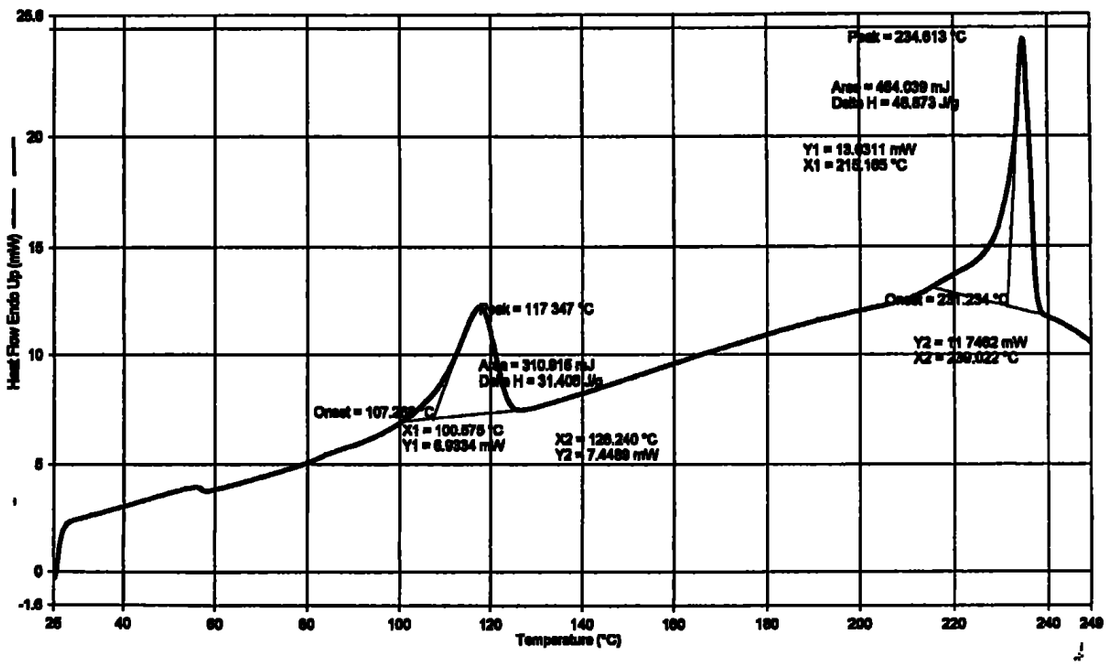
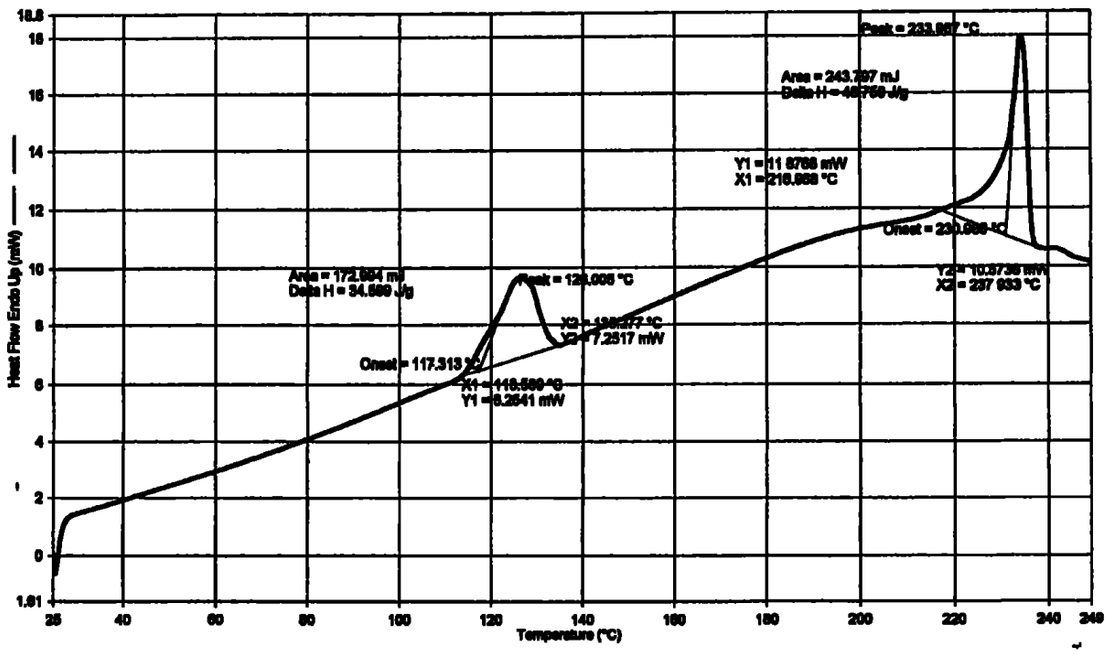


Figure 2-P

[redacted]

1239

Sample weight: 6.000 mg
Comment: butyl acetate solvate
10 °C/min sealed pan



1248

Figure 2-Q

1240

Sample Weight: 6.538 mg
Comment: methyl acetate solvate 1
10 °C/min sealed pan

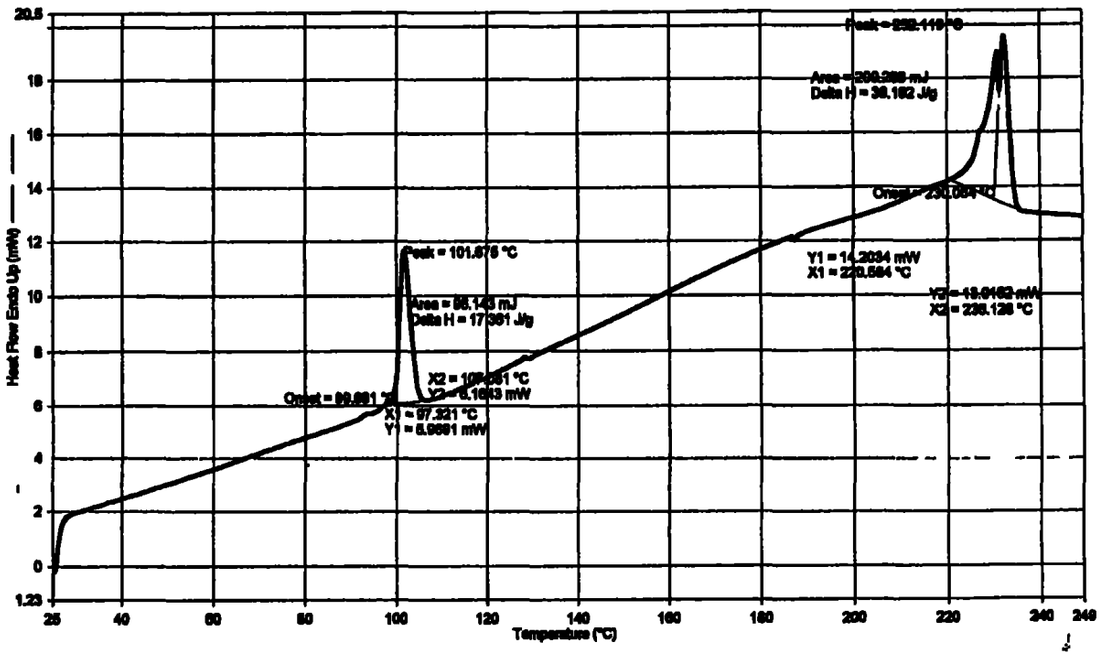
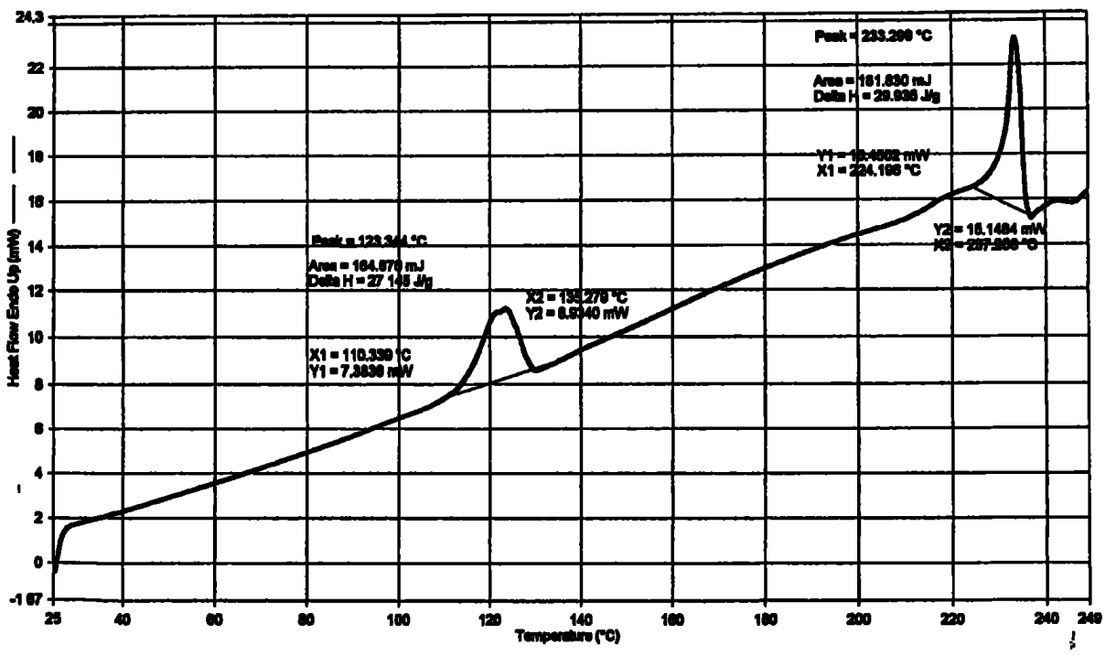


Figure Z-R

[redacted]

1241

Sample Weight: 6.074 mg
Comment:
propyl acetate solvate 1
10 °C/min sealed pan



250

Figure 2-5

n-butanol solvate

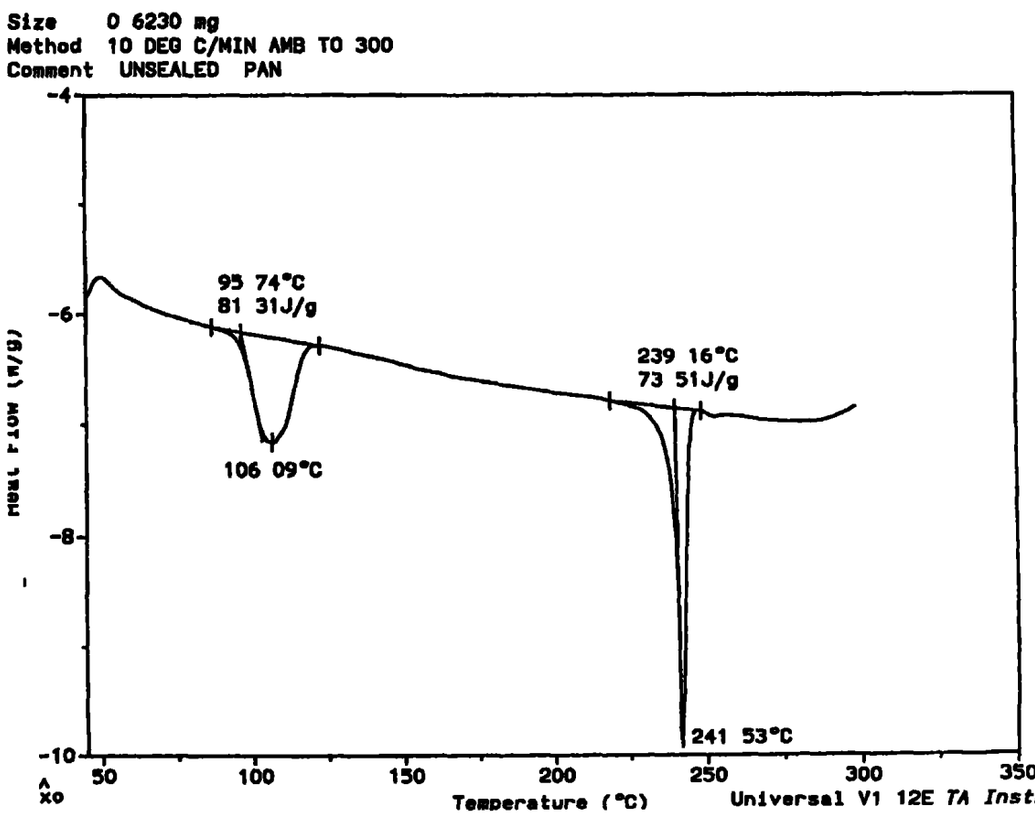


Figure 2-T

n-octanol solvate

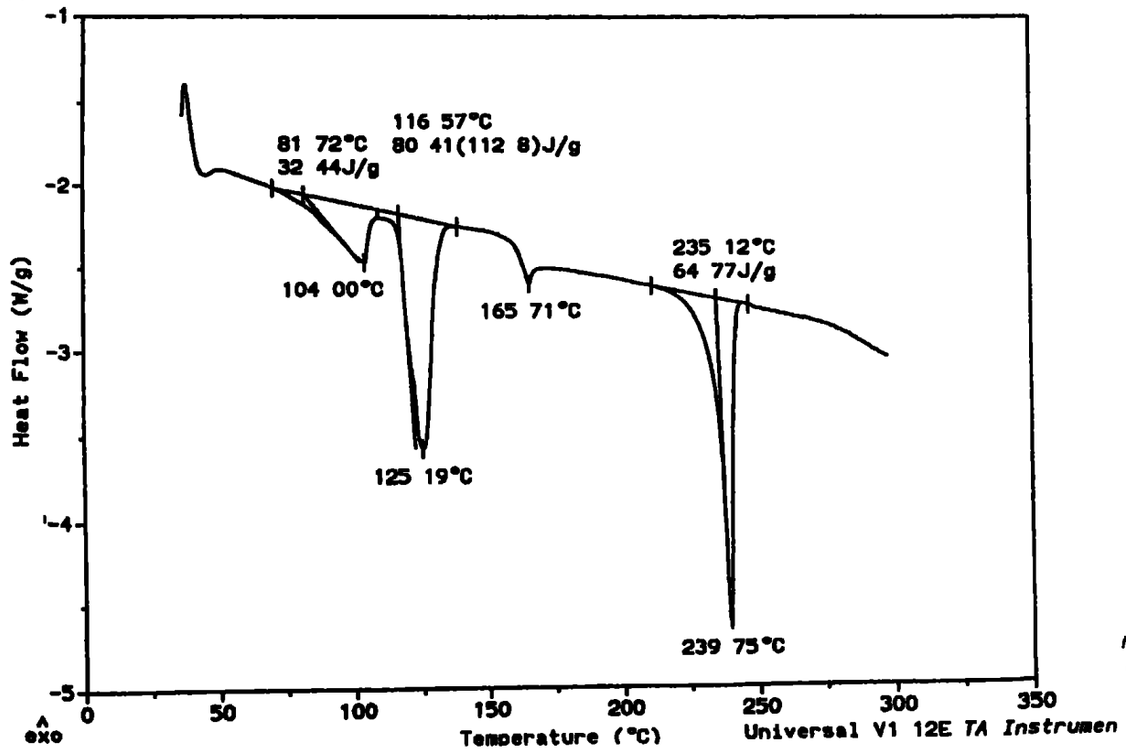


Figure 3-A

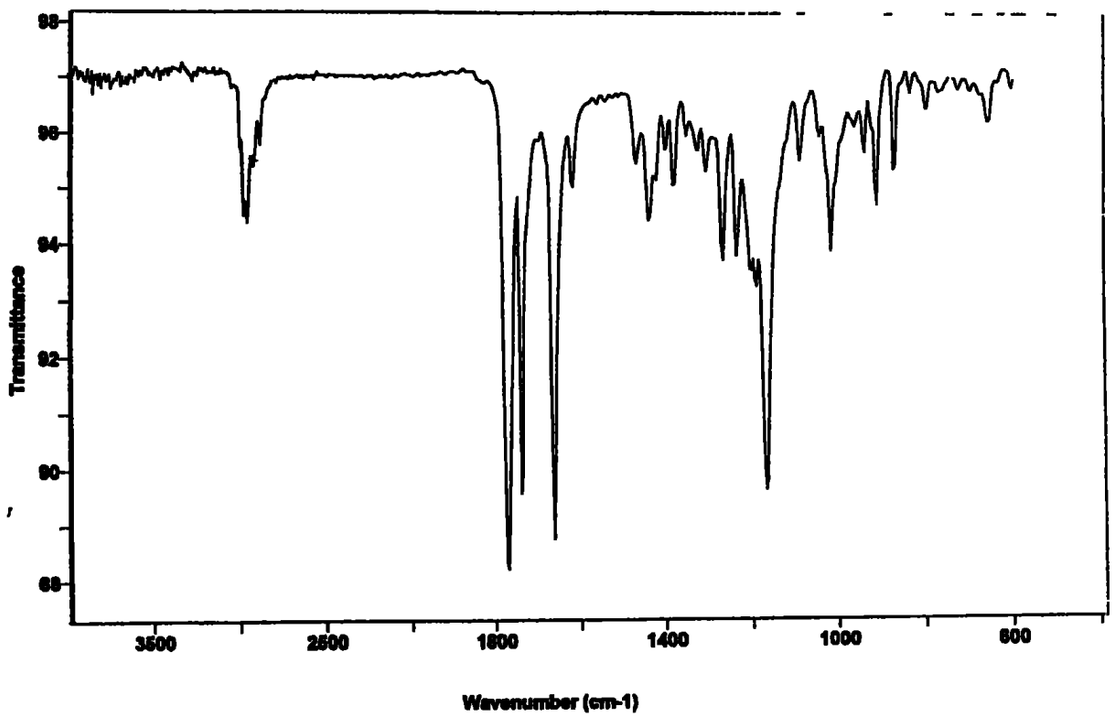

Transmittance
98
96
94
92
90
88
3500
2500
1800
1400
1000
600
Wavenumber (cm-1)

Figure 3-B

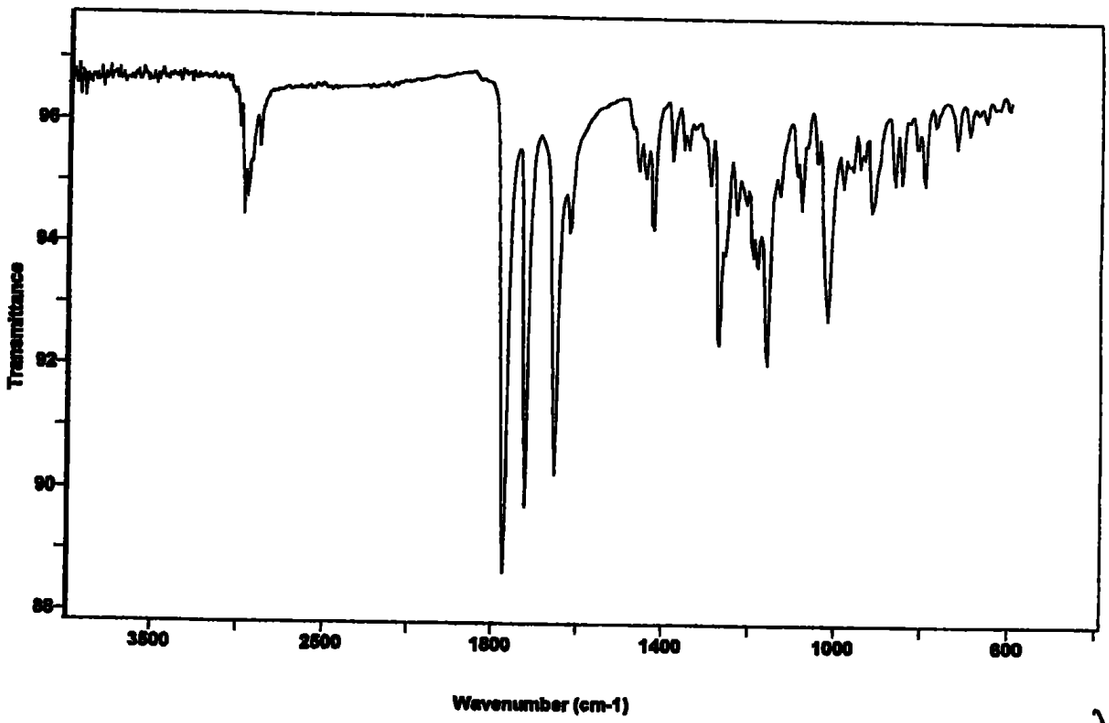

Transmittance
96
94
92
90
88
3500
2500
1800
1400
1000
600
Wavenumber (cm-1)

Figure 3-C

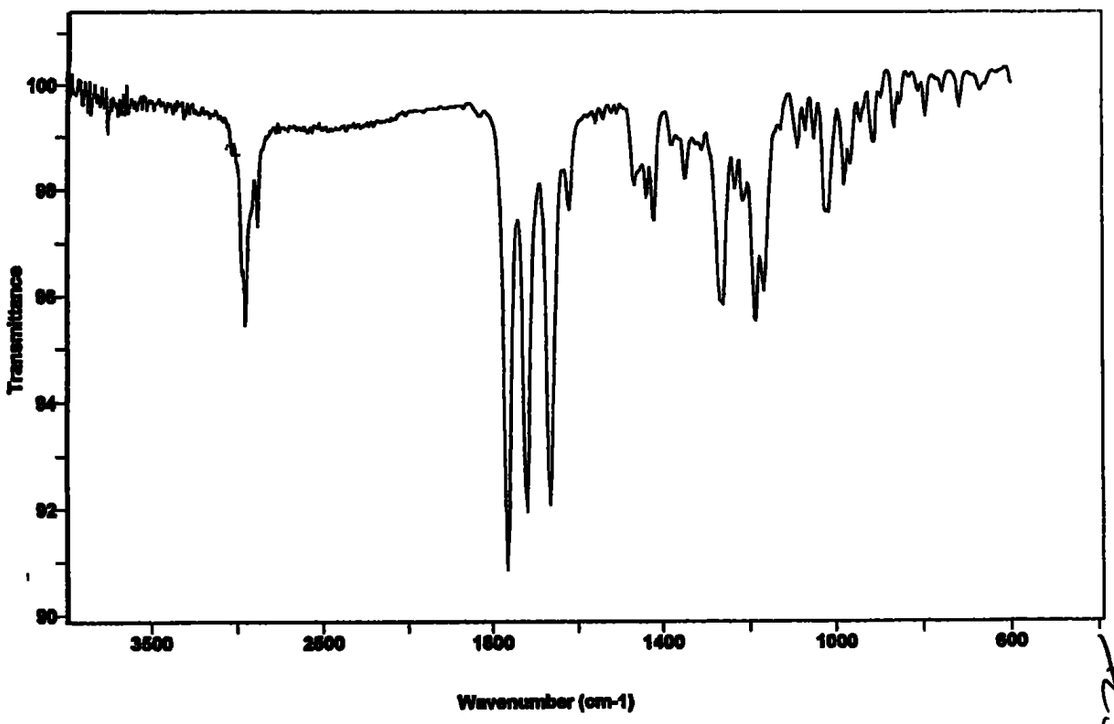
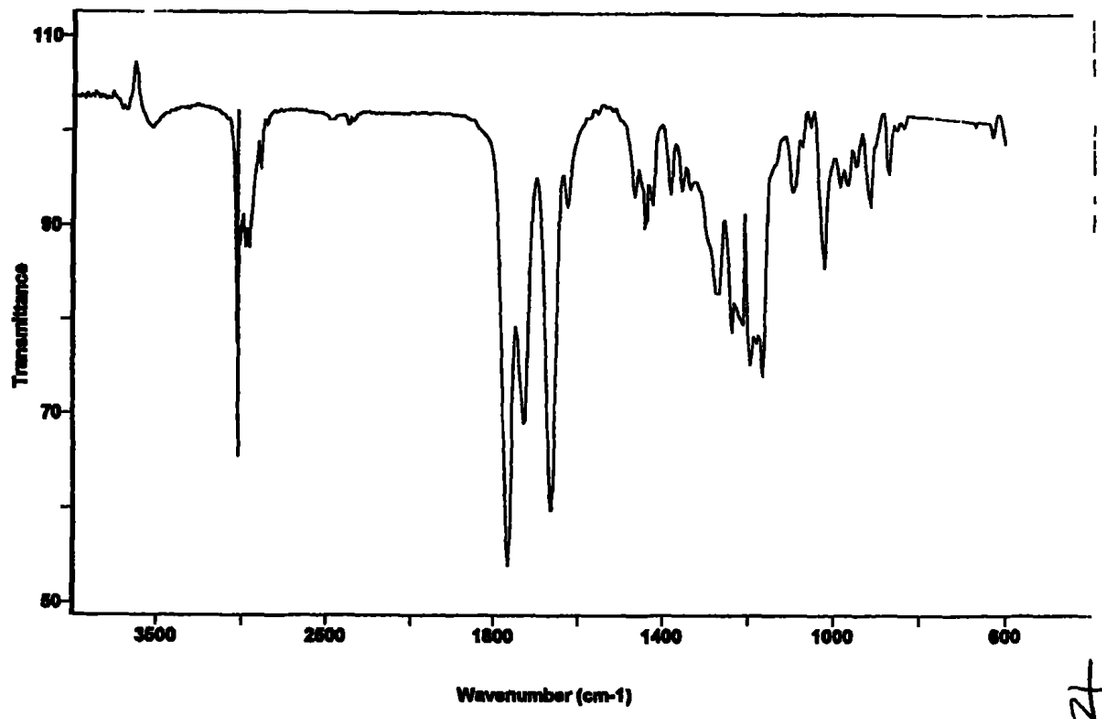

100
98
96
94
92
90
Transmittance
3500
2500
1800
1400
1000
600
Wavenumber (cm-1)

Figure 3-D



1247

Figure 3-E

n-propyl alcohol solvate

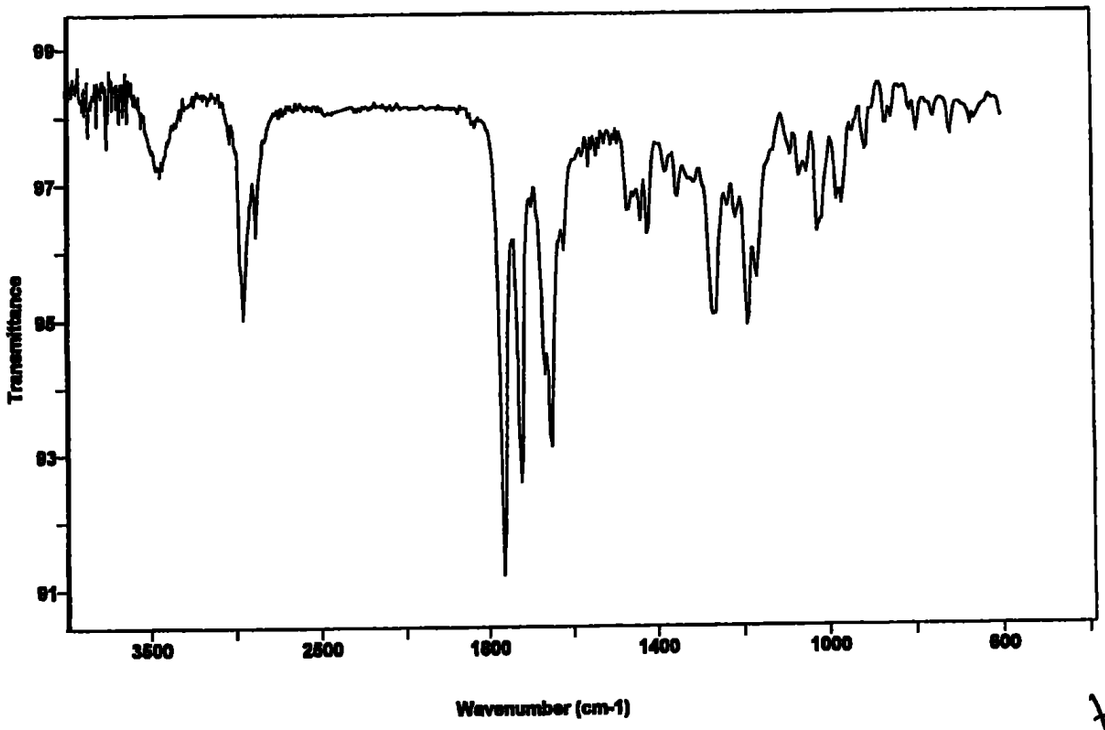


Figure 3-F

1249

tetrahydrofuran solvate

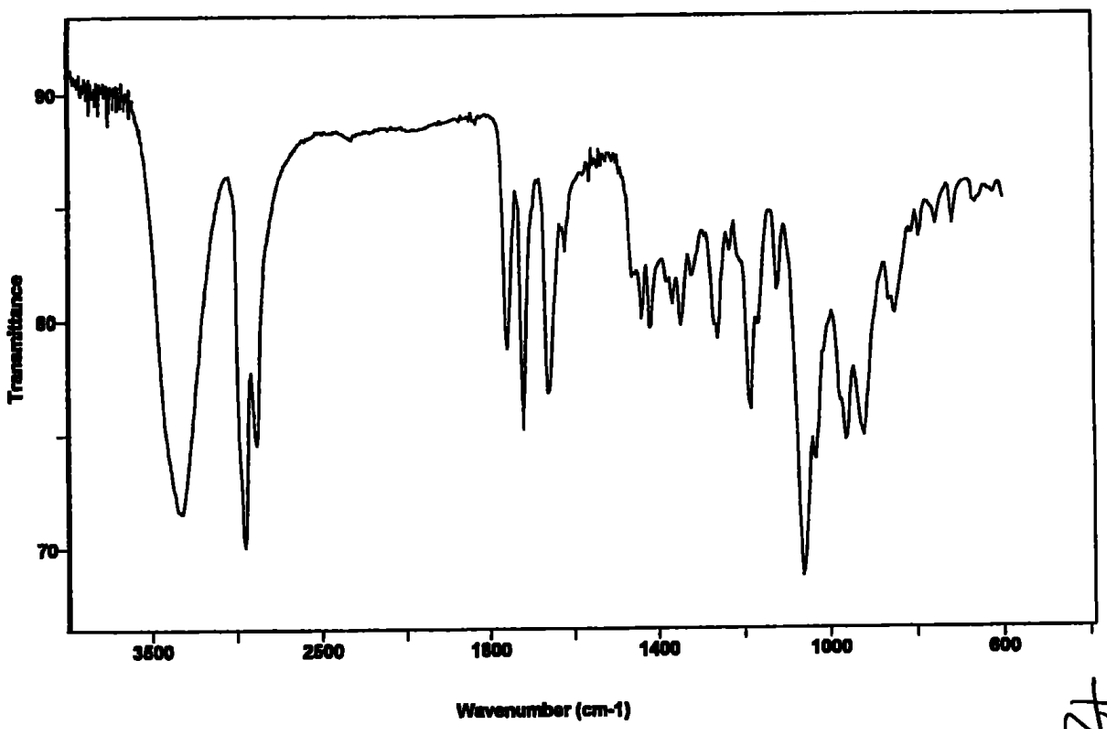


Figure 3 G

ethyl propionate solvate

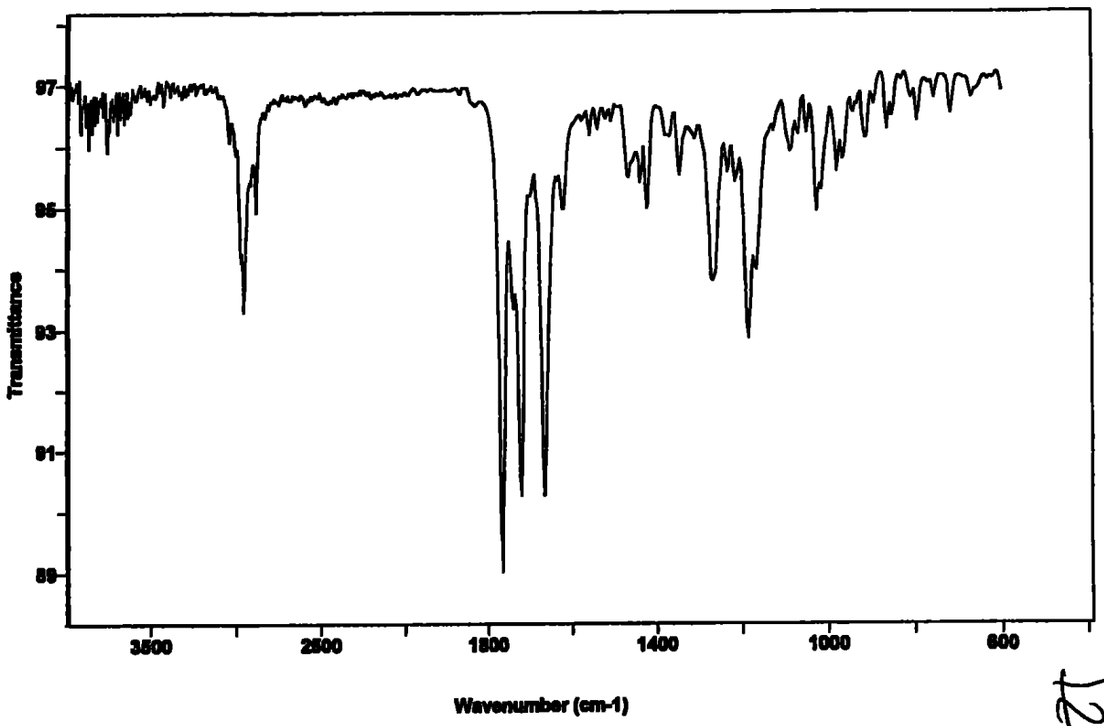


Figure 3-H

6088021 20269109

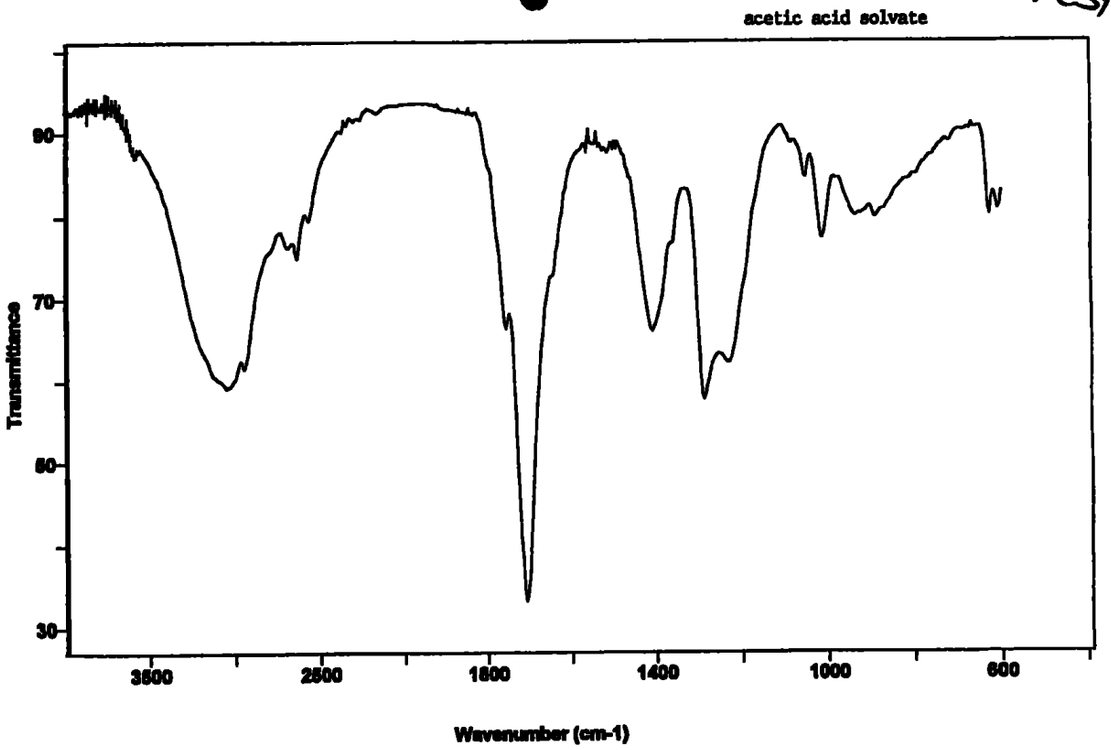


Figure 3-I

6068021 20269709

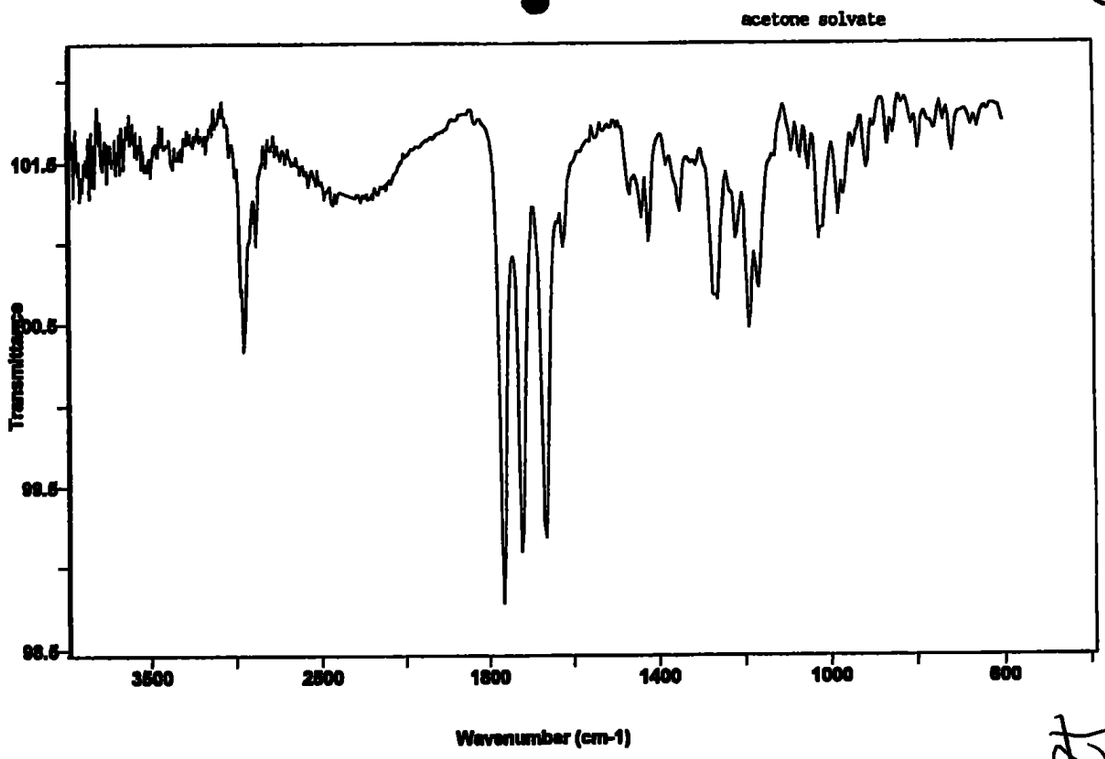


Figure 3-J

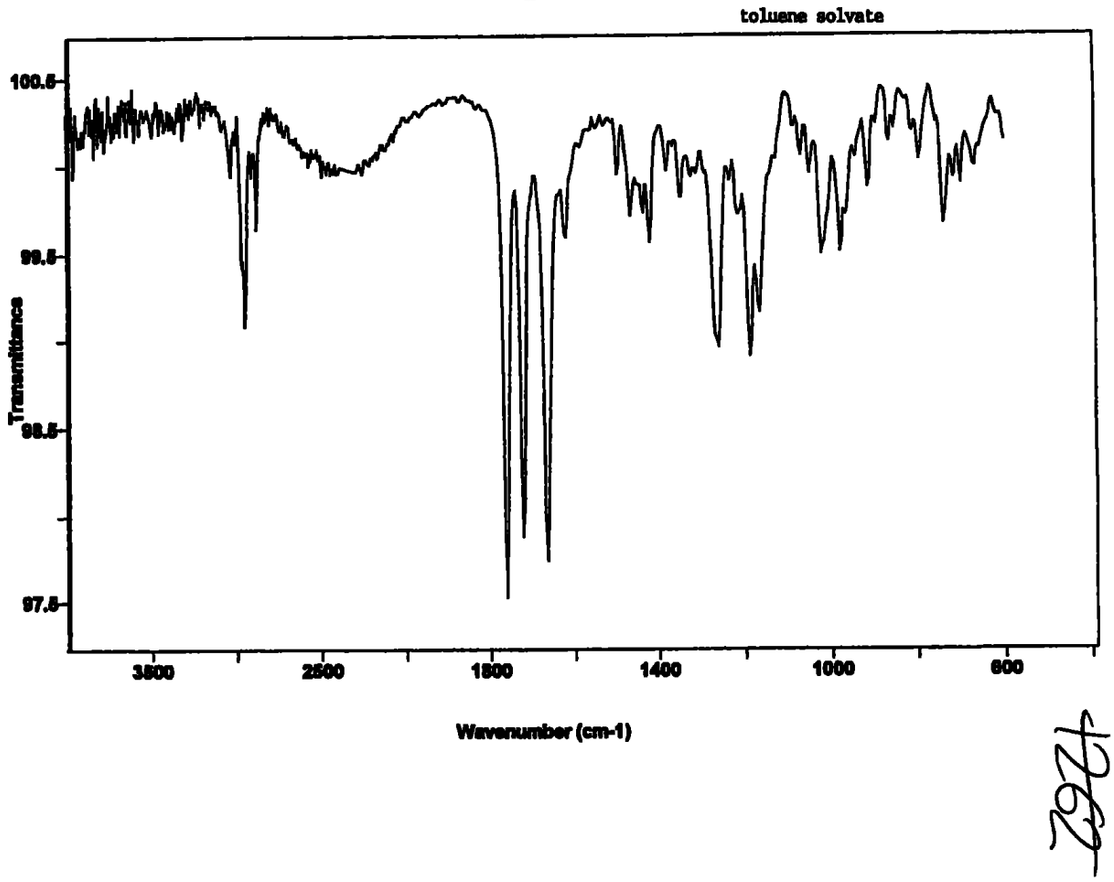

toluene solvate
100.5
99.5
98.5
97.5
Transmittance
3500
2500
1800
1400
1000
600
Wavenumber (cm-1)

Figure 3-K

isopropanol solvate

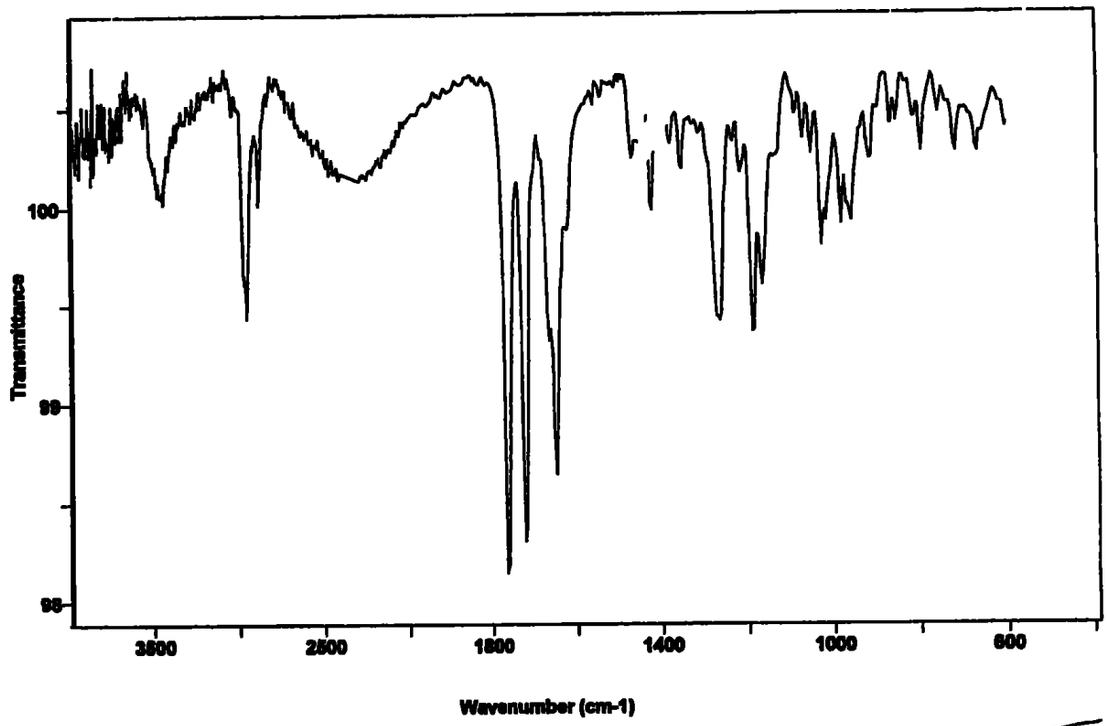
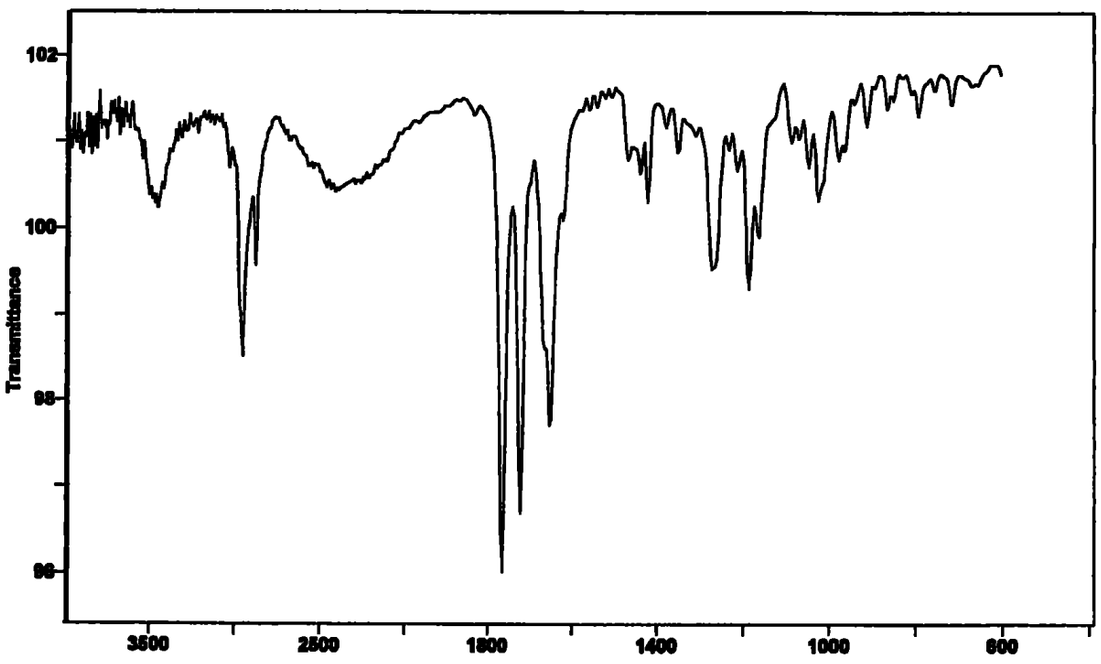


Figure 3-L

ethanol solvate



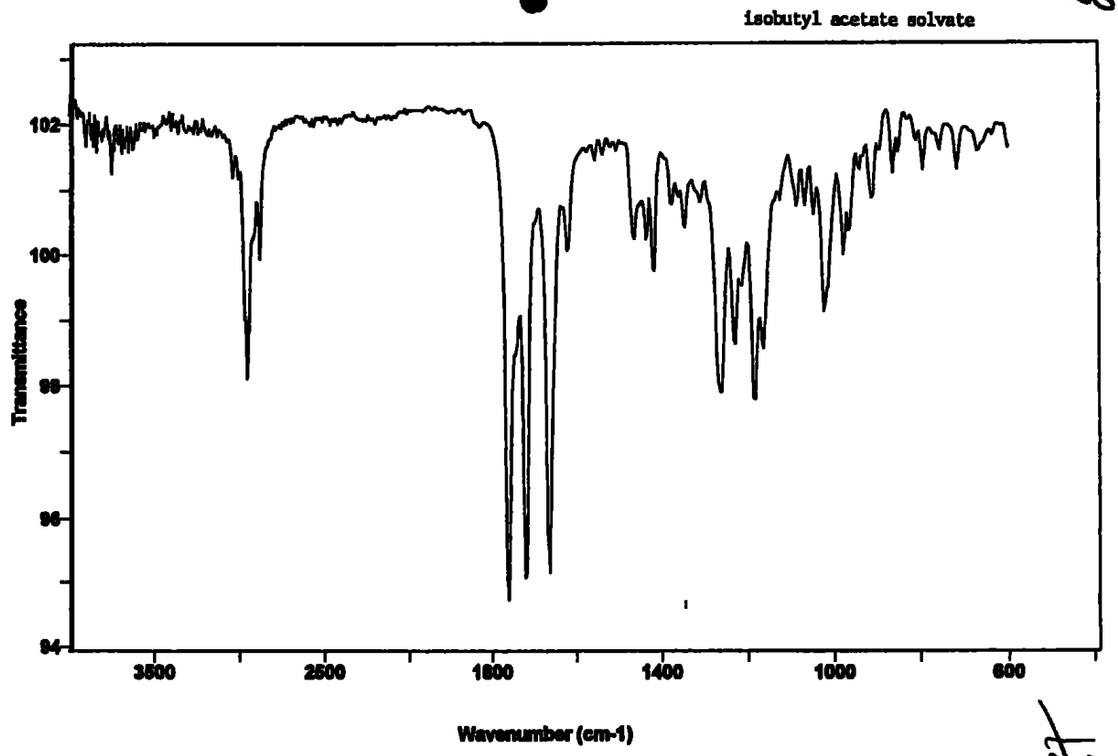

isobutyl acetate solvate
102
100
98
96
94
Transmittance
3500
2500
1800
1400
1000
600
Wavenumber (cm-1)

Figure 3-N

6088021 6269706

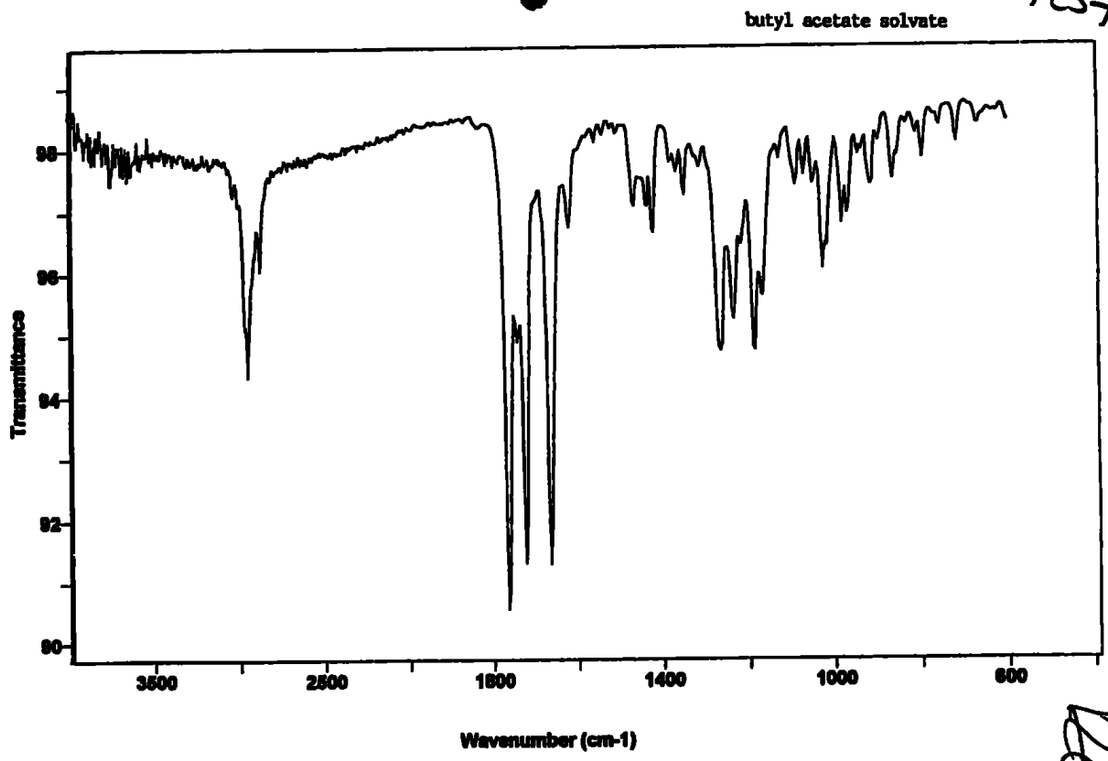
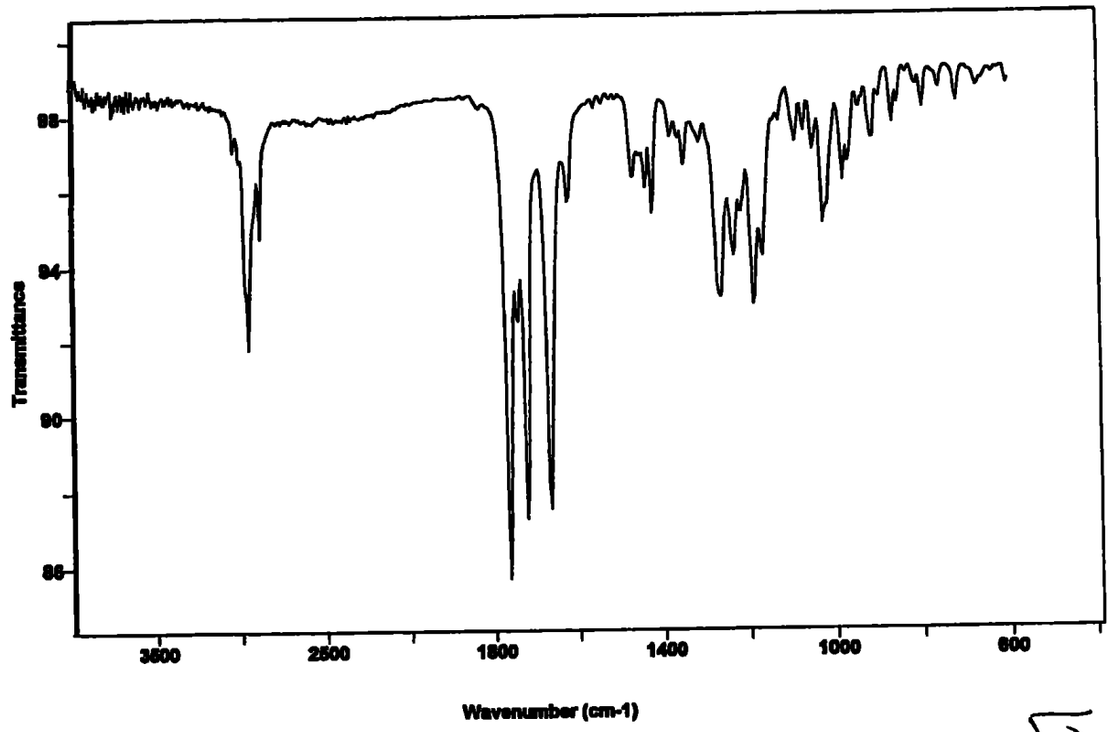


Figure 3-0

propyl acetate solvate



1258

1267

Figure 3-P

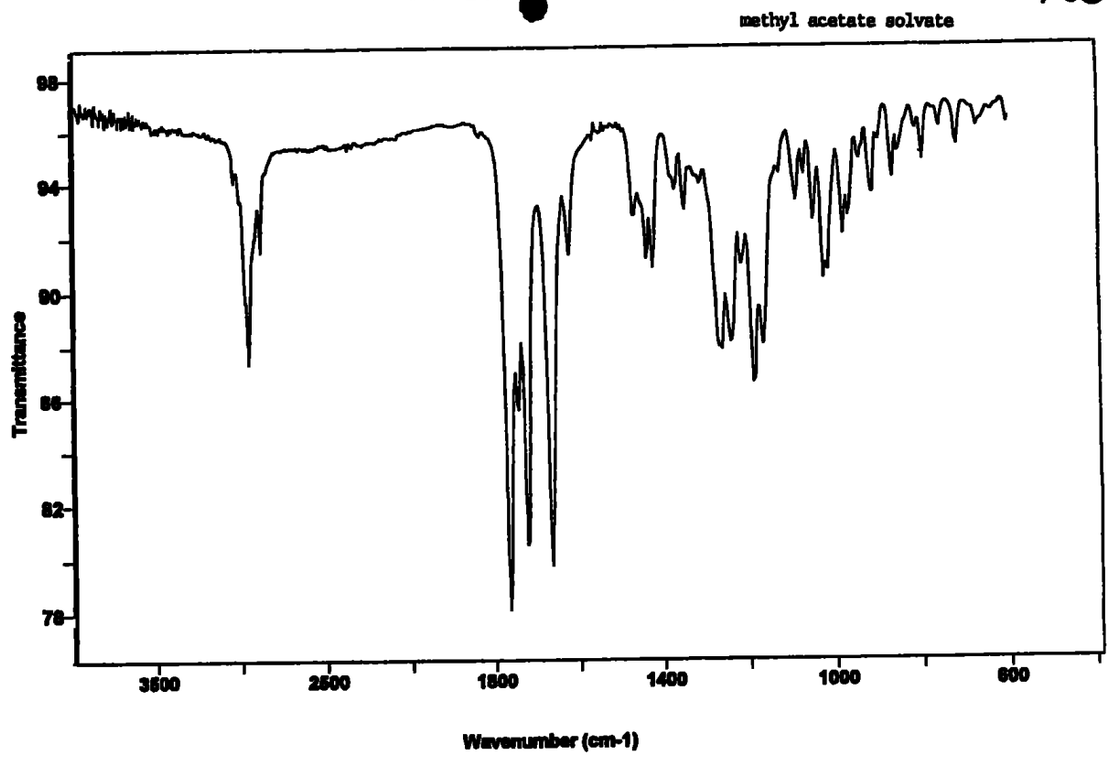

methyl acetate solvate
98
94
90
86
82
78
Transmittance
3500
2500
1800
1400
1000
600
Wavenumber (cm-1)

Figure 3-Q

66802 /J269109

propylene glycol solvate

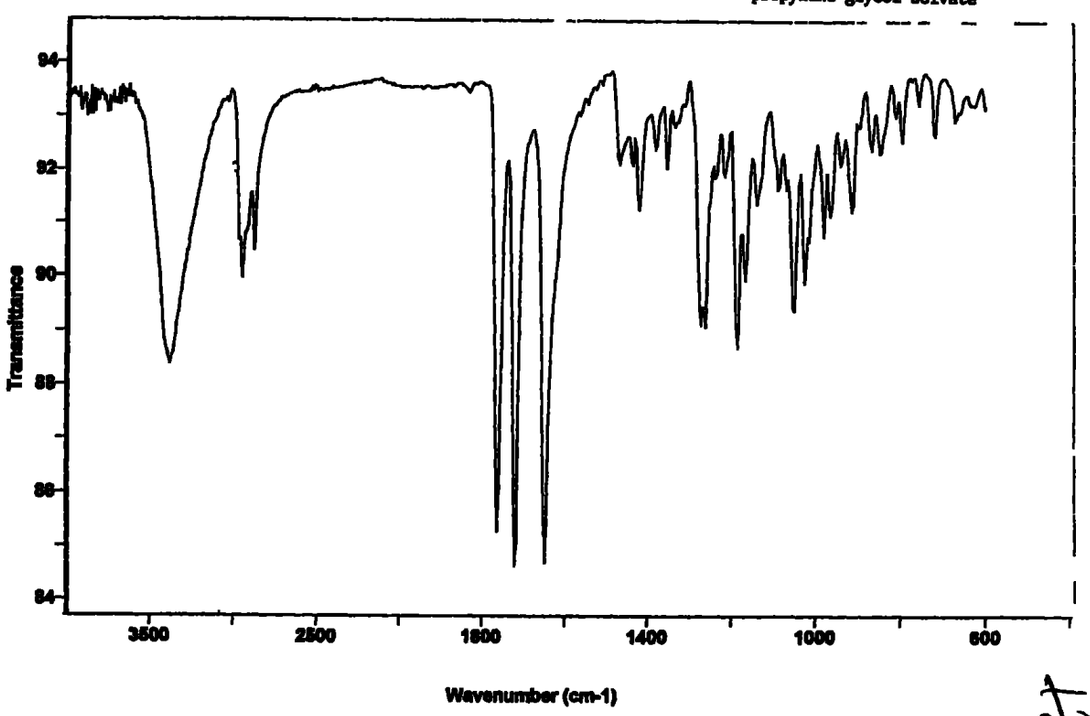
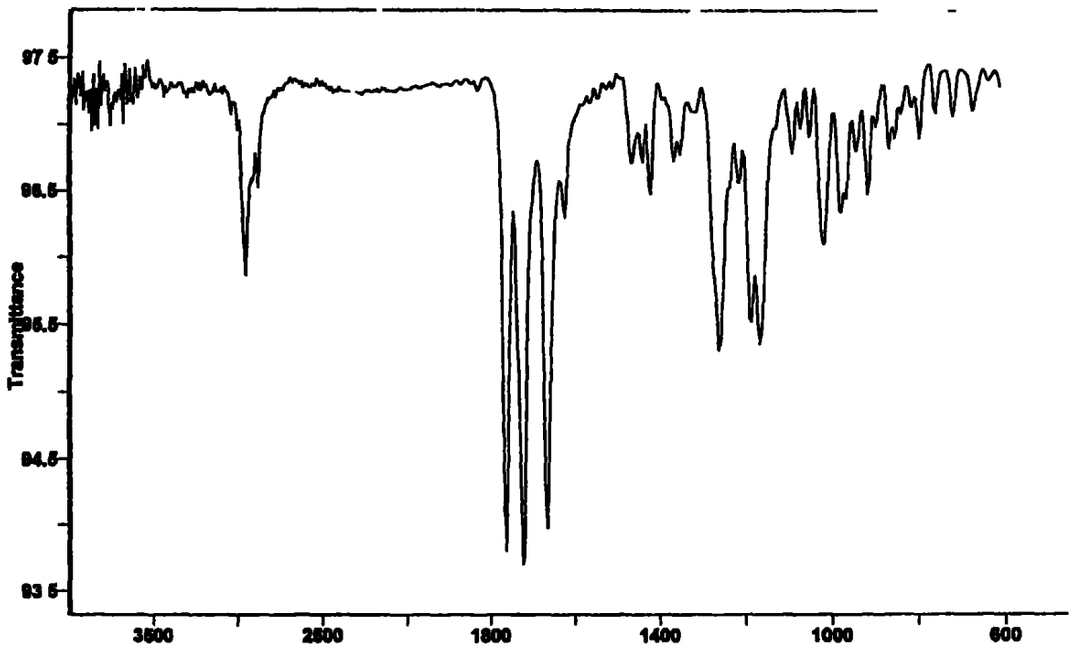


Figure 3-R

6688021 20269709

1261

t-butyl acetate solvate

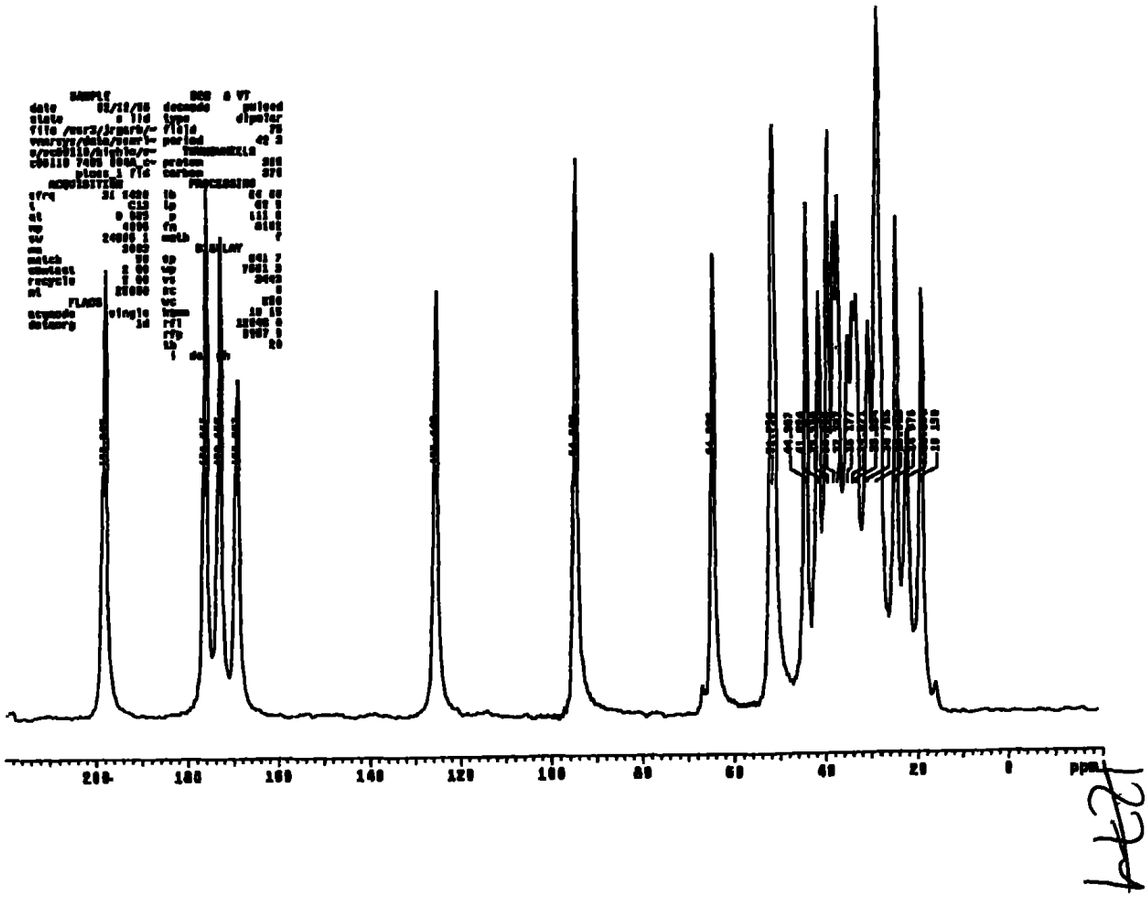


427

Figure 4

6018970 120899

1262



1221

Figure 5

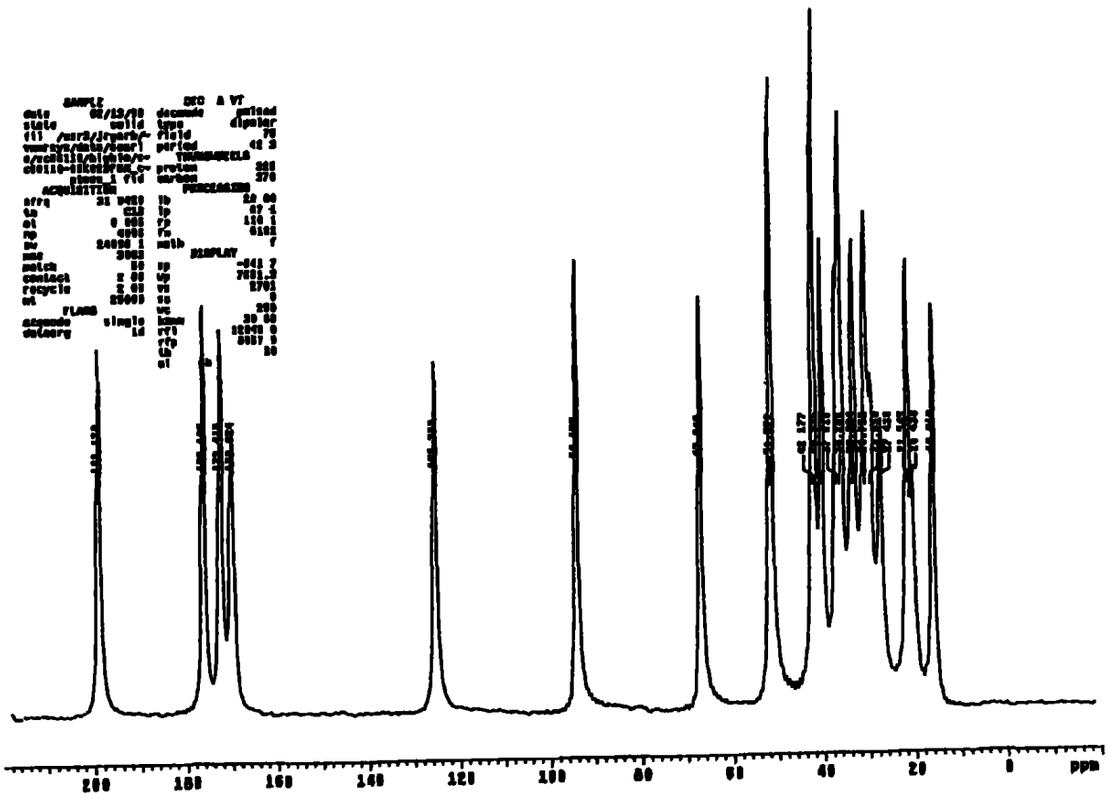


Figure 6-A

Size 3 3010 mg
Method 10 deg ramp to 300 deg

methyl ethyl ketone solvate

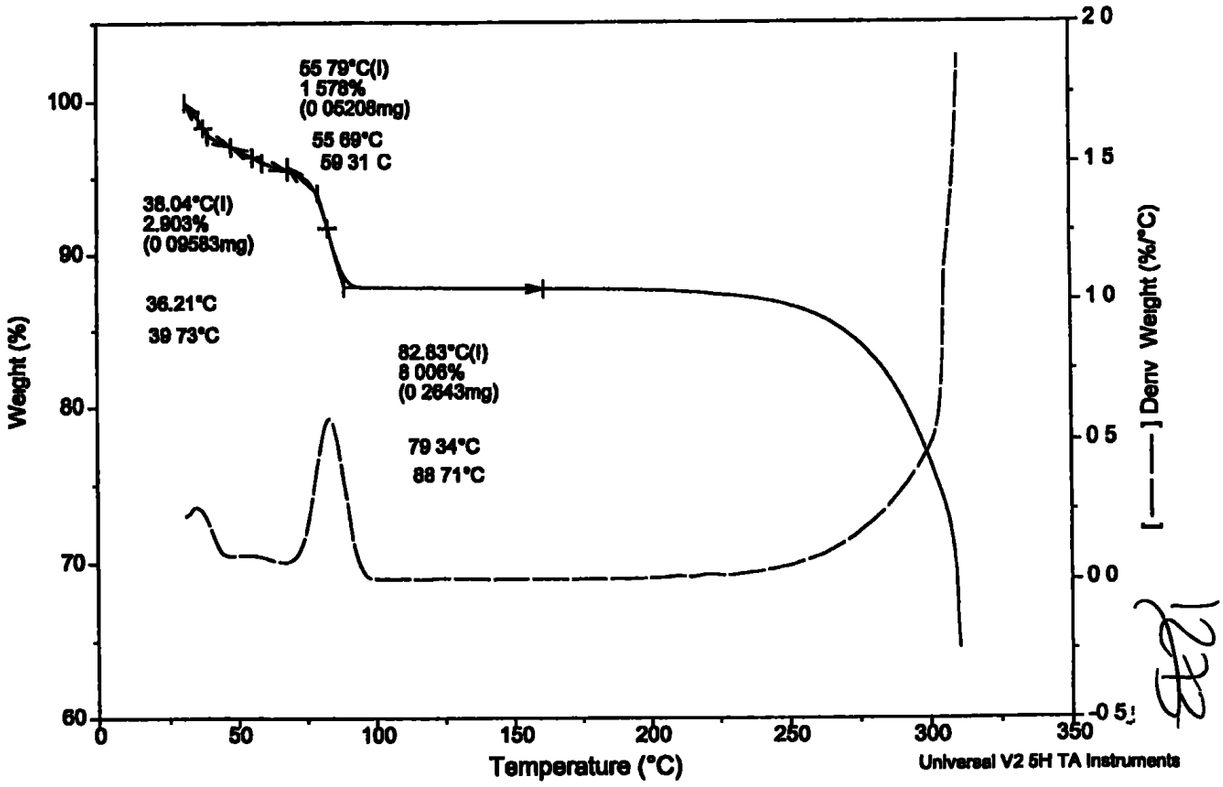


Figure 6-B

6660271 20269709

Size 13.4190 mg
Method 10 deg ramp to 300 deg

n-propyl alcohol solvate

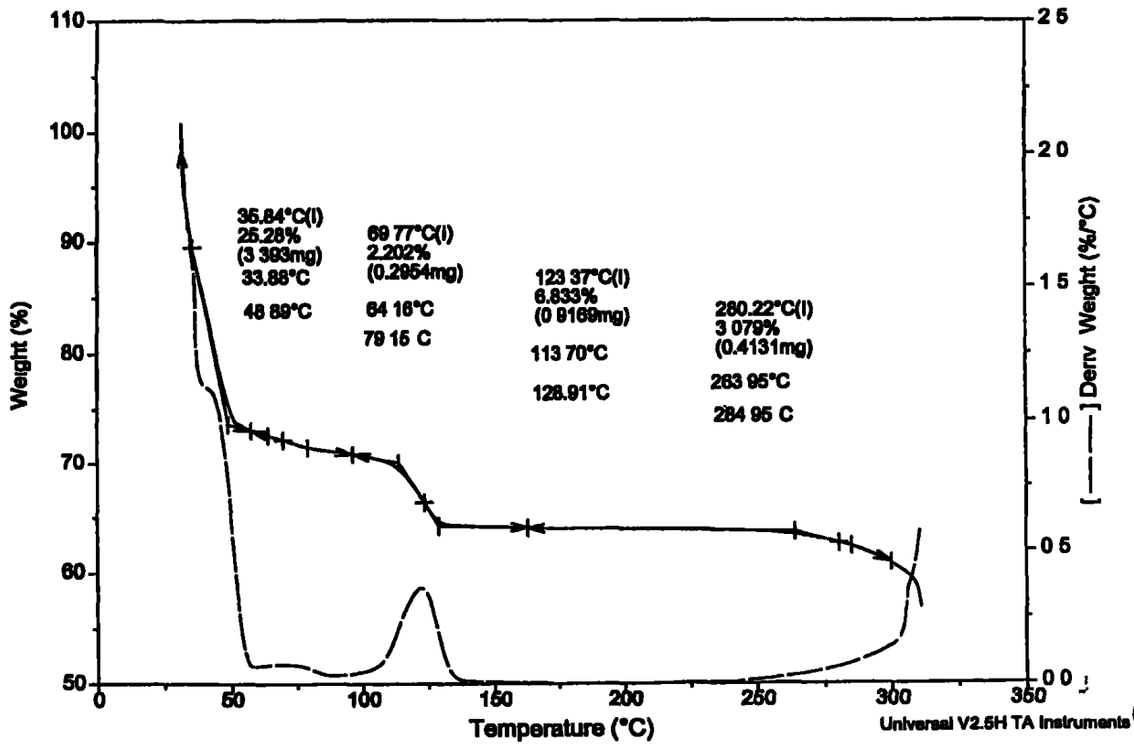


Figure 6-C

Size 14 9110 mg
Method 10 deg ramp to 300 deg

tetrahydrofuran solvate

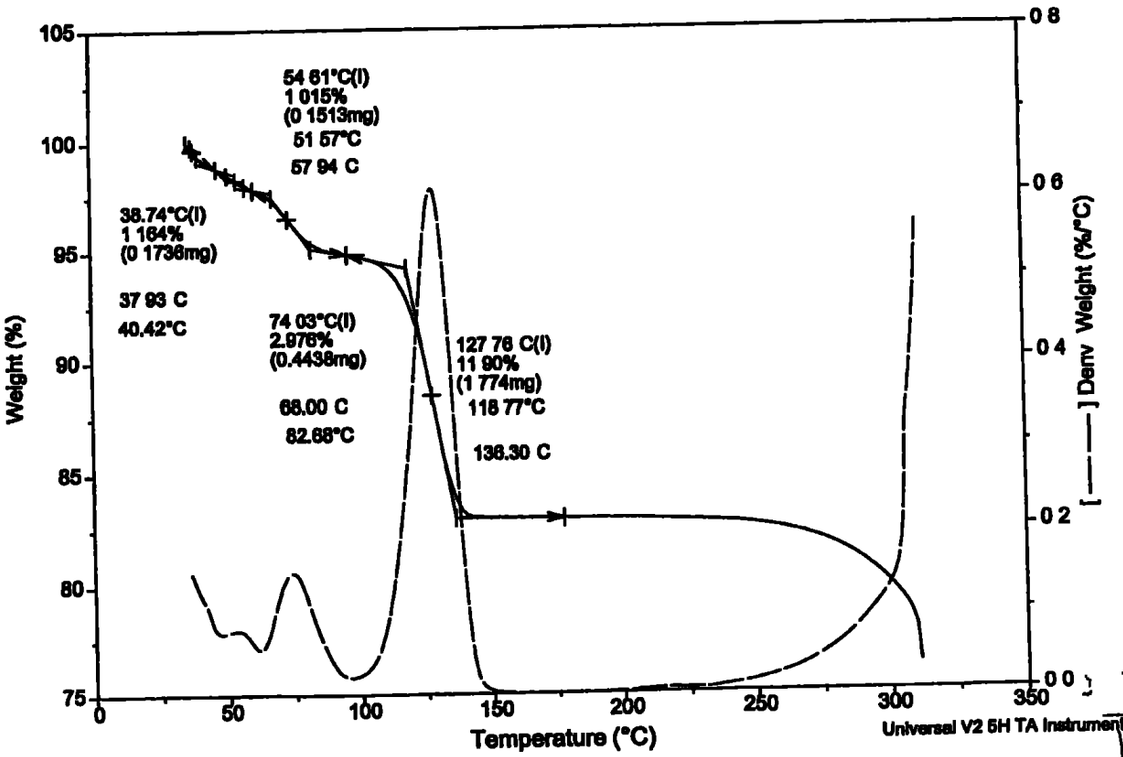


Figure 6-D

1267

Size 9 5030 mg
Method 10 deg ramp to 300 deg

ethyl propionate solvate

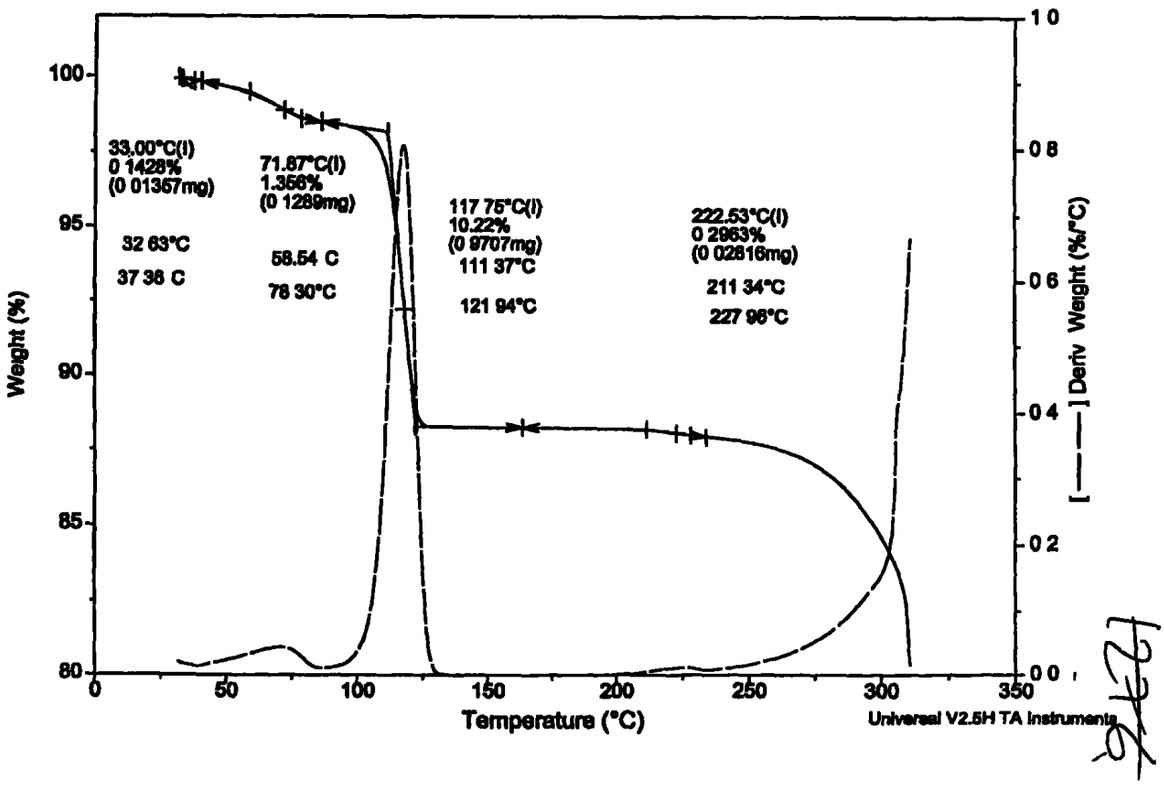


Figure 6-E

bb 021 20269709

Size 8.4270 mg
Method 10 deg ramp to 300 deg

acetic acid solvate

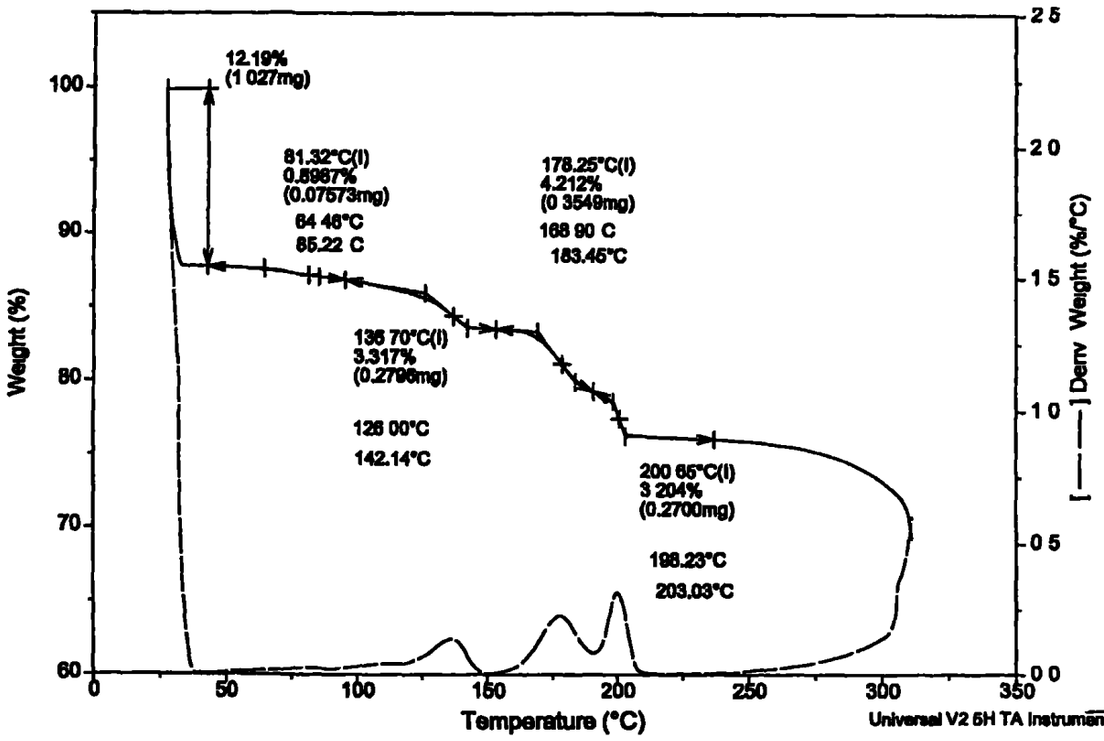


Figure 6-F

1269

Size 3.9730 mg
Method 10 deg ramp to 300 deg

chloroform solvate

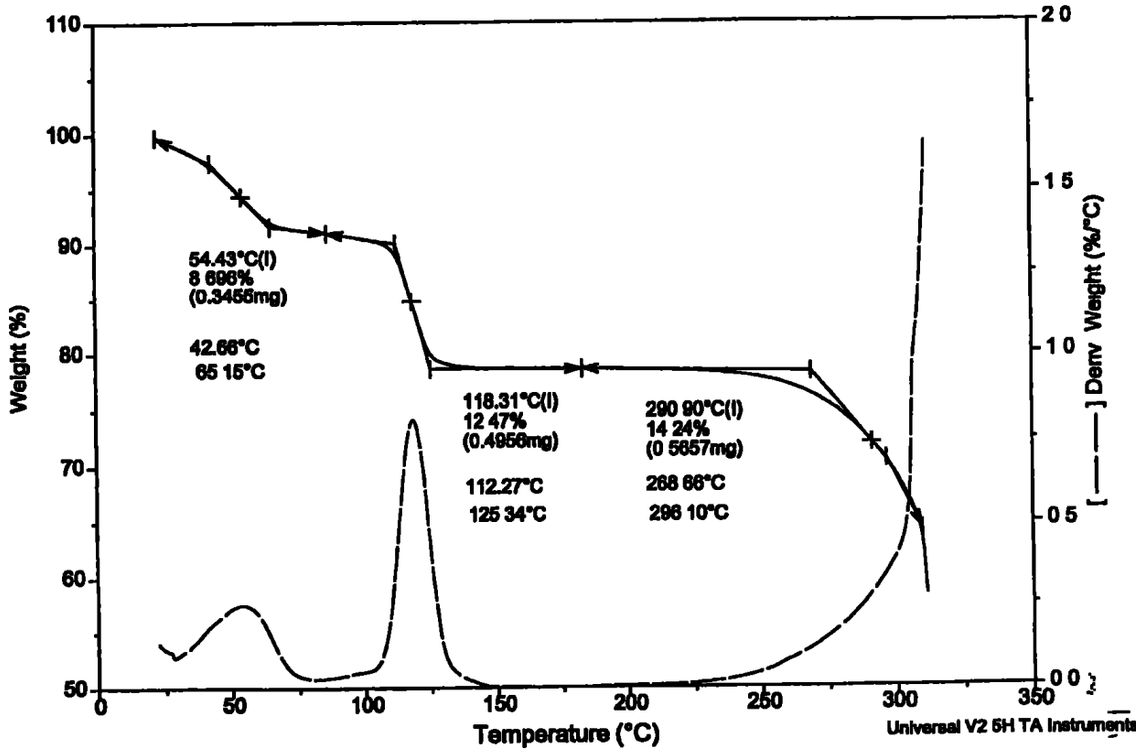


Figure 6-C

6068021 20269T09

Size 2.2040 mg
Method 10 deg ramp to 300 deg

acetone solvate

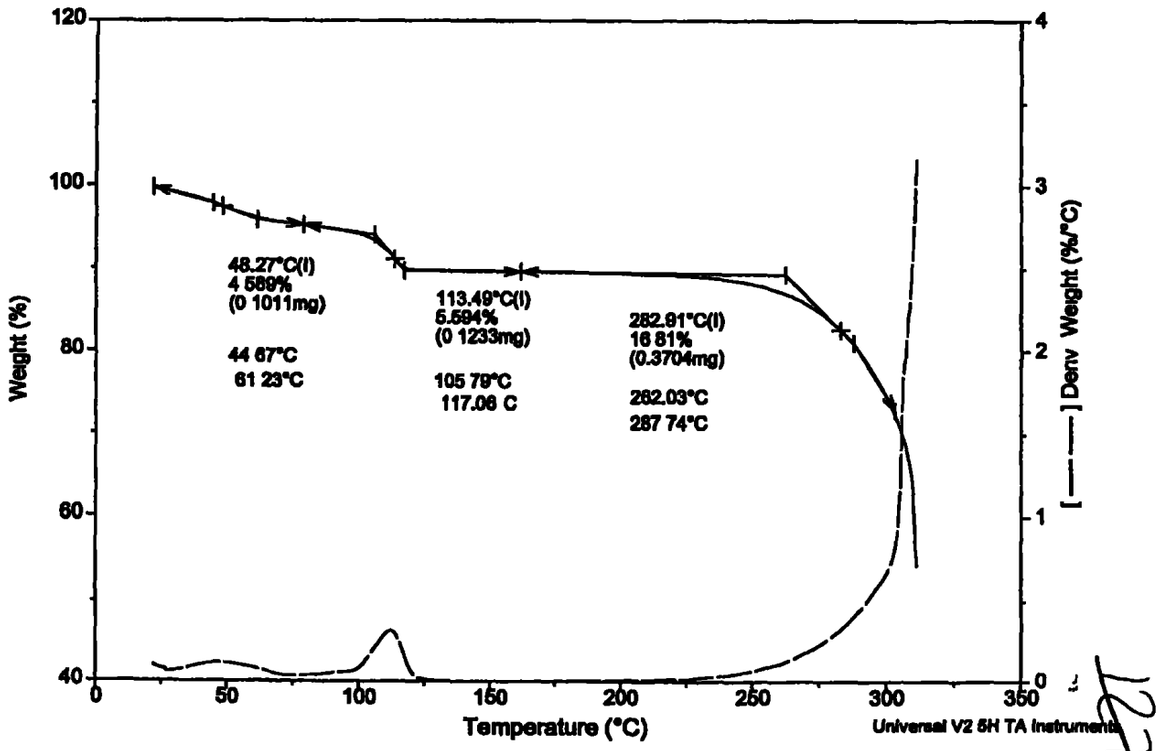


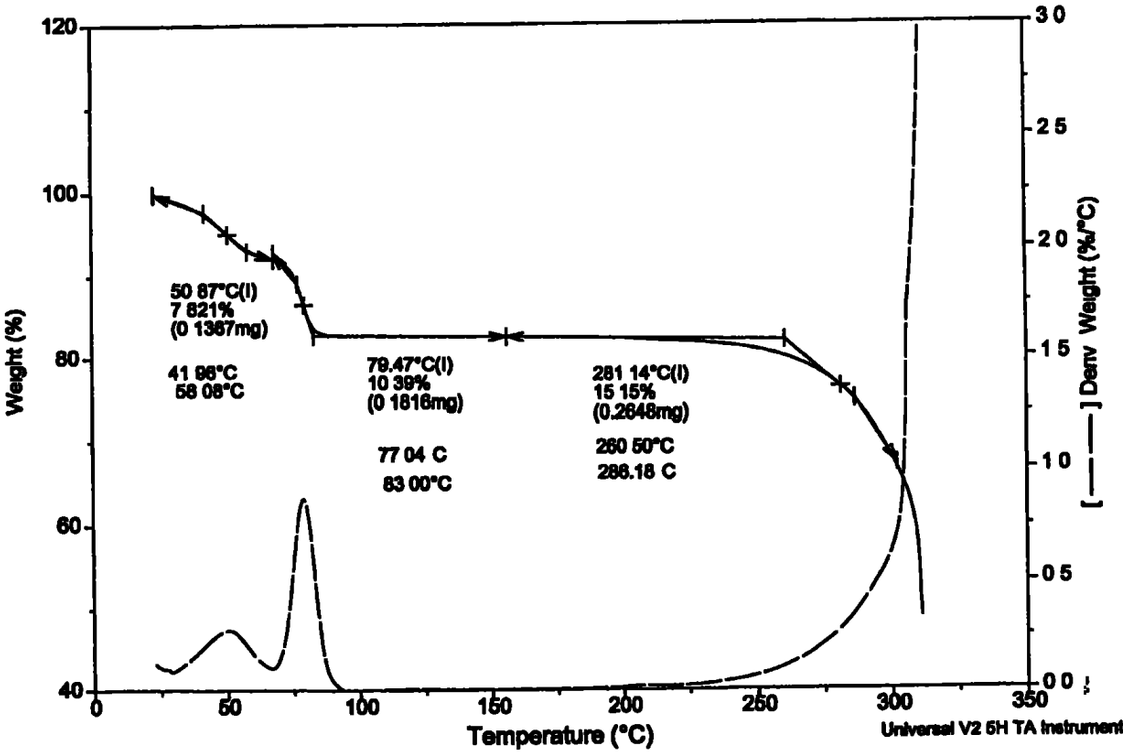
Figure 6-H

6688021 20269109

1271

Size 1 7480 mg
Method 10 deg ramp to 300 deg

toluene solvate



1280

Figure 6-I

Size 6 4350 mg
Method 10 deg ramp to 300 deg

isopropanol solvate

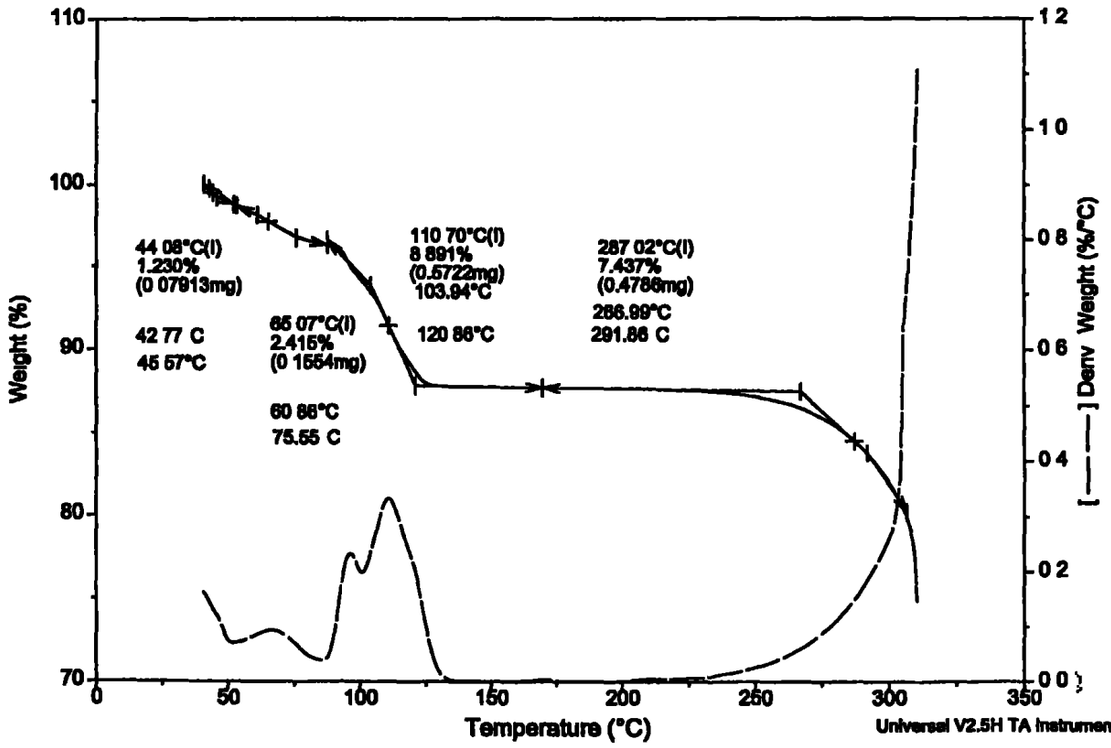


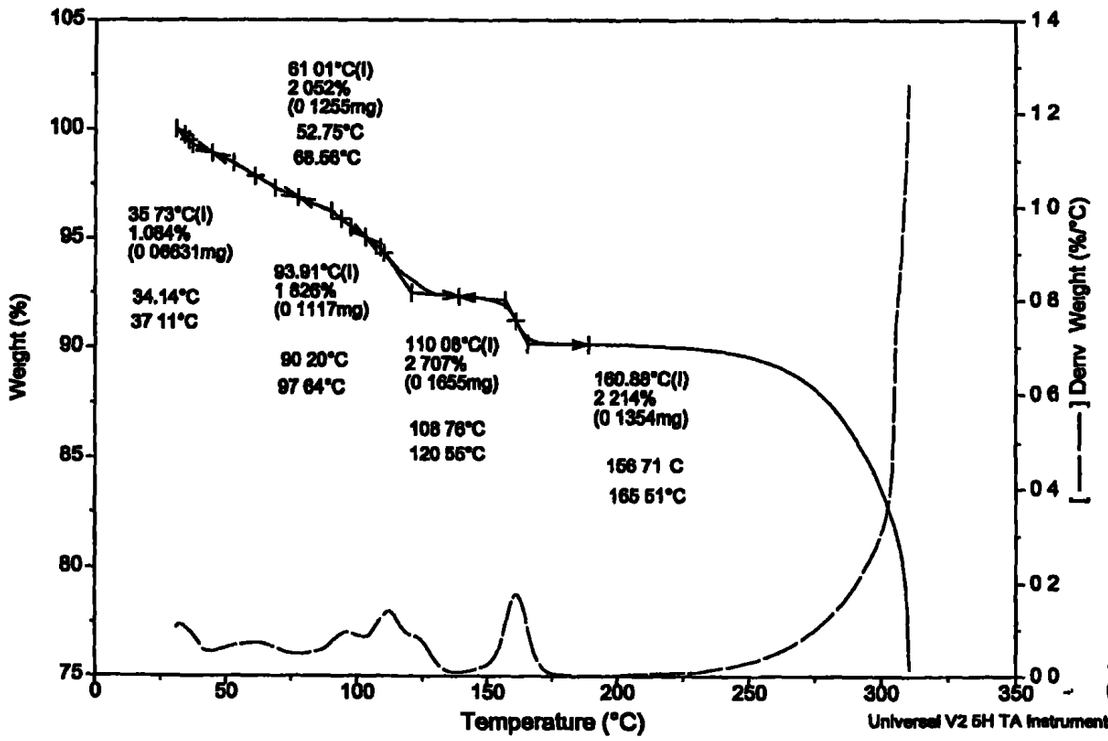
Figure 6-J

6580217 0269709

1273

Size 6 1160 mg
Method 10 deg ramp to 300 deg

ethanol solvate



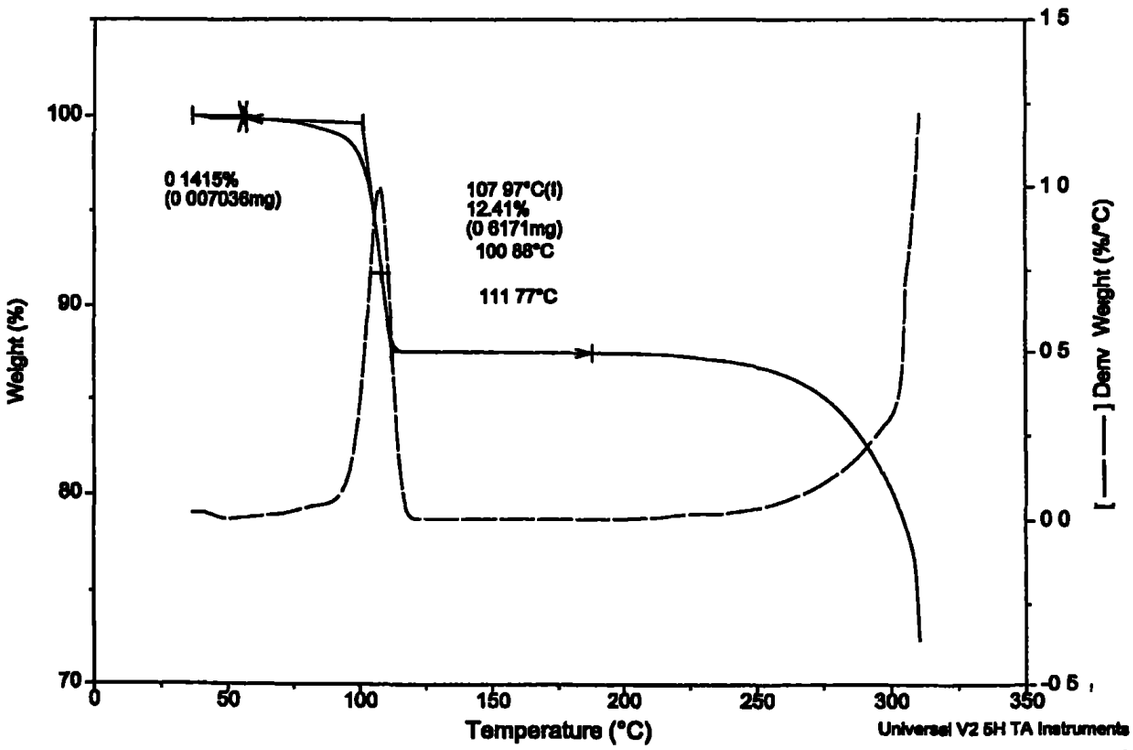
2827

Figure 6-K

1274

Size 4 9710 mg
Method 10 deg ramp to 300 deg

isobutyl acetate solvate



1274

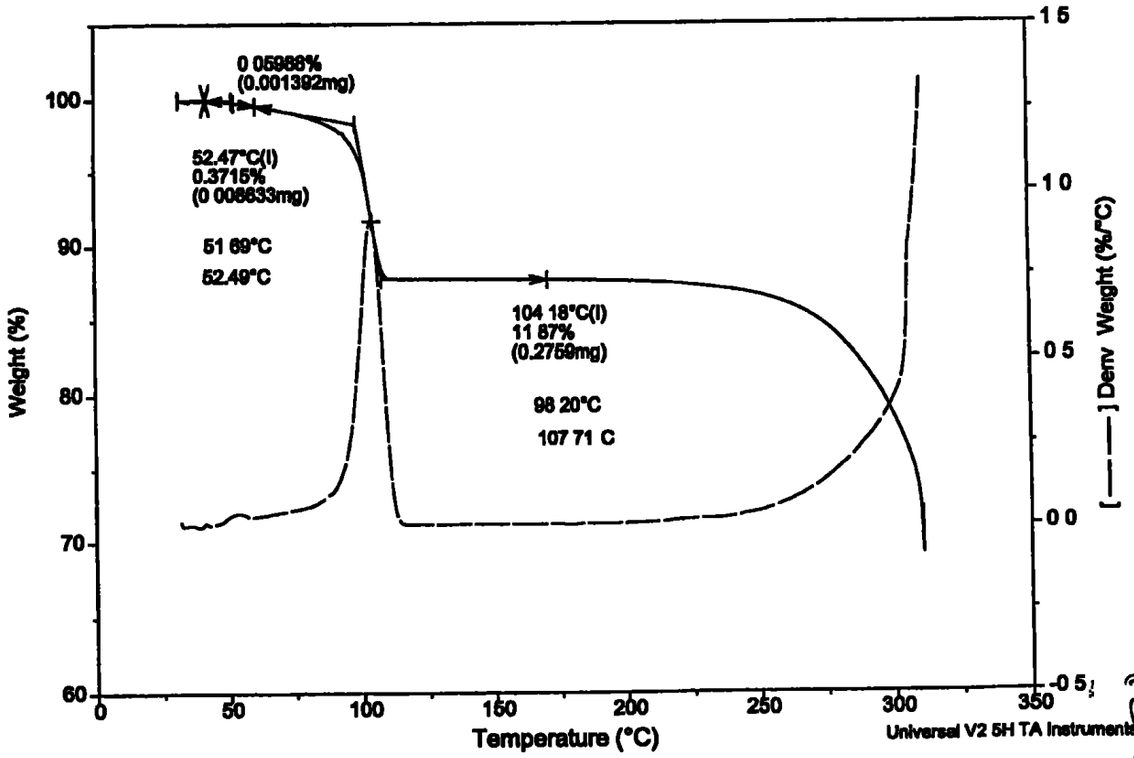
Figure 6-L

668021 0269109

1275

Size 2 3240 mg
Method 10 deg ramp to 300 deg

butyl acetate solvate



1284

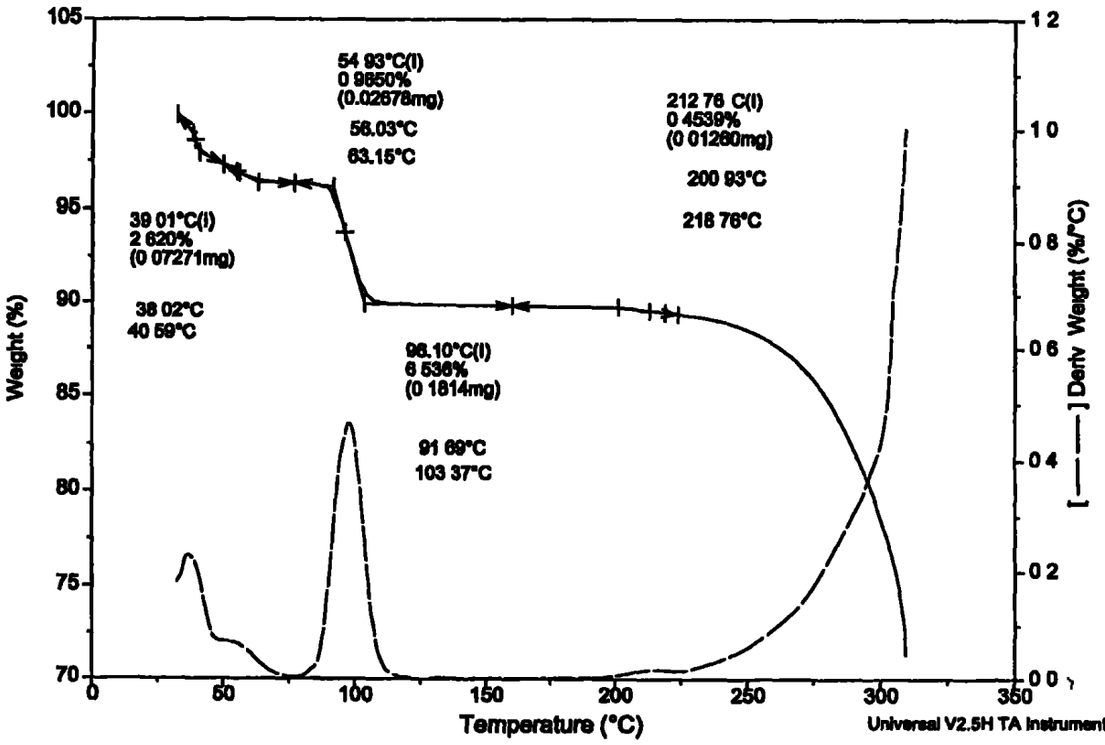
Figure 6-H

6688021 12088

1276

Size 2 7750 mg
Method 10 deg ramp to 300 deg

methyl acetate solvate



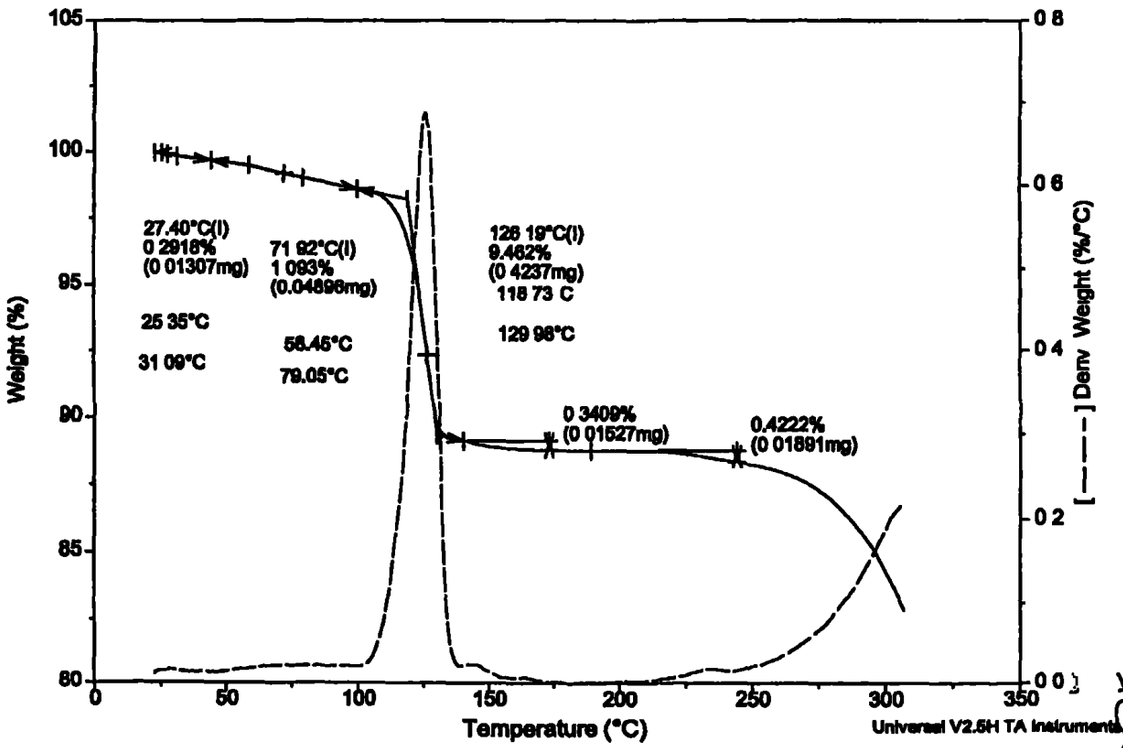
1275

Figure 6-N

1277

Size 4 4780 mg
Method TG 10D/M TO 300

propyl acetate solvate



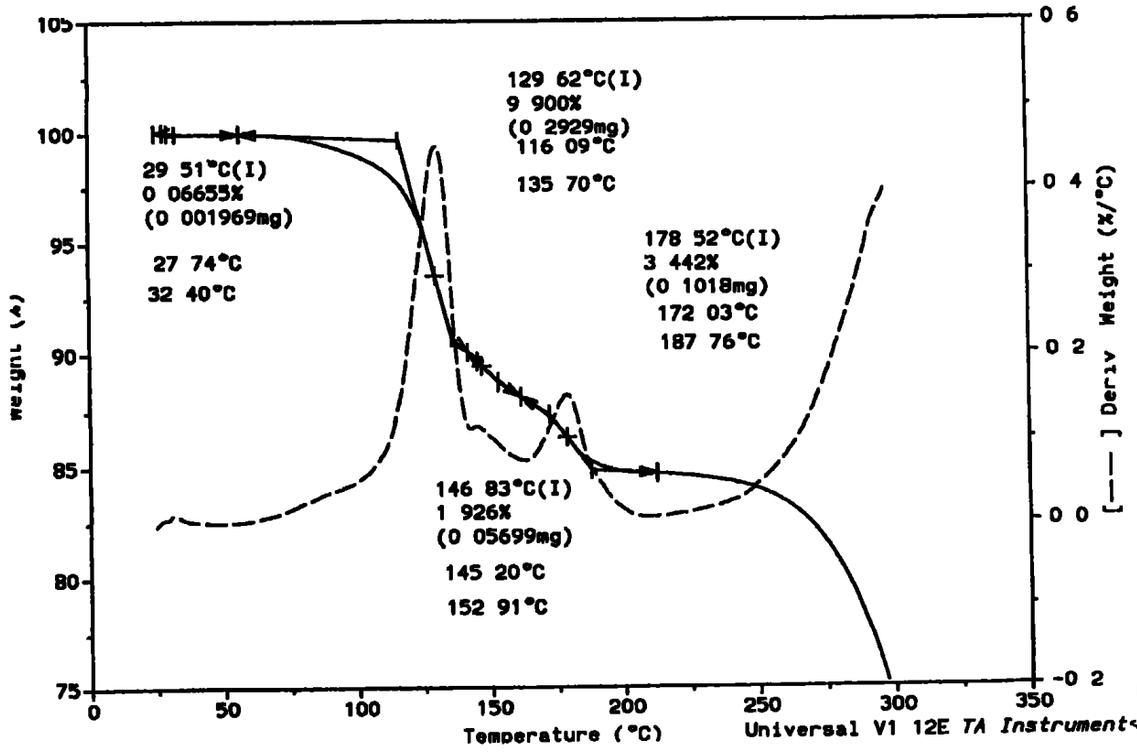
1286

Figure 6-0

1278

propylene glycol solvate

Size 2 9590 mg
Method TG 10D/M TO 300



Size 2 7050 mg
Method TG 10D/M TO 300

n-butanol solvate

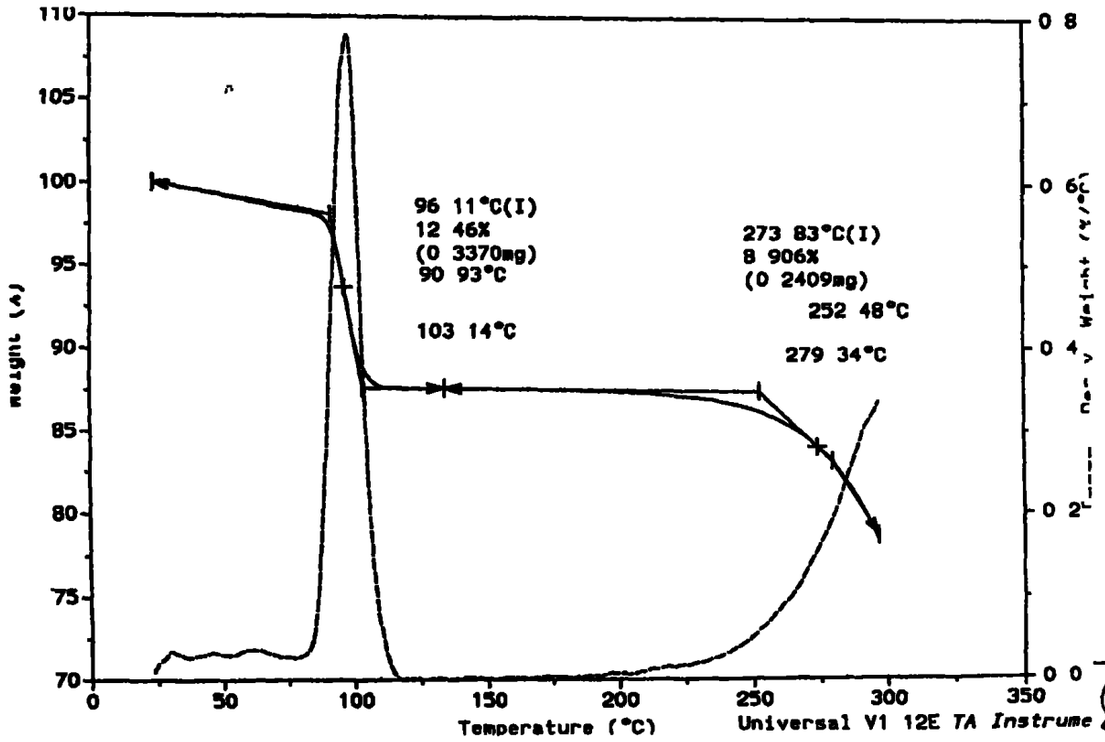


Figure 6-Q

1280

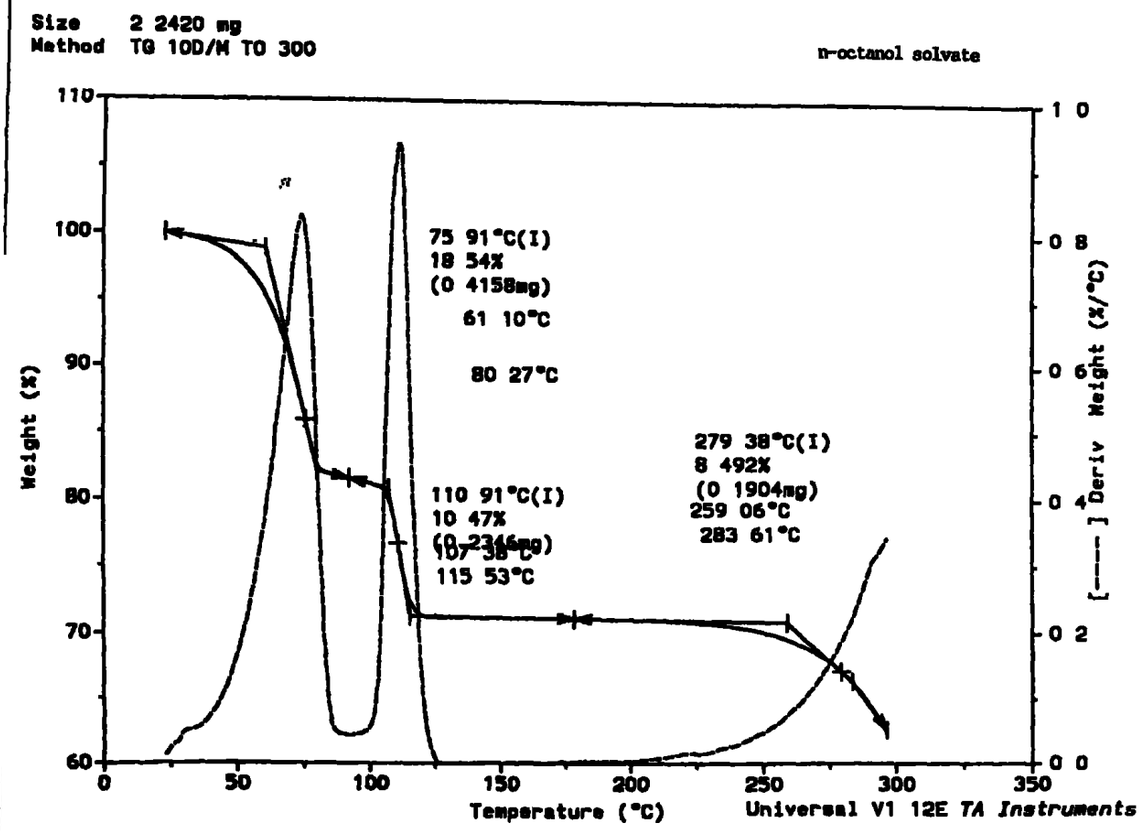


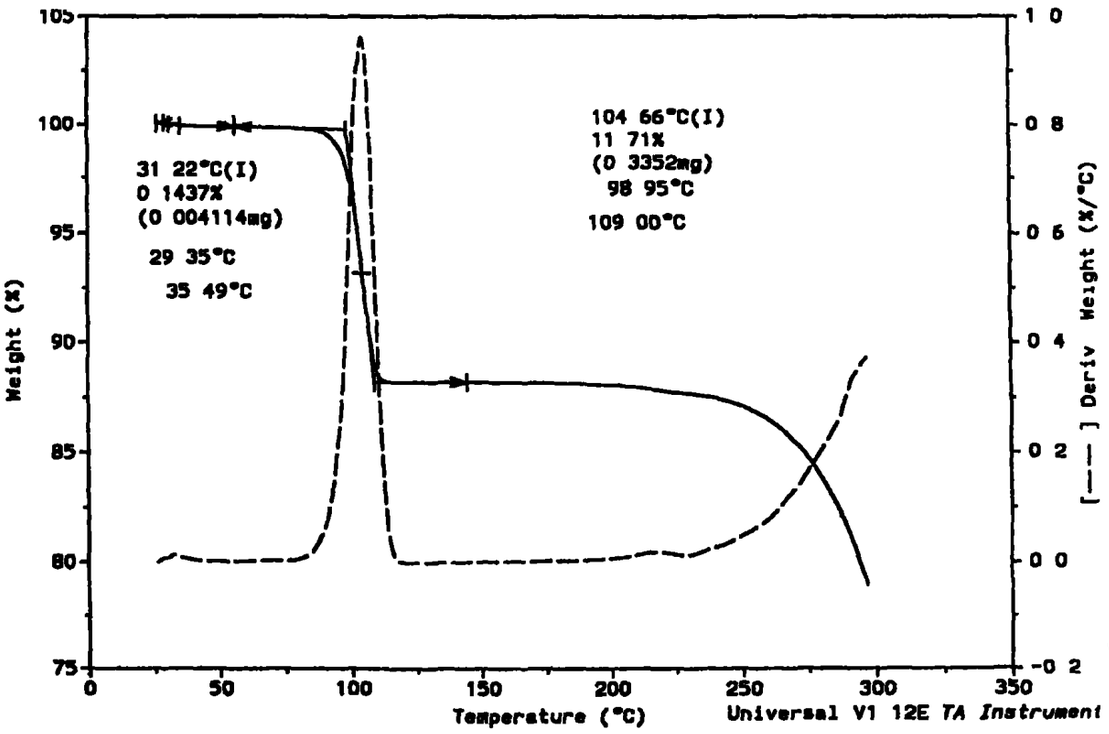
Figure 6-R

6015970 1208899

1281

Size 2 8630 mg
Method TG 10D/M TO 300

t-butyl acetate solvate

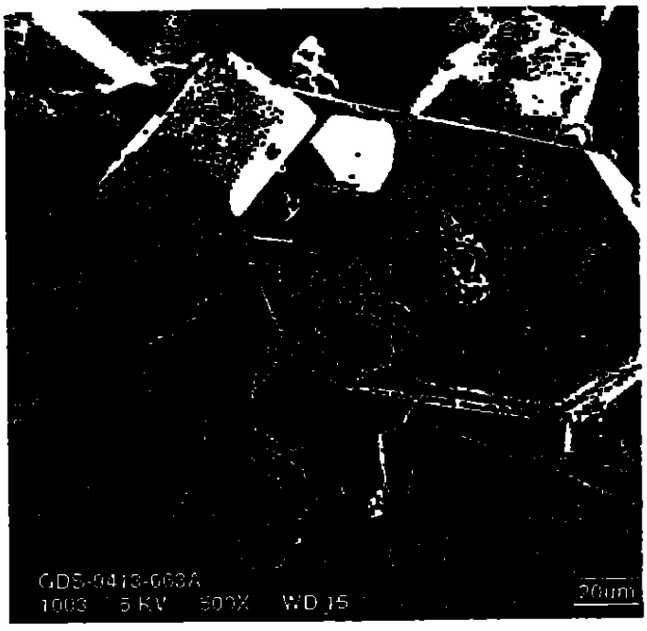


1281

1291
1282

Figure 7

Figure 8



99 0210270 EP 0 659 120

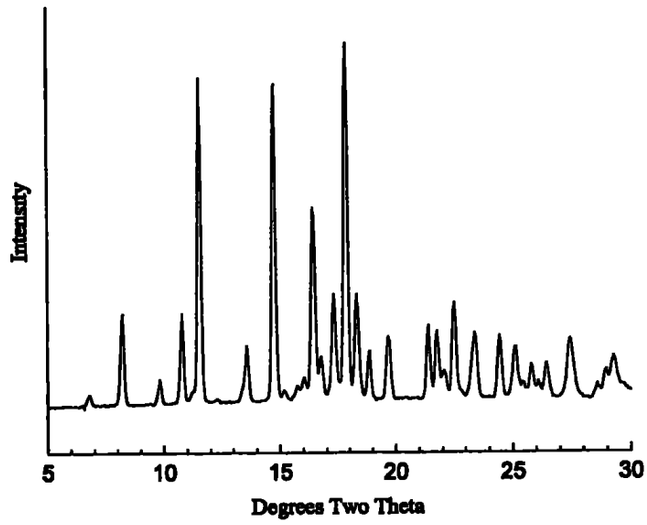


Figure 9

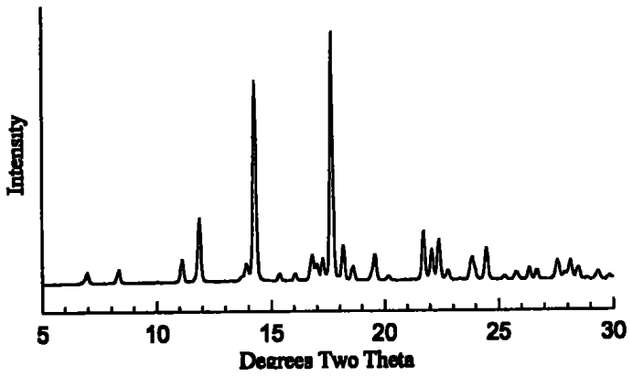


Figure 10

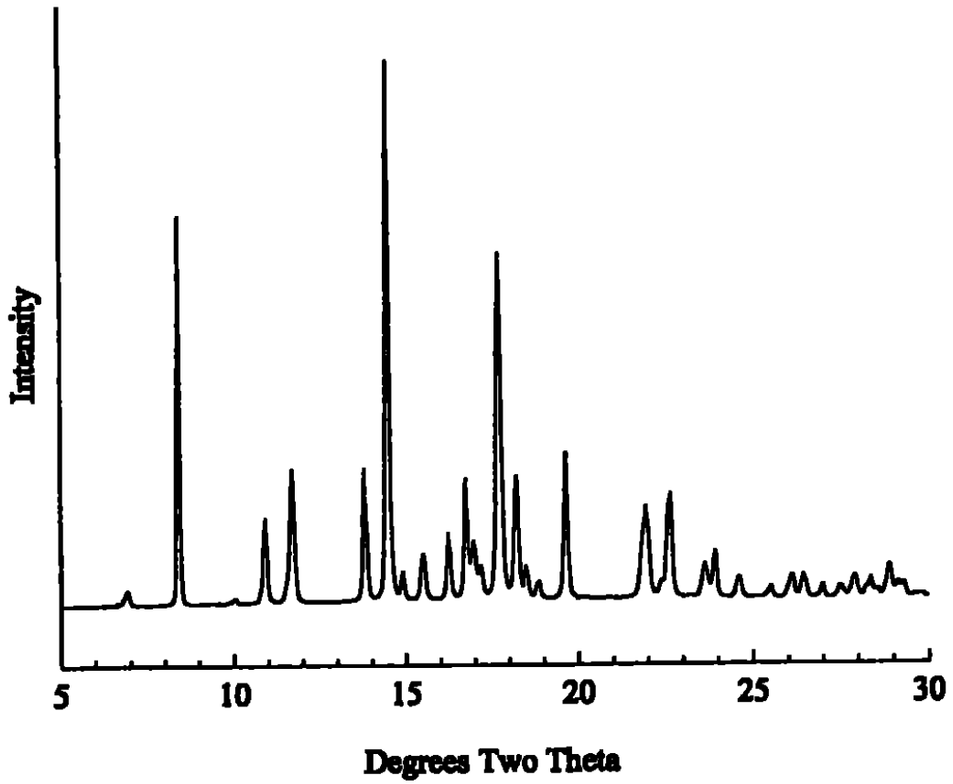


Figure 11

1295
1286

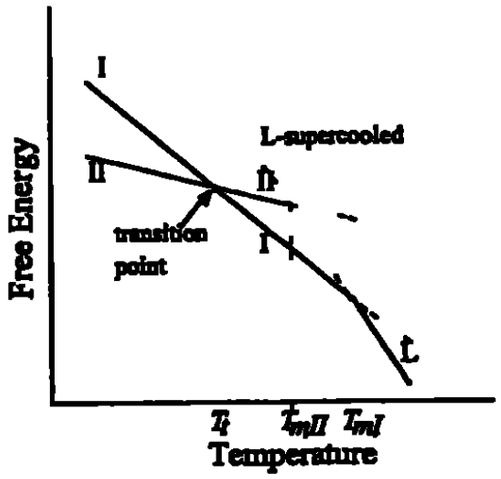


Figure 12

5015974 12/09/94

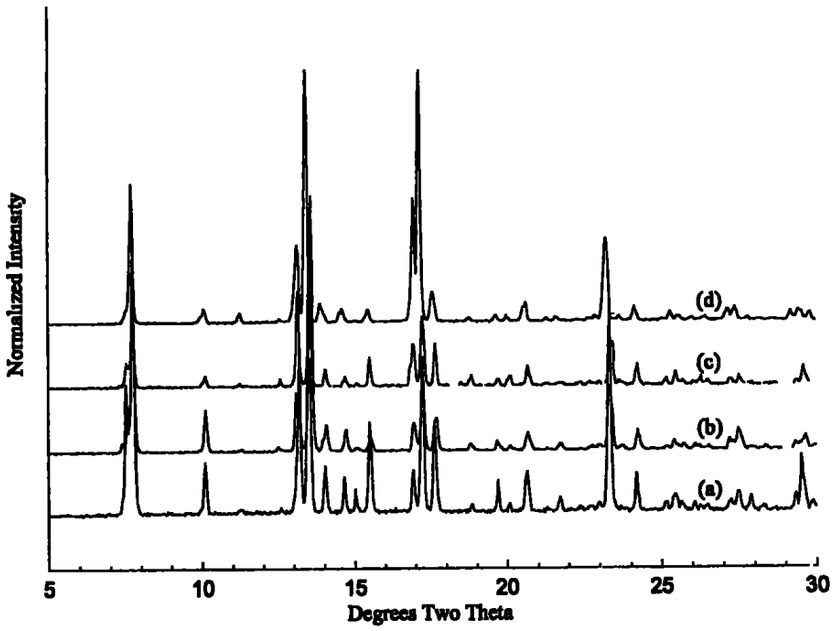


Figure 13

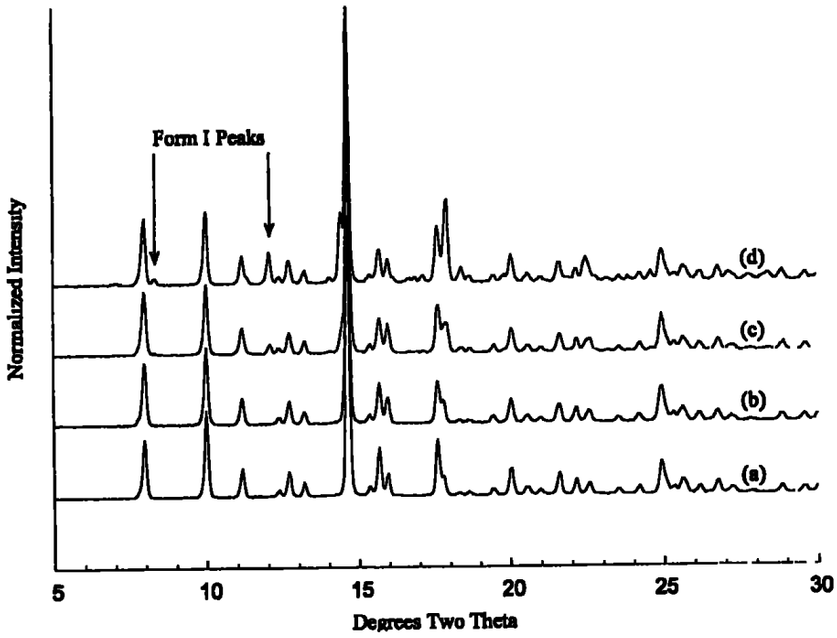

Form I Peaks
Normalized Intensity
(d)
(c)
(b)
(a)
5
10
15
20
25
30
Degrees Two Theta

Figure 14

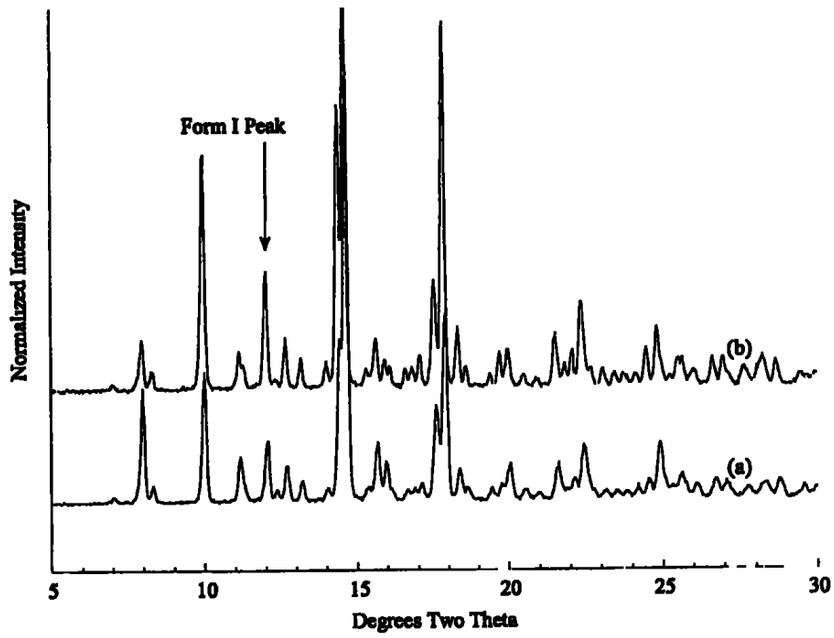

Form I Peak
Normalized Intensity
(b)
(a)
5
10
15
20
25
30
Degrees Two Theta

Figure 15

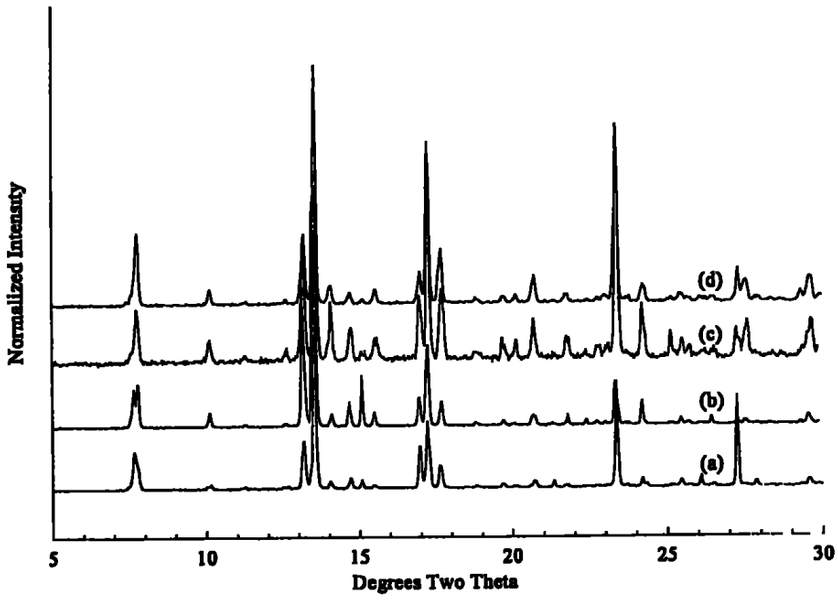


Figure 16

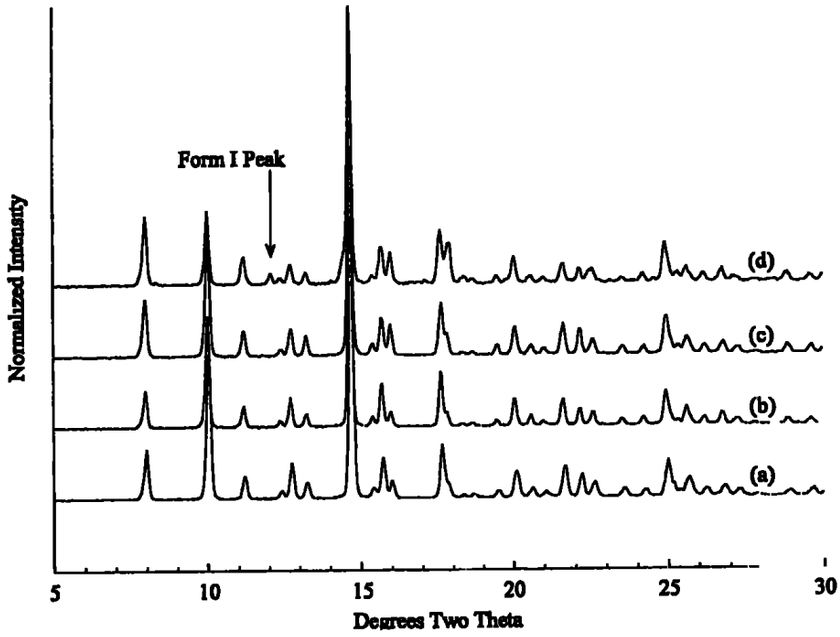

Form I Peak
Normalized Intensity
(d)
(c)
(b)
(a)
5
10
15
20
25
30
Degrees Two Theta

Figure 17

Figure 18

1301
1292

6015970 12/08/99

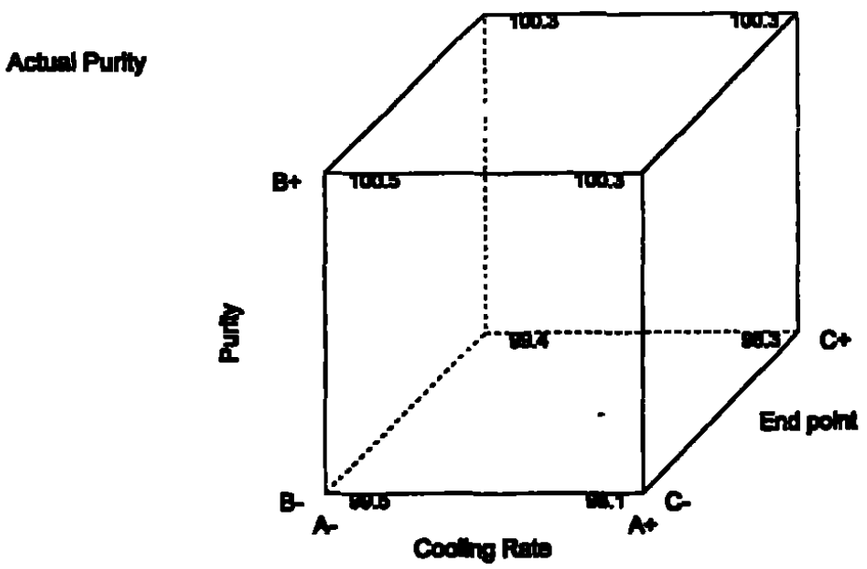
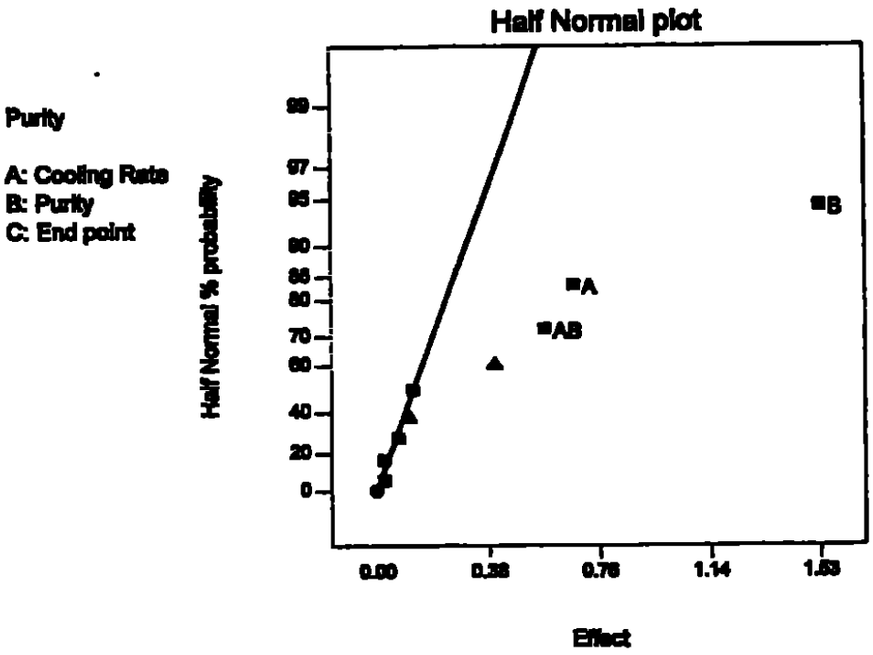


Figure 19

1302

1293



6668012 0169702

Figure 20

1303
1294

6668021 0759109

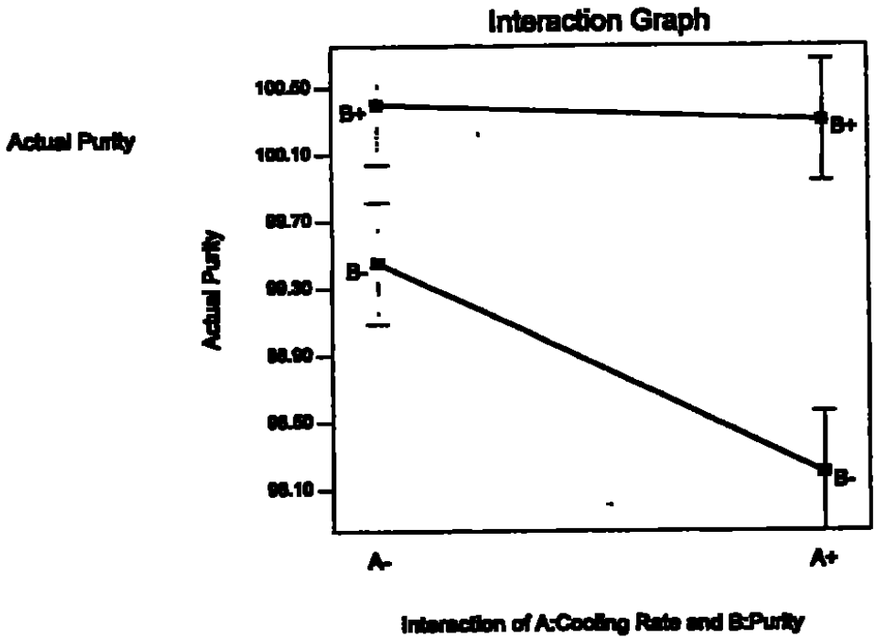


Figure 21

1304

1295

Actual Form

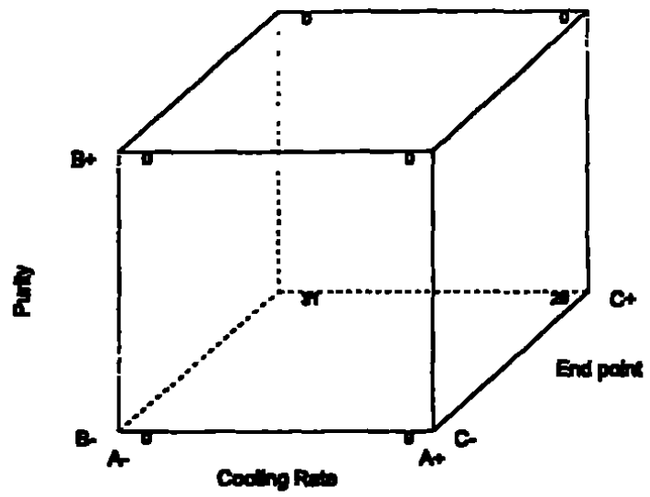


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Figure 22

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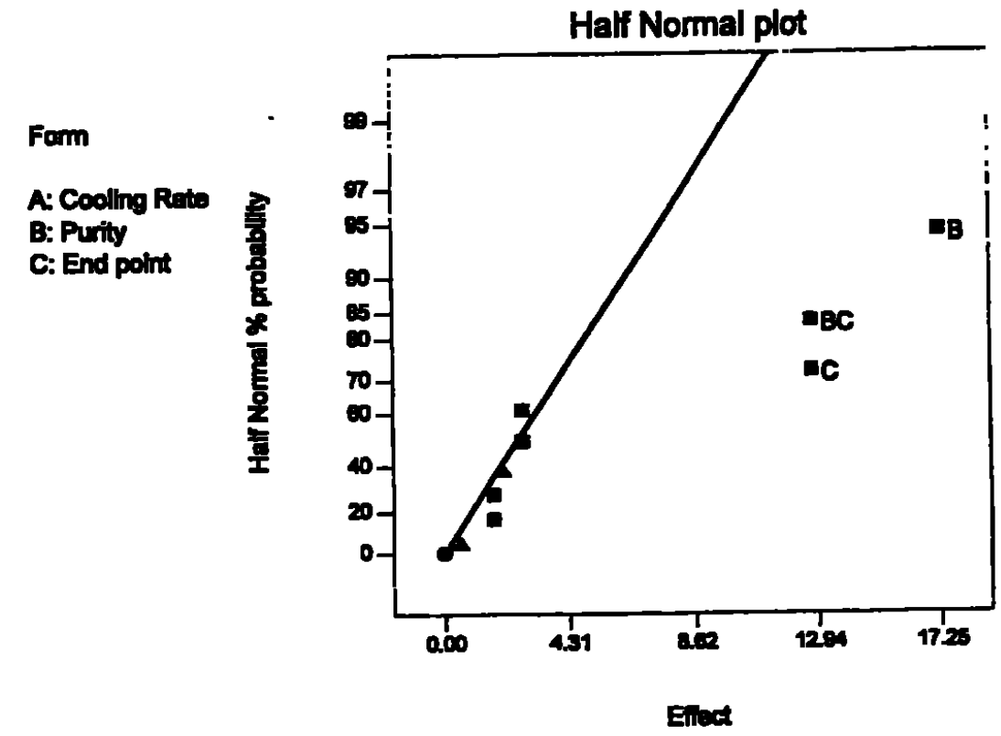

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Figure 23

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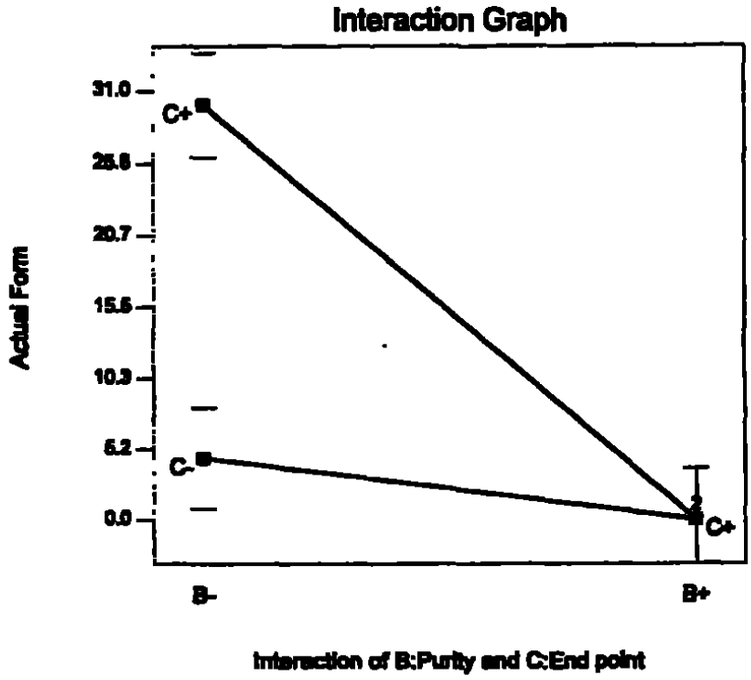
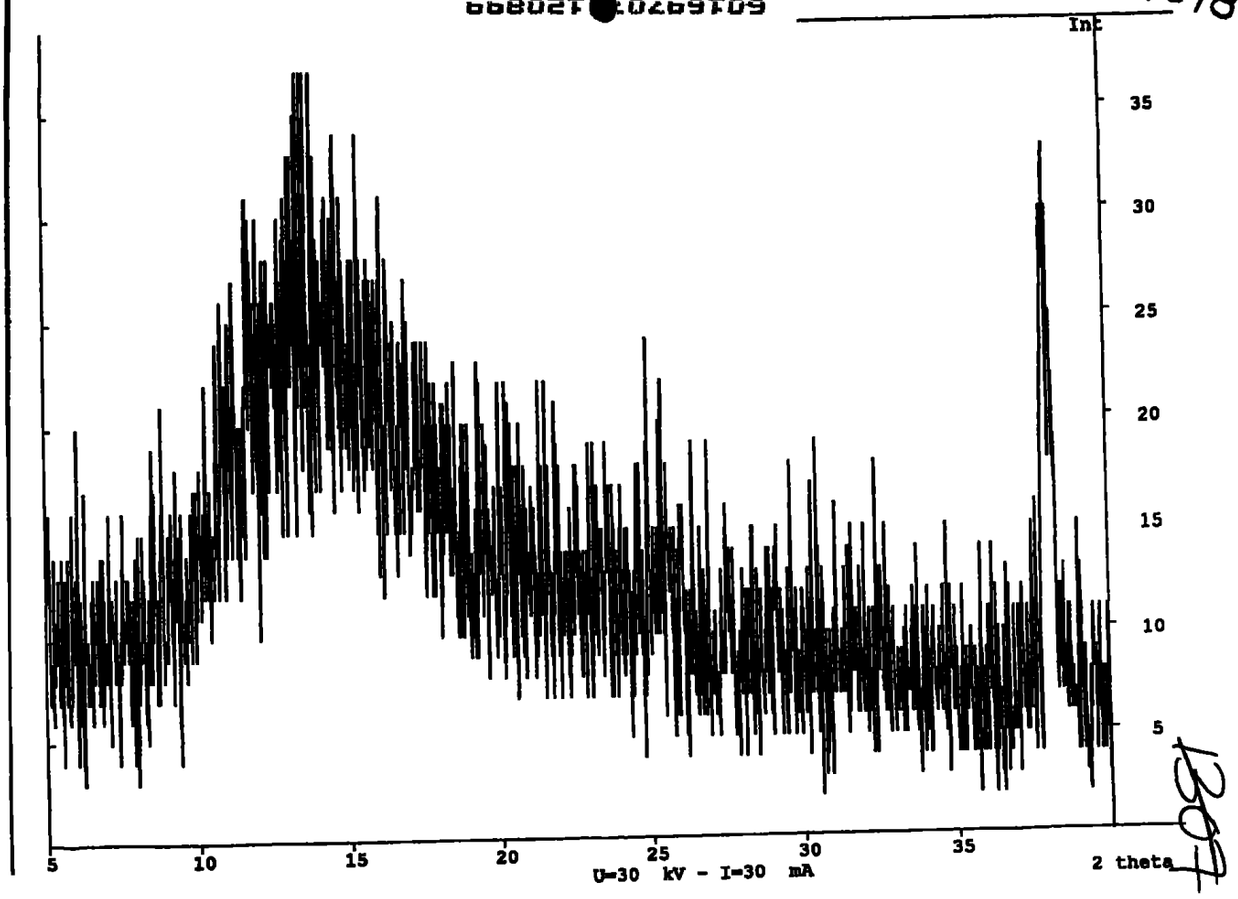
1297



6016970:0269709
6668027:12088

Figure 24

INEL

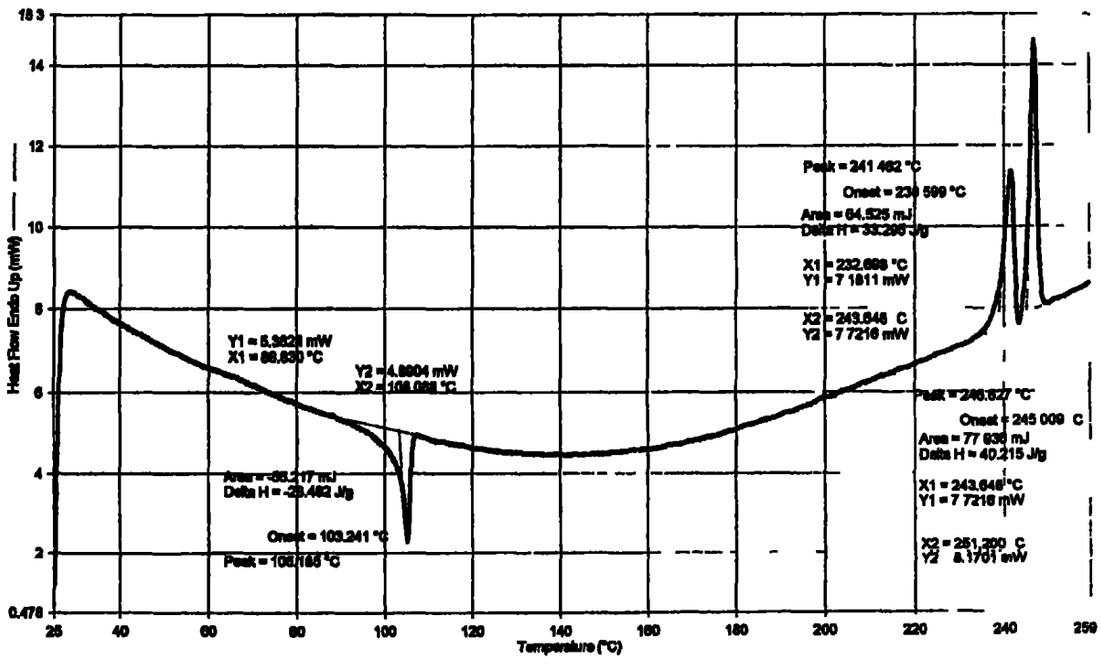


1298

Figure 25

1299

Sample Weight: 1.938 mg
Comment: Eplerenone
Amorphous by freeze dry in water



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LOS ESTADOS UNIDOS DE AMERICA

A TODOS AQUELLOS A QUIENES LA PRESENTE CONCIERNE:
DEPARTAMENTO DE COMERCIO DE LOS ESTADOS UNIDOS

Oficina de Patentes y Marcas de los Estados Unidos

2 de enero de 2001

LA PRESENTE ES PARA CERTIFICAR QUE LA ANEXA A ESTA ES UNA COPIA FIEL DE LOS REGISTROS DE LA OFICINA DE PATENTES Y MARCAS DE LOS ESTADOS UNIDOS DE LOS DOCUMENTOS DE LA SOLICITUD DE PATENTE IDENTIFICADA MAS ADELANTE QUE SATISFACEN LOS REQUISITOS PARA QUE SE OTORGUE UNA FECHA DE PRIORIDAD BAJO 35 USC 111..

SOLICITUD NUMERO: 60/169,707

FECHA DE PRESENTACION: ***8 de diciembre de 1999***

Por la Autoridad del
COMISIONADO DE PATENTES Y MARCAS

Firmado por

E. BORNETT

Funcionario Certificador

REIVINDICACIONES

1. Partículas que comprenden la forma cristalina de forma II de eplerenona, donde al menos 90% de dichas partículas tienen un tamaño de partícula menores a 15 micras.

2. Las partículas de la reivindicación 1 donde la forma cristalina tiene un patrón de difracción de polvos en rayos X con un pico de 8.0 ± 0.2 grados dos theta.

3. Las partículas de la reivindicación 1 donde la forma cristalina tiene un patrón de difracción de polvos en rayos X con un pico de 8.0 ± 0.2, 12.4 ± 0.2, y 13.3 ± 0.2 grados dos tetha.

4. Las partículas de la reivindicación 1 donde la forma cristalina tiene un patrón de difracción de polvos en rayos X sin un pico significante a 12.0 ± 0.2.

5. Las partículas de la reivindicación 1 donde la forma cristalina tiene un patrón de difracción de polvos en rayos X substancialmente como se muestra en la figura 1-B.

6. Las partículas de la reivindicación 1 donde la forma cristalina tiene un rango de fusión de 223°C hasta 234°C.

7. Las partículas de la reivindicación 1 donde la forma cristalina tiene un rango de fusión de entre 237°C hasta 242°C.

8. Las partículas de la reivindicación 1 donde la forma cristalina tiene un espectro infrarrojo con un pico en 1724 cm^{-1}.

9. Las partículas de la reivindicación 1 donde la forma cristalina tiene un espectro infrarrojo sin un pico significante en 1739cm^{-1}.

10. Las partículas de la reivindicación 1 donde la forma cristalina tiene un espectro infrarrojo sin un pico significante en 1399cm^{-1}.

11. Las partículas de la reivindicación 1 donde la forma cristalina tiene un espectro infrarrojo substancialmente como se muestra en la figura 3-B.

12. Las partículas de la reivindicación 1 donde la forma cristalina tiene un espectro ^{13}C NMR con resonancias de carbono en 67.1 ppm.

13. Las partículas de la reivindicación 1 donde la forma cristalina tiene un espectro ^{13}C NMR con resonancias de carbono de 16.0 ppm y hasta 67.1 ppm.

14. Las partículas de la reivindicación 1 donde la forma cristalina tiene un espectro ^{13}C NMR sin resonancias de carbono en 24.7 ppm.

15. Las partículas de la reivindicación 1 donde la forma cristalina tiene un espectro ^{13}C NMR substancialmente como se muestra en la figura 5.

16. Las partículas de la reivindicación 1 donde la forma cristalina tiene un de fusión de 223°C hasta 234°C, y un espectro infrarrojo con un pico de 1724 cm^{-1}.

17. Las partículas de la reivindicación 1 donde la forma cristalina tiene un rango de fusión de 223°C hasta 234°C, y un espectro infrarrojo con un pico de 1724 cm^{-1} y sin picos significantes en 1399 cm^{-1} y en 1739 cm^{-1}.

18. Las partículas de la reivindicación 1 donde la forma cristalina tiene un rango de fusión de 223°C hasta 234°C, y un espectro infrarrojo con un pico de 1724 cm^{-1} y sin picos significantes en 1399 cm^{-1} y en 1739 cm^{-1}; un patrón de difracción de polvos en rayos X con un pico de 8.0 ± 0.2 grados, y un espectro ^{13}C NMR con una resonancia de carbono en 67.1 ppm.

19. Las partículas de la reivindicación 1 donde la forma cristalina tiene un rango de fusión de 237°C hasta 242°C, y un espectro infrarrojo con un pico en 1724 cm^{-1}.

20. Las partículas de la reivindicación 1 donde la forma cristalina tiene un rango de fusión de 237°C hasta 242°C, y un espectro infrarrojo con un pico en 1722 cm^{-1} y sin un pico significante en 1739 cm^{-1}.

21. Las partículas de la reivindicación 1 donde la forma cristalina tiene un rango de fusión de 237°C hasta 242°C, y un espectro

infrarrojo con un pico en 1724 cm^{-1} y sin un pico significante en 1739 cm^{-1} y en 1739 cm^{-1}; un patrón de difracción de polvos en rayos X con un pico de 8.0 ± 0.2 grados, y un espectro ^{13}C NMR con una resonancia de carbono en 67.1 ppm

22. Las partículas de la reivindicación 1 donde la forma cristalina tiene un sistema de cristal monocíclico.

23. Las partículas de la reivindicación 1 donde la forma cristalina tiene un grupo espacial $P2_1$.

24. Las partículas de la reivindicación 1 donde la forma cristalina tiene valores de celda unitaria a, b y c de 8.78 Å, en 11.14 Å y en 11.06 Å, respectivamente.

25. Las partículas de la reivindicación 1 donde la forma cristalina tiene valores de celda unitaria α, β y γ de 90°, de 90.52°, y de 90°, respectivamente.

26. Partículas de forma de la reivindicación 1 donde al menos 90% de dichas partículas tiene un tamaño de partícula menor a 1 micra.

27. Las partículas de la reivindicación 26 donde al menos 90% de dichas partículas tienen un tamaño de partícula menor a 800 nm.

28. Las partículas de la reivindicación 26 donde al menos 90% de dichas partículas tiene un tamaño de partícula entre 10 nm hasta 1 micra.

29. Las partículas de la reivindicación 26 donde al menos 90% de dichas partículas tiene un tamaño de partícula entre 100 nm hasta 800 nm.

30. Las partículas de la reivindicación 26 donde al menos 90% de dichas partículas tiene un tamaño de partícula entre 200 nm hasta 800 nm.

31. Las partículas de la reivindicación 26 donde al menos 90% de dichas partículas tiene un tamaño de partícula entre 400 nm hasta 800 nm.

32. Eplererona que comprende la forma cristalina de acuerdo a la reivindicación 1, donde dicha eplerenona tiene una fase de pureza de al menos 50% de la Forma II.

33. La eplerenona de la reivindicación 32 donde dicha eplerenona tiene una fase de pureza de al menos 90% de la forma II.

34. La eplerenona de la reivindicación 32 donde dicha eplerenona es una fase substancialmente pura de forma II de eplerenona.

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35. Una composición farmacéutica que comprende una cantidad terapéuticamente efectiva de la forma de cualquiera de las reivindicaciones 1 a 35 en asociación con al menos un portador, adyuvante o diluyente farmacéuticamente aceptable.

36. La composición de la reivindicación 35 donde la única forma cristalina de eplerenona presente en la composición es una Forma II de eplerenona.

37. Un método para tratar o prevenir una condición mediada de aldosterona o un desorden en un sujeto, dicho método comprende administrar a un sujeto que tiene o es susceptible a dicha condición o desorden una cantidad terapéuticamente efectiva con una forma de acuerdo a la reivindicación 1.

38. El método de la reivindicación 37 para ser usado en el tratamiento de hiperaldosteronismo.

39. El método de acuerdo a la reivindicación 37 para ser usado en el tratamiento de hipertensión.

40. El método de la reivindicación 37 para ser usado en el tratamiento de fallas de corazón.

41. El método de acuerdo a la reivindicación 37 para ser usado en el tratamiento de cirrosis en el hígado.

42. El método de la reivindicación 37 para ser usado en el tratamiento de una condición seleccionada de un grupo que consiste de exceso de colágeno/fibrosis, hipertrofia benigna prostática y depresión.

COLO 12482-801-001-5

JJC-ID-6

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PR 249535

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

January 05, 2001

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE UNDER 35 USC 111.

APPLICATION NUMBER: ***60/169,807***
FILING DATE: ***December 08, 1999***

By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

H. PHILLIPS
Certifying Officer

12-09-99

BSC
APPROVED

Express Mail Label Number EL523767125US
Attorney Docket Number SRL 6177 (C-3256)

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

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   RESIDENT (CITY AND EITHER STATE OR FOREIGN COUNTRY)

[x] Additional inventors are being named on the 3rd separately numbered sheet(s) attached hereto.

Title of the Invention: SOLVATED CRYSTALLINE FORMS OF EPLERENONE

Correspondence Address: Customer Number 000321

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1311

The application includes:

[x] Specification - Number of Pages 100

[x] Drawings - Number of Sheets 92

[ ] Small Entity Statement

[x] Return Receipt Postcard

[ ] Other _____

Method of Payment of Filing Fee:

[X] A check in the amount of $ 150.00 is enclosed to cover the Provisional Filing Fee.

[ ] The Commissioner is hereby authorized to charge filing fees and credit Deposit Account No. 19-1345.

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

[X] No
[ ] Yes, the name of the U.S. Government agency and the Government contract number are: _____

_____

Respectfully submitted,

Scott A. Williams, Reg. No. 39,876

Date: _____

SAWskg

60/160,807 11/20/99

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INVENTOR(S):

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JC883 U.S. PTO
60/169807
12/08/99

FEE TRANSMITTAL

Application Number TBA
Filing Date December 8, 1999
First Named Inventor Kathleen P. Barton
Group Art Unit TBA
Examiner Name TBA
Attorney Docket Number SRL 6177 (C-3256)

METHOD OF PAYMENT

1. [ ] The Commissioner is hereby authorized to charge the indicated fees to Deposit Account No. 19-1345, in the name of Senniger, Powers, Leavitt & Roedel.

   [ ] The Commissioner is hereby authorized to charge any additional filing and claim fees under 37 CFR 1.16 and application processing fees under 37 CFR 1.17 to Deposit Account No. 19-1345, in the name of Senniger, Powers, Leavitt & Roedel.

2. [x] Check Enclosed. The Commissioner is hereby authorized to charge any under payment or credit any over payment to Deposit Account No. 19-1345, in the name of Senniger, Powers, Leavitt & Roedel.

FEE CALCULATION

1. [x] BASIC FILING FEE $150.00 (Type: Provisional)
   Entity Status: large

2. [ ] CLAIM FEE $_____

   Total Claims _____
   Independent Claims _____
   Multiple Dependent Claims _____

3. [ ] ADDITIONAL FEES $_____

   [ ] Surcharge - late filing fee or oath
   [ ] Surcharge - late provisional filing fee or cover sheet
   [ ] Extension for reply within _____ month
   [ ] Notice of Appeal
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   [ ] Request for Reexamination
   [ ] Petitions to the Commissioner
   [ ] Submission of Information Disclosure Statement
   [ ] Recording each patent assignment per property
   [ ] Other: _____

TOTAL AMOUNT OF PAYMENT $ 150.00

12/8/99

Scott A. Williams, Reg. No. 39,876 Date

SAWskg

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SOLVATED CRYSTALLINE FORMS OF EPLERENONE

Field of the Invention

This invention is in the field of aldosterone receptor antagonist pharmaceutical agents and specifically relates to novel solvated crystalline forms of eplerenone, methods of preparing these solvated crystalline forms of eplerenone, pharmaceutical compositions comprising a solvated crystalline form of eplerenone, and methods for the treatment and/or prophylaxis of aldosterone-mediated conditions and/or disorders, including conditions and disorders associated with hyperaldosteronism such as hypertension.

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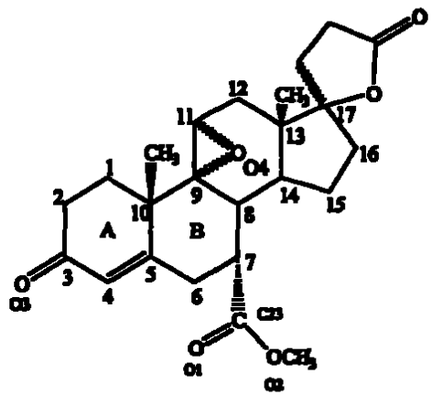
Background of the Invention

The compound methyl hydrogen 9,11-epoxy-17-hydroxy-3-oxopregn-4-ene-7,21-dicarboxylate, γ-lactone 1 (also referred to herein as "eplerenone") was first reported in Grob et al., U.S. Patent 4,559,332 which discloses a class of 9,11-epoxy steroid compounds and their salts. Eplerenone is an aldosterone receptor antagonist that can be administered in a therapeutically effective amount where use of an aldosterone receptor antagonist is indicated, such as in the treatment of pathological conditions associated with hyperaldosteronism including hypertension, heart failure including cardiac insufficiency, and cirrhosis of the liver.



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U.S. Patent 4,559,332 generally discloses the preparation of eplerenone and the preparation of pharmaceutical formulations comprising eplerenone.

Additional processes for the preparation of 9,11-epoxy steroid compounds and their salts, including eplerenone, are disclosed in Ng et al., WO97/21720 and Ng et al., WO98/25948.

Grob et al., "Steroidal Aldosterone Antagonists: Increased Selectivity of 9α,11-Epoxy Derivatives", Helvetica Chimica Acta, Vol. 80, pp. 566-585 (1997), discloses an X-ray crystal structure analysis of a solvate of eplerenone prepared by crystallizing eplerenone from a methylene chloride/diethyl ether solvent system.

De Gasparo et al., "Antialdosterones: Incidence and Prevention of Sexual Side Effects", J. Steroid Biochem., Vol. 32, No. 13, pp. 223-227 (1989), discloses the use of non-formulated eplerenone having a 20 micron particle size in a single dose study of eplerenone.

Spironolactone 2, a 20-spiroxane-steroid having activity as an aldosterone receptor antagonist, is commercially available for the treatment of hypertension. Spironolactone, however, has antiandrogenic activity that can result in gynecomastia and impotence in men. It also has weak progestational activity that can produce menstrual irregularities in women. Accordingly, there is interest in the development of additional active aldosterone receptor antagonists such as eplerenone that do not interact with other steroid receptor systems such as glucocorticoid, progestin and androgen steroid receptor systems and/or that provide for a broader range of treatment.

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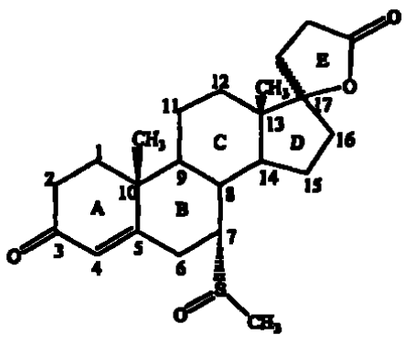

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Agafonov et al., "Polymorphism of Spironolactone", J. Pharm. Sciences, Vol. 80, No. 2, pp 181-185 (1991), discloses an acetonitrile solvate, an ethanol solvate, an ethyl acetate solvate, a methanol solvate and two non-solvated polymorphic crystalline forms of spironolactone. Brittan, Polymorphism in Pharmaceutical Solids, pp. 114-116, 207, 235, and 261 (Marcel Dekker, Inc. 1999), likewise discloses these solid state forms of spironolactone.

Summary of the Invention

A first aspect of the invention comprises novel solvated crystalline forms of eplerenone. These solvated crystalline forms possess activity as aldosterone receptor antagonists and have unique properties relative to other solid state forms of eplerenone. In particular, these solvated crystalline forms are useful as intermediates in the preparation of the novel Form I and Form II crystalline forms of eplerenone.

A second aspect of the invention comprises particles of a solvated crystalline form of eplerenone wherein at least 90% of the particles have a particle size less than about 400 microns.

A third aspect of the invention comprises a substantially phase pure solvated crystalline form of eplerenone.

A fourth aspect of the invention comprises pharmaceutical compositions comprising a solvated crystalline form of eplerenone.

A fifth aspect of the invention comprises a method for the prophylaxis and/or treatment of an aldosterone-mediated condition and/or disorder comprising administering to a subject a therapeutically-effective amount of a solvated crystalline form of eplerenone.

A sixth aspect of the invention comprises methods for the preparation of a solvated crystalline form of eplerenone.

Additional aspects of the invention are discussed throughout the specification of this application.

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Brief Description of the Drawings

Fig. 1-A shows X-ray powder diffraction patterns of Form I eplerenone.

Fig. 1-B shows X-ray powder diffraction patterns of Form II eplerenone.

Fig. 1-C shows X-ray powder diffraction patterns of the methyl ethyl ketone solvate of eplerenone.

Figs. 1-D through 1-O show X-ray powder diffraction patterns for the following eplerenone solvates: n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, acetone solvate, toluene solvate, isopropanol solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, methyl acetate solvate, and propyl acetate solvate, respectively.

Fig. 2-A shows a differential scanning calorimetry (DSC)

thermogram of non-milled Form II directly crystallized from methyl ethyl ketone.

Fig. 2-B shows a differential scanning calorimetry (DSC) thermogram of non-milled Form II prepared by desolvation of a solvate obtained by crystallization of a high purity eplerenone from methyl ethyl ketone.

Fig. 2-C shows a differential scanning calorimetry (DSC) thermogram of Form II prepared by crystallizing a solvate from a solution of high purity eplerenone in methyl ethyl ketone, desolvating the solvate to yield Form II, and milling the resulting Form II.

Fig. 2-D shows a differential scanning calorimetry (DSC) thermogram of non-milled Form I prepared by desolvation of a solvate obtained by digestion of low purity eplerenone from appropriate solvents.

Figs. 2-E through 2-T show DSC thermograms for the following eplerenone solvates: n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, chloroform solvate, acetone solvate, toluene solvate, isopropanol solvate, t-butyl acetate solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, methyl acetate solvate, propyl acetate solvate, n-butanol solvate, and n-octanol solvate, respectively.

Fig. 3-A shows the infrared spectra (diffuse reflectance, DRIFTS) of Form I eplerenone.

Fig. 3-B shows the infrared spectra (diffuse reflectance, DRIFTS) of Form II eplerenone.

Fig. 3-C shows the infrared spectra (diffuse reflectance, DRIFTS) of the methyl ethyl ketone solvate of eplerenone.

Fig. 3-D shows the infrared spectra (diffuse

reflectance, DRIFTS) of eplerenone in chloroform solution.

Figs. 3-E through 3-Q show infrared absorption spectra for the following eplerenone solvates: n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, acetone solvate, toluene solvate, isopropanol solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, propyl acetate solvate, methyl acetate solvate, propylene glycol solvate, and t-butyl acetate solvate respectively.

Fig. 4 shows ^{13}C NMR spectra for Form I of eplerenone.

Fig. 5 shows ^{13}C NMR spectra for Form II of eplerenone.

Figs. 6-A through 6-R show thermogravimetry analysis profiles for the following eplerenone solvates: methyl ethyl ketone solvate, n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, chloroform solvate, acetone solvate, toluene solvate, isopropanol solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, methyl acetate solvate, propyl acetate solvate, propylene glycol solvate, n-butanol solvate, n-octanol solvate, and t-butyl acetate solvate, respectively.

Fig. 7 is a scanning electron micrograph of Form II eplerenone prepared by desolvation of the methyl ethyl ketone solvate.

Fig. 8 is a scanning electron micrograph of Form II eplerenone prepared by direct crystallization from ethyl acetate.

Fig. 9 shows an X-ray powder diffraction pattern of a crystalline form of 7-methyl hydrogen 4α,5α:9α,11α-diepoxy-17-hydroxy-3-oxo-17α-pregnane-7α,21-dicarboxylate, γ-lactone isolated from methyl ethyl ketone.

Fig. 10 shows an X-ray powder diffraction pattern of the crystalline form of 7-methyl hydrogen 11α,12α-epoxy-17-hydroxy-3-oxo-17α-pregn-4-ene-7α,21-dicarboxylate, γ-lactone isolated from isopropanol.

Fig. 11 shows an X-ray powder diffraction pattern of the crystalline form of 7-methyl hydrogen 17-hydroxy-3-oxo-17α-pregna-4,9(11)-diene-7α,21-dicarboxylate, γ-lactone isolated from n-butanol.

Fig. 12 illustrates the Gibbs free energy-temperature relationship for enantiotropically related polymorphs.

Fig. 13 shows the X-ray powder diffraction patterns for the wet cake (methyl ethyl ketone solvate) obtained from (a) 0%, (b) 1%, (c) 3%, and (d) 5% diepoxide-doped methyl ethyl ketone crystallizations.

Fig. 14 shows the X-ray powder diffraction patterns for the dried solids obtained from (a) 0%, (b) 1%, (c) 3%, and (d) 5% diepoxide-doped methyl ethyl ketone crystallizations.

Fig. 15 shows the X-ray powder diffraction patterns for the dried solids from the methyl ethyl ketone crystallization with 3% doping of diepoxide (a) without grinding of the solvate prior to drying, and (b) with grinding of the solvate prior to drying.

Fig. 16 shows the X-ray powder diffraction patterns for the wet cake (methyl ethyl ketone solvate) obtained from (a) 0%, (b) 1%, (c) 5%, and (d) 10% 11,12-epoxide-doped methyl ethyl ketone crystallizations.

Fig. 17 shows the X-ray powder diffraction patterns for the dried solids obtained from (a) 0%, (b) 1%, (c) 5%, and (d) 10% 11,12-epoxide-doped methyl ethyl ketone crystallizations.

Fig. 18 shows a cube plot of product purity, starting material purity, cooling rate and endpoint temperature based on the data reported in Table X-7A.

Fig. 19 shows a half normal plot prepared using the cube plot of Fig. 18 to determine those variables having a statistically significant effect on the purity of the final material.

Fig. 20 is an interaction graph based on the results reported in Table X-7A showing the interaction between starting material purity and cooling rate on final material purity.

Fig. 21 shows a cube plot of Form I weight fraction, starting material purity, cooling rate and endpoint temperature based on the data reported in Table X-7A.

Fig. 22 shows a half normal plot prepared using the cube plot of Fig. 21 to determine those variables having a statistically significant effect on the purity of the final material.

Fig. 23 is an interaction graph based on the results reported in Table X-7A showing the interaction between starting material purity and endpoint temperature on final material purity.

Fig. 24 shows an X-ray diffraction pattern of amorphous eplerenone.

Fig. 25 shows a DSC thermogram of amorphous eplerenone.

Fig. 26 shows the dissolution rates measured for four polymorph samples.

Detailed Description of the Preferred Embodiments

As with all pharmaceutical compounds and compositions, the chemical and physical properties of eplerenone are

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important in its commercial development. These properties include, but are not limited to: (1) packing properties such as molar volume, density and hygroscopicity, (2) thermodynamic properties such as melting temperature, vapor pressure and solubility, (3) kinetic properties such as dissolution rate and stability (including stability at ambient conditions, especially to moisture and under storage conditions), (4) surface properties such as surface area, wettability, interfacial tension and shape, (5) mechanical properties such as hardness, tensile strength, compactibility, handling, flow and blend; and (6) filtration properties. These properties can affect, for example, the processing and storage of pharmaceutical compositions comprising eplerenone. Solid state forms of eplerenone that provide an improvement in one or more of these properties relative to other solid state forms of eplerenone are desirable.

The present invention comprises such novel solid state forms of eplerenone. Specifically, these novel solid state forms include solvated crystalline forms, non-solvated and non-hydrated crystalline forms (designated "Form I" and "Form II"), and an amorphous form of eplerenone. Each solid state form of eplerenone described in the present application possesses one or more of the above-described advantageous chemical and/or physical properties relative to the other solid state forms described herein or otherwise disclosed in the literature.

In one embodiment of the invention, the solid state form comprises Form I eplerenone. Form I exhibits a faster dissolution rate (approximately 30% higher) at lower temperatures (i.e., temperatures below the enantiotropic

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transition temperature as later discussed) than, for example, Form II. Where dissolution of eplerenone in the gastrointestinal tract is the rate-controlling step for delivery of the eplerenone to the target cells, faster dissolution generally results in improved bioavailability. Form I, therefore, can provide an improved bioavailability profile relative to Form II. In addition, selection of a solid state form of eplerenone having a faster dissolution rate likewise provides greater flexibility in the selection of excipients for, and in the formulation of, immediate release pharmaceutical compositions relative to other solid state forms having a slower dissolution rate.

In another embodiment of the invention, the solid state form comprises Form II eplerenone. Form II possesses greater physical stability at lower temperatures (i.e., at temperatures below the enantiotropic transition temperature as later discussed) than, for example, Form I. Solid state forms of eplerenone such as Form II that do not require the use of special processing or storage conditions, and that avoid the need for frequent inventory replacement, are desirable. For example, selection of a solid state form of eplerenone that is physically stable during the manufacturing process (such as during milling of eplerenone to obtain a material with reduced particle size and increased surface area) can avoid the need for special processing conditions and the increased costs generally associated with such special processing conditions. Similarly, selection of a solid state form of eplerenone that is physically stable at different conditions of storage (especially considering the different possible storage conditions during the lifetime of the eplerenone product) can help avoid polymorphic or other

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degradative changes in the eplerenone that can lead to product loss or deterioration of product efficacy. Therefore, the selection of a solid state form of eplerenone such as Form II having greater physical stability provides a meaningful benefit over less stable eplerenone forms.

In another embodiment of the invention, the solid state form of eplerenone comprises a solvated crystalline form of eplerenone. Such solvated crystalline forms are useful as intermediates in the preparation of Form I and Form II eplerenone. An additional benefit from the use of a solvated crystalline form as an intermediate is the "intrinsic micronizing" of the crystal that results upon desolvation as discussed later in this application. Such "intrinsic micronizing" can reduce or eliminate milling requirements. Further, where additional milling is still required, it is easier to mill certain solvates before the desolvation step than to mill Form I or Form II after desolvation of the solvate.

Pharmaceutically acceptable solvated crystalline forms also can be used directly in pharmaceutical compositions. These solvated crystalline forms preferably do not comprise methylene chloride, isopropanol or ethyl ether; and, in yet another embodiment, do not comprise methylene chloride, isopropanol, ethyl ether, methyl ethyl ketone or ethanol; and, in yet another embodiment, do not comprise methylene chloride, isopropanol, ethyl ether, methyl ethyl ketone, ethanol, ethyl acetate or acetone. More preferably, the solvated crystalline forms are substantially exclusive of solvents that are not pharmaceutically-acceptable solvents. Because Form I and Form II typically are more physically stable than the crystalline solvates at room temperature and under atmospheric pressure,

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the solvated crystalline forms used in such compositions generally comprise a pharmaceutically acceptable higher boiling and/or hydrogen bonding solvent such as, but not limited to, butanol. It is believed that the solvated crystalline forms collectively can offer a range of different dissolution rates and, where dissolution of eplerenone in the gastrointestinal tract is the rate-controlling step for delivery of the eplerenone to the target cells, bioavailabilities relative to Form I and Form II.

In another embodiment of the invention, the solid state form of eplerenone comprises an amorphous form of eplerenone. Amorphous eplerenone is useful as an intermediate in the preparation of Form I and Form II eplerenone. In addition, it is believed that amorphous eplerenone possesses a different dissolution rate and, where dissolution of eplerenone in the gastrointestinal tract is the rate-controlling step for delivery of the eplerenone to the target cells, bioavailability relative to Form I and Form II.

In another embodiment, the invention comprises combinations of solid state eplerenone forms selected from the group consisting of Form I eplerenone, Form II eplerenone, solvated crystalline eplerenone and amorphous eplerenone. Such combinations are useful, for example, in the preparation of pharmaceutical compositions having differing dissolution profiles, including controlled release compositions.

In addition, depending upon the intended use of the solid state form of eplerenone, processing considerations may favor the selection of a specific solid state form of eplerenone or a specific combination of such solid state forms. Pure phase Form II, for example, generally is more easily prepared than

pure phase Form I. A mixture of Form I and Form II, however, generally is more easily prepared than phase pure Form II and allows for the use of a low purity eplerenone starting material. Use of a solvated crystalline form instead of Form I or Form II in a composition eliminates a processing step, desolvation, for those processes that otherwise would proceed by desolvation of the solvated crystalline form. Alternatively, the desolvation step can be eliminated, for example, if Form II is directly crystallized from an appropriate solvent without the intervening preparation and desolvation of the intermediate solvated crystalline form. These processes are described in greater detail later in the application.

Although each of the above solid state eplerenone embodiments can embrace a broad range of eplerenone particle sizes, it has been discovered that reduction of the particle size of the solid state form of eplerenone to a D_{90} particle size (later defined herein) between about 25 microns to about 400 microns can improve the bioavailability of unformulated eplerenone and pharmaceutical compositions comprising that solid state form of eplerenone. Accordingly, the D_{90} particle size of the unformulated eplerenone or the eplerenone used as a starting material in the pharmaceutical composition generally is less than about 400 microns, preferably less than about 200 microns, more preferably less than about 150 microns, still more preferably less than about 100 microns, and still more preferably less than about 90 microns. In one embodiment, the D_{90} particle size is between about 40 microns to about 100 microns. In another embodiment, the D_{90} particle size is between about 30 microns to about 50 microns. In another embodiment, the D_{90} particle size is between about 50

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microns to about 150 microns. In another embodiment, the D_{90} particle size is between about 75 microns to about 125 microns.

Definitions

The term "amorphous" as applied to eplerenone refers to a solid state wherein the eplerenone molecules are present in a disordered arrangement and do not form a distinguishable crystal lattice or unit cell. When subjected to X-ray powder diffraction, amorphous eplerenone does not produce any characteristic crystalline peaks.

Where reference is made in this application to the "boiling point" of a substance or solution, the term "boiling point" means the boiling point of the substance or solution under the applicable process conditions.

The term "crystalline form" as applied to eplerenone refers to a solid state form wherein the eplerenone molecules are arranged to form a distinguishable crystal lattice (i) comprising distinguishable unit cells, and (ii) yielding diffraction peaks when subjected to X-ray radiation.

The term "crystallization" as used throughout this application can refer to crystallization and/or recrystallization depending upon the applicable circumstances relating to the preparation of the eplerenone starting material.

The term "digestion" means a process in which a slurry of solid eplerenone in a solvent or mixture of solvents is heated at the boiling point of the solvent or mixture of solvents under the applicable process conditions.

The term "direct crystallization" as used herein refers to the crystallization of eplerenone directly from a suitable

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solvent without the formation and desolvation of an intermediate solvated crystalline solid state form of eplerenone.

The term "particle size" as used herein refers to particle size as measured by conventional particle size measuring techniques well known in the art, such as laser light scattering, sedimentation field flow fractionation, photon correlation spectroscopy, or disk centrifugation. The term "D_{90} particle size" means the particle size of at least 90% of the particles as measured by such conventional particle size measuring techniques.

The term "DSC" means differential scanning calorimetry.

The term "HPLC" means high pressure liquid chromatography.

The term "IR" means infra red.

The term "min" means minute.

The term "psi" means pounds per square inch absolute.

The term "purity" means the chemical purity of eplerenone according to conventional HPLC assay. As used herein, "low purity eplerenone" generally means eplerenone that contains an effective amount of a Form I growth promoter and/or a Form II growth inhibitor. As used herein, "high purity eplerenone" generally means eplerenone that does not contain, or contains less than an effective amount of, a Form I growth promoter and/or a Form II growth inhibitor.

The term "phase purity" means the solid state purity of eplerenone with regard to a particular crystalline or amorphous form of the eplerenone as determined by the infrared spectroscopy analytical methods described herein.

The term "XPRD" means X-ray powder diffraction.

The term "rpm" means revolutions per minute.

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The term "TGA" means thermogravimetric analysis.

The term "T_m" means melting temperature.

Characterization of Crystalline Form

1. Molecular Conformation

Single crystal X-ray analysis indicates that the eplerenone molecular conformation differs between Form I and Form II, particularly with respect to the orientation of the ester group at the 7-position of the steroid ring. The orientation of the ester group can be defined by the C8-C7-C23-02 torsion angle.

In the Form I crystal lattice, the eplerenone molecule adopts a conformation in which the methoxy group of the ester is approximately aligned with the C-H bond at the 7-position and the carbonyl group is approximately positioned over the center of the B-steroid ring. The C8-C7-C23-02 torsion angle is approximately -73.0° in this conformation. In this orientation, the carbonyl oxygen atom of the ester group (01) is in close contact with the oxygen atom of the 9,11-epoxide ring (04). The 01-04 distance is about 2.97 Å, which is just below the van der Waal's contact distance of 3.0 Å (assuming van der Waal's radii of 1.5Å for the oxygen).

In the Form II crystal lattice, the eplerenone molecule adopts a conformation in which the ester group is rotated approximately 150° relative to that of Form I and has a C8-C7-C23-02 torsion angle of approximately +76.9°. In this orientation, the methoxy group of the ester is directed toward the 4,5-alkene segment of the A-steroid ring. In this orientation, the distance between either oxygen atom of the ester group (01,02) and the oxygen atom of the 9,11-epoxide

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ring is increased relative to the distance determined for Form I. The 02-04 distance is approximately 3.04Å, falling just above the van der Waal's contact distance. The 01-04 distance is about 3.45Å.

The eplerenone molecule appears to adopt a conformation characteristic of Form II in the solvated crystalline forms analyzed by single crystal X-ray diffraction to date.

2. X-Ray Powder Diffraction

The various crystalline forms of eplerenone were analyzed with either a Siemens D5000 powder diffractometer or an Inel Multipurpose Diffractometer. For the Siemens D500 powder diffractometer, the raw data was measured for 2q values from 2 to 50, with steps of 0.020 and step periods of two seconds. For the Inel Multipurpose Diffractometer, samples were placed in an aluminum sample holder and raw data was collected for 30 minutes at all two theta values simultaneously.

Tables 1A, 1B and 1C set out the significant parameters of the main peaks in terms of 2q values and intensities for the Form I (prepared by desolvation of the ethanol solvate obtained by digestion of low purity eplerenone), Form II (prepared by desolvation of the methyl ethyl ketone solvate obtained by recrystallization of high purity eplerenone), and methyl ethyl ketone solvate (prepared by room temperature slurry conversion of high purity eplerenone in methyl ethyl ketone) crystalline forms of eplerenone, respectively (X-ray radiation at a wavelength of 1.54056 Angstroms).

Minor shifts in peak positioning may be present in the diffraction patterns of Form I and Form II as a result of imperfections in the spacing of the crystal diffraction planes

due to the route of manufacture of Form I and Form II (i.e. desolvation of a solvate). In addition, Form I is isolated from a solvate prepared by digestion of crude eplerenone. This method results in a lower overall chemical purity (approximately 90%) of the Form I. Finally, the solvated forms of eplerenone are expected to show some shifting in the positioning of the diffraction peaks due to the increased mobility of the solvent molecules within the solvent channels in the crystal lattice.

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TABLE 1A: FORM I DATA

Angle 2-theta	d-spacing Angstrom	Intensity	Intensity %
6.994	12.628	1188	7.2
8.291	10.655	2137	13
10.012	8.827	577	3.5
11.264	7.849	1854	11.3
12.04	7.344	7707	46.8
14.115	6.269	3121	19
14.438	6.13	15935	96.8
15.524	5.703	637	3.9
16.169	5.477	1349	8.2
16.699	5.305	1663	10.1
16.94	5.23	1692	10.3
17.147	5.167	2139	13
17.66	5.018	6883	41.8
17.91	4.949	16455	100
18.379	4.823	3106	18.9
18.658	4.752	1216	7.4
19.799	4.48	1499	9.1
20.235	4.385	383	2.3
21.707	4.091	1267	7.7
21.8	4.073	1260	7.7
21.959	4.044	1279	7.8
22.481	3.955	4264	25.9
23.191	3.832	1026	6.2
23.879	3.723	1000	6.1
24.599	3.616	1688	10.3
25.837	3.445	931	5.7
26.034	3.42	686	4.2
26.868	3.316	912	5.5
27.093	3.288	1322	8
27.762	3.209	1236	7.5
28.34	3.147	1845	11.2
28.881	3.091	957	5.8
29.866	2.9892	745	4.5
30.627	2.9168	992	6
31.108	2.8726	1205	7.3
33.215	2.6951	1287	7.8
33.718	2.656	802	4.9
34.434	2.6024	914	5.6

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TABLE 1B: FORM II DATA

Angle 2-Theta	d-spacing Angstrom	Intensity Cps	Intensity %
7.992	11.054	11596	26.6
10.044	8.799	12048	27.6
11.206	7.889	4929	11.3
12.441	7.109	1747	4
12.762	6.936	4340	9.9
13.257	6.673	2444	5.6
14.705	6.019	43648	100
15.48	5.727	2670	6.1
15.727	6.63	7982	18.3
16.016	5.529	3519	8.1
17.671	5.015	8897	20.4
17.9	4.951	2873	6.6
18.352	4.83	612	1.4
18.703	4.74	689	1.6
19.524	4.543	1126	2.6
20.103	4.413	3753	8.6
20.83	4.302	1451	3.3
21.067	4.214	876	2
21.675	4.097	2760	6.3
22.232	3.995	1951	4.5
22.852	3.922	1657	3.8
23.624	3.763	827	1.9
24.279	3.663	1242	2.8
25.021	3.556	5144	11.8
25.485	3.492	1702	3.9
25.707	3.463	2493	5.7
26.251	3.392	1371	3.1
26.85	3.318	1970	4.5
27.319	3.262	1029	2.4
27.931	3.192	440	1
27.969	3.187	440	1
28.937	3.083	1128	2.6
29.703	3.005	1211	2.8
30.173	2.9594	1506	3.5
30.584	2.9206	1602	3.7
30.885	2.8928	1550	3.6
31.217	2.8626	1068	2.4
31.605	2.8285	1038	2.4
32.059	2.7895	1211	2.8
32.64	2.7412	684	1.6
32.747	2.7324	758	1.7
33.46	2.6759	508	1.2
34.194	2.6201	1085	2.5
34.545	2.5943	915	2.1

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TABLE 1C: METHYL ETHYL KETONE DATA

Angle 2-Theta	d-spacing Angstrom	Intensity Cps	Intensity %
7.584	11.648	5629	32.6
7.753	11.393	15929	92.3
10.151	8.707	2877	16.7
11.31	7.817	701	4.1
12.646	6.994	1027	5.9
13.193	6.705	15188	88
13.556	6.528	14225	82.4
14.074	6.287	1966	11.4
14.748	6.002	2759	16
15.165	5.837	801	4.6
15.548	5.694	1896	11
17.031	5.202	7980	46.2
17.26	5.127	17267	100
17.706	5.005	6873	39.8
18.555	4.776	545	3.2
18.871	4.699	1112	6.4
19.766	4.488	1704	9.9
20.156	4.401	1398	8.1
20.725	4.282	2844	16.3
21.787	4.076	1127	6.5
22.06	4.026	451	2.6
22.864	3.886	1542	8.9
23.412	3.796	14185	82.2
23.75	3.743	1154	6.7
24.288	3.662	3063	17.7
25.253	3.524	1318	7.6
25.503	3.49	1736	10.1
25.761	3.455	1225	7.1
26.176	3.402	1348	7.8
26.548	3.355	1098	6.4
27.357	3.257	1944	11.3
27.605	3.229	2116	12.3
27.9	3.195	858	5
28.378	3.142	583	3.4
28.749	3.103	763	4.4
29.3	3.046	1182	6.8
29.679	3.006	2806	16.1
30.402	2.9377	2184	12.6
30.739	2.9063	648	3.8

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Graphical examples of the x-ray diffraction patterns for Form I, Form II, and the methyl ethyl ketone solvate

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crystalline forms of eplerenone are shown in Figs. 1-A, 1-B, and 1-C, respectively. Form I shows distinguishing peaks at 7.0 ± 0.2, 8.3 ± 0.2, and 12.0 ± 0.2 degrees two theta. Form II shows distinguishing peaks at 8.0 ± 0.2, 12.4 ± 0.2, 12.8 ± 0.2, and 13.3 ± 0.2 degrees two theta. The methyl ethyl ketone solvated crystalline form shows distinguishing peaks at 7.6 ± 0.2, 7.8 ± 0.2, and 13.6 ± 0.2 degrees two theta.

Examples of the x-ray diffraction patterns are shown in Figs. 1-D through 1-O for the following solvate crystalline forms of eplerenone: n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, acetone solvate, toluene solvate, isopropanol solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, methyl acetate solvate, and propyl acetate solvate, respectively.

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3. Melting/Decomposition Temperature

The temperatures of melting and/or decomposition of non-solvated eplerenone crystalline forms were determined using a TA Instruments 2920 differential scanning calorimeter. Each sample (1-2 mg) was placed in either a sealed or unsealed aluminum pan and heated at 10°C/minute. Melting/decomposition ranges were defined from the extrapolated onset to the maximum of the melting/decomposition endotherm.

The melting of the non-solvated eplerenone crystals forms (Form I and Form II) was associated with chemical decomposition and loss of trapped solvent from the crystal lattice. The melting/decomposition temperature also was affected by the manipulation of the solid prior to analysis. For example, non-milled Form II (approximate D_{90} particle size of about 180-450 microns) prepared by direct crystallization

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from an appropriate solvent or from desolvation of a solvate obtained from crystallization of high purity eplerenone in an appropriate solvent or mixture of solvents generally had a melting range of about 237-242°C. Milled Form II (approximate D_{90} particle size of about 80-100 microns) (Form II prepared by crystallizing a solvate from a solution of high purity eplerenone in an appropriate solvent or mixture of solvents, desolvating the solvate to yield Form II, and milling the resulting Form II) generally had a lower and broader melting/decomposition range of about 223-234°C. Non-milled Form I (approximate D_{90} particle size of about 180-450 microns) prepared by desolvation of a solvate obtained by digestion of low purity eplerenone generally had a higher melting/decomposition range of about 247-251°C. Examples of the DSC thermograms of (a) non-milled Form II directly crystallized from methyl ethyl ketone, (b) non-milled Form II prepared by desolvation of a solvate obtained by crystallization of a high purity eplerenone from methyl ethyl ketone, (c) Form II prepared by milling a desolvated solvate obtained by crystallization of high purity eplerenone from methyl ethyl ketone, and (d) non-milled Form I prepared by desolvation of a solvate obtained by digestion of low purity eplerenone from methyl ethyl ketone are given in Figures 2-A, 2-B, 2-C and 2-D, respectively.

DSC thermograms of solvated forms of eplerenone were determined using a Perkin Elmer Pyris 1 differential scanning calorimeter. Each sample (1-10 mg) was placed in an unsealed aluminum pan and heated at 10°C/minute. One or more endothermal events at lower temperatures were associated with enthalpy changes that occurred as solvent was lost from the solvate crystal lattice. The highest temperature endotherm

or endotherms were associated with the melting/decomposition of Form II or Form I eplerenone. Examples of the DSC thermograms are shown in Figs. 2-E through 2-T for the following solvated crystalline forms of eplerenone: n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, chloroform solvate, acetone solvate, toluene solvate, isopropanol solvate, t-butyl acetate solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, methyl acetate solvate, propyl acetate solvate, n-butanol solvate, and n-octanol solvate, respectively.

4. Infrared Absorption Spectroscopy

Infrared absorption spectra of the non-solvated forms of eplerenone (Form I and Form II) were obtained with a Nicolet DRIFT (diffuse reflectance infrared fourier transform) Magna System 550 spectrophotometer. A Spectra-Tech Collector system and a microsample cup were used. Samples (5%) were analyzed in potassium bromide and scanned from 400-4000 cm^{-1}. Infrared absorption spectra of eplerenone in dilute chloroform solution (3%) or in the solvated crystal forms were obtained with a Bio-rad FTS-45 spectrophotometer. Chloroform solution samples were analyzed using a solution cell of 0.2 mm path length with sodium chloride salt plates. Solvate FTIR spectra were collected using an IBM micro-MIR (multiple internal reflectance) accessory. Samples were scanned from 400-4000 cm^{-1}. Examples of the infrared absorption spectra of (a) Form I, (b) Form II, (c) the methyl ethyl ketone solvate, and (d) eplerenone in chloroform solution are shown in Figures 3-A, 3-B, 3-C and 3-D, respectively.

Table 2 discloses illustrative absorption bands for

eplerenone in the Form I, Form II, and methyl ethyl ketone solvate crystal forms. Illustrative absorption bands for eplerenone in chloroform solution are also disclosed for comparison. Differences between Form I and either Form II or the methyl ethyl ketone solvate were observed, for example, in the carbonyl region of the spectrum. Form I has an ester carbonyl stretch of approximately 1739 cm^{-1} while both Form II and the methyl ethyl ketone solvate have the corresponding stretch at approximately 1724 and 1722 cm^{-1}, respectively. The ester carbonyl stretch occurs at approximately 1727 cm^{-1} in the eplerenone in chloroform solution. The change in stretching frequency of the ester carbonyl between Form I and Form II reflects the change in orientation of the ester group between the two crystal forms. In addition, the stretch of the ester of the conjugated ketone in the A-steroid ring shifts from approximately 1664-1667 cm^{-1} in either Form I or the methyl ethyl ketone solvate to approximately 1655 cm^{-1} in Form II. The corresponding carbonyl stretch occurs at approximately 1665 cm^{-1} in dilute solution.

Another difference between Form I and Form II was seen in the C-H bending region. Form I has an absorption at approximately 1399 cm^{-1} which is not observed in Form II, the methyl ethyl ketone solvate, or the eplerenone in chloroform solution. The 1399 cm^{-1} stretch occurs in the region of CH_2 scissoring for the C2 and C21 methylene groups adjacent to carbonyl groups.

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Table 2

Absorption Region	Form I (cm^{-1})	Form II (cm^{-1})	Methyl Ethyl Ketone Solvate (cm^{-1})	Eplerenone in Chloroform (cm^{-1})
ν C=O(lactone)	1773	1775	1767	1768
ν C=O(ester)	1739	1724	1722	1727
ν C=O(3keto)	1664	1655	1667	1665
ν C=C (3,4-olefin)	1619	1619	1622	1623
δ_{as}CH3, δCH2, δCH2 (α to carbonyl)	1460, 1444, 1426	1467, 1438, 1422, 1399	1467, 1438, 1422	1464, 1438, 1422
δ_{s}CH3	1380	1381	~1380	1378

Examples of infrared absorption spectra are shown in Figs. 3-E through 3-S for the following solvated crystalline forms of eplerenone: n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, acetone solvate, toluene solvate, isopropanol solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, propyl acetate solvate, methyl acetate solvate, propylene glycol solvate, and t-butyl acetate solvate respectively.

5. Nuclear Magnetic Resonance

^{13}C NMR spectra were obtained at a field of 31.94 MHz. Examples of the ^{13}C NMR spectra of Form I and Form II eplerenone are shown in Figs. 4 and 5, respectively. The Form I eplerenone analyzed to obtain the data reflected in Fig. 4 was not phase pure and included a small amount of Form II eplerenone. Form I is most clearly distinguished by the carbon resonances at around 64.8 ppm, 24.7 ppm and 19.2 ppm.

Form II is most clearly distinguished by the carbon resonances at around 67.1 ppm and 16.0 ppm.

6. Thermogravimetry

Thermogravimetric analysis was performed using a TA Instruments TGA 2950 thermogravimetric analyzer. Samples were placed in an unsealed aluminum pan under nitrogen purge. Starting temperature was 25°C with the temperature increased at a rate of about 10°C/minute.

Examples of thermogravimetry analysis profiles are shown in Figs. 6-A through 6-R for the following solvated crystalline forms of eplerenone: methyl ethyl ketone solvate, n-propyl alcohol solvate, tetrahydrofuran solvate, ethyl propionate solvate, acetic acid solvate, chloroform solvate, acetone solvate, toluene solvate, isopropanol solvate, ethanol solvate, isobutyl acetate solvate, butyl acetate solvate, methyl acetate solvate, propyl acetate solvate, propylene glycol solvate, n-butanol solvate, n-octanol solvate, and t-butyl acetate solvate, respectively.

7. Microscopy

Hot-stage microscopy was performed on single crystals of the methyl ethyl ketone solvate using a Linkam THMS 600 Hot Stage with Zeiss Universal Polarized Light Microscope. Under polarized light at room temperature the solvate crystal was birefringent and translucent indicating that the crystal lattice was highly ordered. As the temperature increased to about 60°C, noticeable defects began to emerge along the long crystal dimension. A scanning electron micrograph of Form II eplerenone obtained by desolvation of the methyl ethyl ketone solvate is shown in Fig. 7 and reveals surface defects,

pores, cracks and fractures within the crystal lattice. A scanning electron micrograph of Form II eplerenone obtained by direct crystallization from ethyl acetate is shown in Fig. 8 and does not exhibit similar surface defects, pores, cracks and fractures within the crystal lattice.

8. Unit Cell Parameters

Tables 3A, 3B and 3C below summarize the unit cell parameters determined for Form I, Form II, and several solvated crystalline forms.

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TABLE 3A

Parameter	Form I	Form II	Methyl ethyl ketone Solvate
Crystal system	Ortho-rhombic	Monoclinic	Orthorhombic
Space group	$P2_12_12_1$	$P2_1$	$P2_12_12_1$
a	21.22 Å	8.78 Å	23.53 Å
b	15.40 Å	11.14 Å	8.16 Å
c	6.34 Å	11.06 Å	13.08 Å
α	90°	90°	90°
β	90°	93.52°	90°
γ	90°	90°	90°
z	4	2	4
Volume (Å)	2071.3	1081.8	2511.4
ρ (calculated)	1.329 g/cm³	1.275 g/cm³	1.287 g/cm³
R	0.0667	0.062	0.088

TABLE 3B

Parameter	Acetone Solvate	Toluene Solvate	Butyl Acetate Solvate[1]
Crystal system	Ortho-rhombic	Ortho-rhombic	Ortho-rhombic
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
a	23.31 Å	23.64 Å	23.07 Å
b	13.13 Å	13.46 Å	13.10 Å
c	8.28 Å	8.16 Å	8.24 Å
α	90°	90°	90°
β	90°	90°	90°
γ	90°	90°	90°
z	4	4	4
Volume (Å)	2533.7	2596.6	2490.0
ρ (calculated)	1.239 g/cm³	1.296 g/cm³	1.334 g/cm³
R	0.058	0.089	0.093

[1]The solvate molecules were not completely refined due to disorder of the solvent molecules in the channels.

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TABLE 3C

Parameter	Isobutyl Acetate Solvate[1]	Isopropanol Solvate[1]	Ethanol Solvate[1]
Crystal system	Ortho-rhombic	Ortho-rhombic	Ortho-rhombic
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
a	23.19 Å	23.15 Å	23.51 Å
b	12.95 Å	12.73 Å	13.11 Å
c	8.25 Å	8.25 Å	8.27 Å
α	90°	90°	90°
β	90°	90°	90°
γ	90°	90°	90°
z	4	4	4
Volume (Å)	2476.4	2433.2	2548.6
ρ (calculated)	1.337 g/cm³	1.296 g/cm³	1.234 g/cm³
R	0.098	0.152	0.067

[1]The solvate molecules were not refined completely due to disorder of the solvent molecules in the channels.

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Additional information on selected solvated crystalline forms of eplerenone is reported in Table 4 below. The unit cell data reported in Table 3A above for the methyl ethyl ketone solvate also are representative of the unit cell parameters for many of these additional eplerenone crystalline solvates. Most of the eplerenone crystalline solvates tested are substantially isostructural to each other. While there may be some minor shifting in the X-ray powder diffraction peaks from one solvated crystalline form to the next due to the size of the incorporated solvent molecule, the overall diffraction patterns are substantially the same and the unit cell parameters and molecular positions are substantially identical for most of the solvates tested.

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TABLE 4

Solvent	Stoichiometry (Solvent: Eplerenone)	Isostructural to Methyl Ethyl ketone Solvate?	Desolvation Temperature[1] (°C)
Methyl Ethyl Ketone	1:1	N/A	89
2-Pentanone	---	---	---
Acetic Acid	1:2	Yes	203
Acetone	1:1	Yes	117
Butyl Acetate	1:2	Yes	108
Chloroform	---	Yes	125
Ethanol	1:1	Yes	166
Isobutanol	---	---	---
Isobutyl Acetate	1:2	Yes	112
Isopropanol	1:1	Yes	121
Methyl Acetate	1:1	Yes	103
Ethyl Propionate	1:1	Yes	122
n-Butanol	1:1	Yes	103
n-Octanol	---	Yes	116
n-Propanol	1:1	Yes	129
Propyl Acetate	1:1	Yes	130
Propylene Glycol	---	Yes	188
t-Butanol	---	---	---
Tetrahydrofuran	1:1	Yes	136
Toluene	1:1	Yes	83
t-Butyl Acetate	---	Yes	109

[1]Defined as the extrapolated desolvation temperature from the final solvent weight loss step as determined by thermogravimetric analysis at a heating rate of 10°C/minute under nitrogen purge. Desolvation temperatures, however, can be affected by the method of manufacture of the solvate. Different methods can produce different numbers of nucleation sites capable of initiating desolvation in the solvate at lower temperatures.

The unit cell of the solvate is composed of four eplerenone molecules. The stoichiometry of the eplerenone molecules and solvent molecules in the unit cell is also reported in Table 4 above for a number of solvates. The unit cell of Form I is composed of four eplerenone molecules. The unit cell of Form II is composed of two eplerenone molecules. The solvate unit cells are converted during desolvation into

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Form I and/or Form II unit cells when the eplerenone molecules undergo translation and rotation to fill the spaces left by the solvent molecules. Table 4 also reports the desolvation temperatures for a number of different solvates.

9. Crystal Properties of Impurity Molecules

Selected impurities in eplerenone can induce the formation of Form I during the desolvation of the solvate. In particular, the effect of the following two impurity molecules was evaluated: 7-methyl hydrogen 4α,5α:9α,11α-diepoxy-17-hydroxy-3-oxo-17α-pregnane-7α,21-dicarboxylate, γ-lactone 3 (the "diepoxide"); and 7-methyl hydrogen 11α,12α-epoxy-17-hydroxy-3-oxo-17α-pregn-4-ene-7α,21-dicarboxylate, γ-lactone 4 (the "11,12-epoxide").

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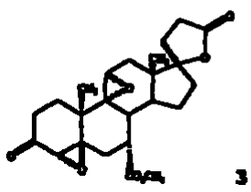

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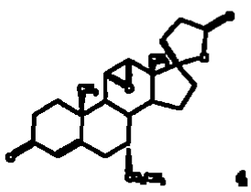

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The effect of these impurity molecules on the eplerenone crystalline form resulting from desolvation is described in greater detail in the examples of this application.

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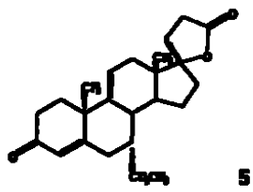
Given the similarity in single crystal structure of 7-methyl hydrogen 17-hydroxy-3-oxo-17α-pregna-4,9(11)-diene-7α,21-dicarboxylate, γ-lactone 5 (the "9,11-olefin") and Form I, it is hypothesized that the 9,11-olefin also can induce the formation of Form I during the desolvation of the solvate.



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A single crystal form was isolated for each impurity compound. Representative X-ray powder diffraction patterns for the crystal forms isolated for the diepoxide, 11,12-epoxide and 9,11-olefin are given in Figs. 9, 10 and 11, respectively. The X-ray powder diffraction pattern of each impurity molecule is similar to the X-ray powder diffraction pattern of Form I, suggesting that Form I and the three impurity compounds have similar single crystal structures.

Single crystals of each impurity compound also were isolated and subjected to X-ray structure determination to verify that these three compounds adopt single crystal structures similar to that of Form I. Single crystals of the diepoxide were isolated from methyl ethyl ketone. Single crystals of the 11,12-epoxide were isolated from isopropanol. Single crystals of the 9,11-olefin were isolated from n-butanol. Crystal structure data determined for the crystalline form of each impurity compound are given in Table 5. The resulting crystal system and cell parameters were substantially the same for the Form I, diepoxide, 11,12-epoxide, and 9,11-olefin crystalline forms.

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TABLE 5

Parameter	Form I	Diepoxide	11,12 Epoxide	9,11 olefin
Crystal system	Ortho-rhombic	Ortho-rhombic	Ortho-rhombic	Ortho-rhombic
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
a	21.22 Å	21.328 Å	20.90 Å	20.90 Å
b	15.40 Å	16.16 Å	15.55 Å	15.74 Å
c	6.34 Å	6.15 Å	6.38 Å	6.29 Å
α	90°	90°	90°	90°
β	90°	90°	90°	90°
γ	90°	90°	90°	90°
Z	4	4	4	4
Volume (Å)	2071.3	2119.0	2073.2	2069.3
ρ (calculated)	1.329 g/cm³	1.349 g/cm³	1.328 g/cm³	1.279 g/cm³
R	0.0667	0.0762	0.0865	0.0764

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The four compounds reported in Table 5 crystallize into the same space group and have similar cell parameters (i.e., they are isostructural). It is hypothesized that the diepoxide, 11,12-epoxide and 9,11-olefin adopt a Form I conformation. The relative ease of isolation of a Form I packing (directly from solution) for each impurity compound, indicates that the Form I lattice is a stable packing mode for this series of structurally similar compounds.

Preparation of Eplerenone

The eplerenone starting material used to prepare the novel crystalline forms of the present invention can be prepared using the methods set forth in Ng et al., WO97/21720; and Ng et al., WO98/25948, particularly scheme 1 set forth in WO97/21720 and WO98/25948.

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Preparation of Crystalline Forms

1. Preparation of Solvated Crystalline Form

The solvated crystalline forms of eplerenone can be prepared by crystallization of eplerenone from a suitable solvent or a mixture of suitable solvents. A suitable solvent or mixture of suitable solvents generally comprises an organic solvent or a mixture of organic solvents that solubilizes the eplerenone together with any impurities at an elevated temperature, but upon cooling, preferentially crystallizes the solvate. The solubility of eplerenone in such solvents or mixtures of solvents generally is about 5 to about 200 mg/mL at room temperature. The solvent or mixtures of solvents preferably are selected from those solvents previously used in the process to prepare the eplerenone starting material, particularly those solvents that would be pharmaceutically acceptable if contained in the final pharmaceutical composition comprising the eplerenone crystalline form. For example, a solvent system comprising methylene chloride that yields a solvate comprising methylene chloride generally is not desirable.

Each solvent used preferably is a pharmaceutically acceptable solvent, particularly a Class 2 or Class 3 solvent as defined in "Impurities: Guideline For Residual Solvents", International Conference On Harmonisation Of Technical Requirements For Registration Of Pharmaceuticals For Human Use (Recommended for Adoption at Step 4 of the ICH Process on July 17, 1997 by the ICH Steering Committee). Still more preferably, the solvent or mixture of solvents is selected from the group consisting of methyl ethyl ketone, 1-propanol,

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2-pentanone, acetic acid, acetone, butyl acetate, chloroform, ethanol, isobutanol, isobutyl acetate, methyl acetate, ethyl propionate, n-butanol, n-octanol, isopropanol, propyl acetate, propylene glycol, t-butanol, tetrahydrofuran, toluene, methanol and t-butyl acetate. Still more preferably, the solvent is selected from the group consisting of methyl ethyl ketone and ethanol.

In another embodiment of this process, the solvent or mixture of solvents is selected from the group consisting of 1-propanol, 2-pentanone, acetic acid, acetone, butyl acetate, chloroform, isobutanol, isobutyl acetate, methyl acetate, ethyl propionate, n-butanol, n-octanol, propyl acetate, propylene glycol, t-butanol, tetrahydrofuran, toluene, methanol and t-butyl acetate.

In another embodiment of this process, the solvent or mixture of solvents is selected from the group consisting of 1-propanol, 2-pentanone, acetic acid, acetone, butyl acetate, chloroform, isobutanol, isobutyl acetate, methyl acetate, ethyl propionate, n-butanol, n-octanol, n-propanol, propyl acetate, propylene glycol, t-butanol, tetrahydrofuran, toluene, methanol and t-butyl acetate.

To prepare the solvated crystalline form of eplerenone, an amount of the eplerenone starting material is solubilized in a volume of the solvent and cooled until crystals form. The solvent temperature at which the eplerenone is added to the solvent generally will be selected based upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, this solvent temperature typically is at least about 25°C, preferably from about 30°C to the boiling point of the solvent, and more preferably from about 25°C below the

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boiling point of the solvent to the boiling point of the solvent.

Alternatively, hot solvent may be added to the eplerenone and the mixture can be cooled until crystals form. The solvent temperature at the time it is added to the eplerenone generally will be selected based upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, the solvent temperature typically is at least 25°C, preferably from about 50°C to the boiling point of the solvent, and more preferably from about 15°C below the boiling point of the solvent to the boiling point of the solvent.

The amount of the eplerenone starting material mixed with a given volume of solvent likewise will depend upon the solubility curve of the solvent or mixture of solvents. Typically, the amount of eplerenone added to the solvent will not completely solubilize in that volume of solvent at room temperature. For most of the solvents described herein, for example, the amount of eplerenone starting material mixed with a given volume of solvent usually is at least about 1.5 to about 4.0 times, preferably about 2.0 to about 3.5 times, and more preferably about 2.5 times, the amount of eplerenone that will solubilize in that volume of solvent at room temperature.

After the eplerenone starting material has completely solubilized in the solvent, the solution typically is cooled slowly to crystallize the solvated crystalline form of eplerenone. For most of the solvents described herein, for example, the solution is cooled at a rate slower than about 20°C/minute, preferably at a rate of about 10°C/minute or slower, more preferably at a rate of about 5°C/minute or

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slower, and still more preferably at a rate of about 1°C/minute or slower.

The endpoint temperature at which the solvated crystalline form is harvested will depend upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, the endpoint temperature typically is less than about 25°C, preferably less than about 5°C, and more preferably less than about -5°C. Decreasing the endpoint temperature generally favors the formation of the solvated crystalline form.

Alternatively, other techniques may be used to prepare the solvate. Examples of such techniques include, but are not limited to, (i) dissolving the eplerenone starting material in one solvent and adding a co-solvent to aid in the crystallization of the solvate crystalline form, (ii) vapor diffusion growth of the solvate, (iii) isolation of the solvate by evaporation, such as rotary evaporation, and (iv) slurry conversation.

The crystals of the solvated crystalline form prepared as described above can be separated from the solvent by any suitable conventional means such as by filtration or centrifugation. Increased agitation of the solvent system during crystallization generally results in smaller crystal particle sizes.

2. Preparation of Form II From Solvate

Form II eplerenone can be prepared directly from the solvated crystalline form by desolvation. Desolvation can be accomplished by any suitable desolvation means such as, but not limited to, heating the solvate, reducing the ambient pressure surrounding the solvate, or combinations thereof.

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If the solvate is heated to remove the solvent, such as in an oven, the temperature of the solvate during this process typically does not exceed the enantiotropic transition temperature for Form I and Form II. This temperature preferably does not exceed about 150°C.

The desolvation pressure and time of desolvation are not narrowly critical. The desolvation pressure preferably is about one atmosphere or less. As the desolvation pressure is reduced, however, the temperature at which the desolvation can be carried out and/or the time of desolvation likewise is reduced. Particularly for solvates having higher desolvation temperatures, drying under vacuum will permit the use of lower drying temperatures. The time of desolvation need only be sufficient to allow for the desolvation, and thus the formation of Form II, to reach completion.

To ensure the preparation of a product that comprises substantially all Form II, the eplerenone starting material typically is a high purity eplerenone, preferably substantially pure eplerenone. The eplerenone starting material used to prepare Form II eplerenone generally is at least 90% pure, preferably at least 95% pure, and more preferably at least 99% pure. As discussed in greater detail elsewhere in this application, certain impurities in the eplerenone starting material can adversely affect the yield and Form II content of the product obtained from the process.

The crystallized eplerenone product prepared in this manner from a high purity eplerenone starting material generally comprises at least 10% Form II, preferably at least 50% Form II, more preferably at least 75% Form II, still more preferably at least 90% Form II, still more preferably at

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least about 95% Form II, and still more preferably substantially phase pure Form II.

3. Preparation of Form I From Solvate

A product comprising Form I can be prepared in substantially the same manner as set forth above for the preparation of Form II by (i) using a low purity eplerenone starting material instead of a high purity eplerenone starting material, (ii) seeding the solvent system with phase pure Form I crystals, or (iii) a combination of (i) and (ii).

A. Use Of Impurities As Growth Promoters and Inhibitors

The presence and amount of selected impurities in the eplerenone starting material, rather than the total amount of all impurities in the eplerenone starting material, affect the potential for Form I crystal formation during the desolvation of the solvate. The selected impurity generally is a Form I growth promoter or Form II growth inhibitor. It may be contained in the eplerenone starting material, contained in the solvent or mixture of solvents before the eplerenone starting material is added, and/or added to the solvent or mixture of solvents after the eplerenone starting material is added. Bonafede et al., "Selective Nucleation and Growth of an Organic Polymorph by Ledge-Directed Epitaxy on a Molecular Crystal Substate", J. Amer. Chem. Soc., Vol. 117, No. 30 (August 2, 1995) discusses the use of growth promoters and growth inhibitors in polymorph systems and is incorporated by reference herein. For the present invention, the impurity generally comprises a compound having a single crystal structure substantially identical to the single crystal structure of Form I. The impurity preferably is a compound

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having an X-ray powder diffraction pattern substantially identical to the X-ray powder diffraction pattern of Form I, and more preferably is selected from the group consisting of the diepoxide, the 11,12-epoxide, the 9,11-olefin and combinations thereof.

The amount of impurity needed to prepare Form I crystals typically can depend, in part, upon the solvent or mixture of solvents and the solubility of the impurity relative to eplerenone. In the crystallization of Form I from a methyl ethyl ketone solvent, for example, the weight ratio of diepoxide to low purity eplerenone starting material typically is at least about 1:100, preferably at least about 3:100, more preferably between about 3:100 and about 1:5, and still more preferably between about 3:100 and about 1:10. The 11,12-epoxide has a higher solubility in methyl ethyl ketone than the diepoxide and generally requires a larger amount of the 11,12-epoxide generally is necessary to prepare Form I crystals. Where the impurity comprises the 11,12-epoxide, the weight ratio of the diepoxide to the low purity eplerenone starting material typically is at least about 1:5, more preferably at least about 3:25, and still more preferably between about 3:25 and about 1:5. Where both the diexpoxide and the 11,12-epoxide impurities are used in the preparation of the Form I crystals, the weight ratio of each impurity to the eplerenone starting material may be lower than the corresponding ratio when only that impurity is used in the preparation of the Form I crystals.

A mixture of Form I and Form II is generally obtained when a solvate comprising the selected impurity is desolvated. The weight fraction of Form I in the product resulting from the initial desolvation of the solvate

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typically is less than about 50%. Further treatment of this product by crystallization or digestion, as discussed below, generally will increase the weight fraction of Form II in the product.

B. Seeding

Form I crystals also can be prepared by seeding the solvent system with phase pure Form I crystals (or a Form I growth promoter and/or Form II growth inhibitor as previously discussed above) prior to crystallization of the eplerenone. The eplerenone starting material can be either a low purity eplerenone or a high purity eplerenone. When the resulting solvate prepared from either starting material is desolvated, the weight fraction of Form I in the product typically is at least about 70% and may be as great as about 100%.

The weight ratio of Form I seed crystals added to the solvent system to the eplerenone starting material added to the solvent system generally is at least about 0.75:100, preferably between about 0.75:100 to about 1:20, and more preferably between about 1:100 to about 1:50. The Form I seed crystals can be prepared by any of the methods discussed in this application for the preparation of Form I crystals, particularly the preparation of Form I crystals by digestion as discussed below.

The Form I seed crystals may be added at one time, in multiple additions or substantially continually over a period of time. The addition of the Form I seed crystals, however, generally is completed before the eplerenone begins to crystallize from solution, i.e., the seeding is completed before the cloud point (the lower end of the metastable zone) is reached. Seeding typically is performed when the solution

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temperature ranges from about 0.5°C above the cloud point to about 10°C above the cloud point, preferably within about 2°C to about 3°C above the cloud point. As the temperature above the cloud point at which the seeds are added increases, the amount of seeding needed for crystallization of Form I crystals generally increases.

The seeding preferably occurs not only above the cloud point, but within the metastable zone. Both the cloud point and the metastable zone are dependent on the eplerenone solubility and concentration in the solvent or mixture of solvents. For a 12 volume dilution of methyl ethyl ketone, for example, the high end of the metastable zone generally is between about 70°C to about 73°C and the lower end of the metastable zone (i.e., the cloud point) is between about 57°C and 63°C. For a concentration of 8 volumes of methyl ethyl ketone, the metastable zone is even narrower because the solution is supersaturated. At this concentration, the cloud point of the solution occurs at about 75°C to about 76°C. Because the boiling point of methyl ethyl ketone is about 80° C under ambient conditions, seeding for this solution typically occurs between about 76.5°C and the boiling point.

An illustrative non-limiting example of seeding with Form I is set forth below in Example 7.

The crystallized eplerenone product obtained using a Form I growth promoter or Form II growth inhibitor, and/or Form I seeding generally comprises at least 2% Form I, preferably at least 5% Form I, more preferably at least 7% Form I, and still more preferably at least about 10% Form I.

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The remaining crystallized eplerenone product generally is Form II.

C. Form I Prepared By Grinding Eplerenone

In yet another alternative, it has been discovered that a small amount of Form I can be prepared by suitable grinding eplerenone. Concentrations of Form I in ground eplerenone as high as about 3% have been observed.

4. Preparation of Form II From Solvate Prepared From Low Purity Eplerenone

As discussed above, crystallization of low purity eplerenone to form a solvate followed by desolvation of the solvate generally yields a product comprising both Form I and Form II. A product having a greater Form II content can be prepared from low purity eplerenone in substantially the same manner as set forth above for the preparation of Form I by seeding the solvent system with phase pure Form II crystals, or by using a Form II growth promoter and/or Form I growth inhibitor. The seeding protocol and the weight ratio of the amount of Form II seed crystals added to the solvent system to the amount of the eplerenone starting material added to the solvent system generally are similar to those ratios previously discussed above for the preparation of Form I eplerenone by seeding with phase pure Form I crystals.

The crystallized eplerenone product prepared in this manner generally comprises at least 10% Form II, preferably at least 50% Form II, more preferably at least 75% Form II, more preferably at least 90% Form II, still more preferably at least about 95% Form II, and still more preferably substantially phase pure Form II.

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The seeding protocols described in this section and in the prior section relating to the preparation of Form I eplerenone also may allow for improved control of the particle size of the crystallized eplerenone.

5. Crystallization of Form II Directly From Solution

Form II eplerenone also can be prepared by the direct crystallization of eplerenone from a suitable solvent or mixture of solvents without the formation of an intermediate solvate and the accompanying need for desolvation. Typically, (i) the solvent has a molecular size that is incompatible with the available channel space in the solvate crystal lattice, (ii) the eplerenone and any impurities are soluble in the solvent at elevated temperatures, and (iii) upon cooling, results in the crystallization of the non-solvated Form II eplerenone. The solubility of eplerenone in the solvent or mixture of solvents generally is about 5 to about 200 mg/mL at room temperature. The solvent or mixture of solvents preferably comprises one or more solvents selected from the group consisting of methanol, ethyl acetate, isopropyl acetate, acetonitrile, nitrobenzene, water and ethyl benzene.

To crystallize Form II eplerenone directly from solution, an amount of the eplerenone starting material is solubilized in a volume of the solvent and cooled until crystals form. The solvent temperature at which the eplerenone is added to the solvent generally will be selected based upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, this solvent temperature typically is at least about 25°C, preferably from about 30°C to the boiling point of the

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solvent, and more preferably from about 25°C below the boiling point of the solvent to the boiling point of the solvent.

Alternatively, hot solvent may be added to the eplerenone and the mixture can be cooled until crystals form. The solvent temperature at the time it is added to the eplerenone generally will be selected based upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, the solvent temperature typically is at least 25°C, preferably from about 50°C to the boiling point of the solvent, and more preferably from about 15°C below the boiling point of the solvent to the boiling point of the solvent.

The amount of the eplerenone starting material mixed with a given volume of solvent likewise will depend upon the solubility curve of the solvent or mixture of solvents. Typically, the amount of eplerenone added to the solvent will not completely solubilize in that volume of solvent at room temperature. For most of the solvents described herein, for example, the amount of eplerenone starting material mixed with a given volume of solvent usually is at least about 1.5 to about 4.0 times, preferably about 2.0 to about 3.5 times, and more preferably about 2.5 times, the amount of eplerenone that will solubilize in that volume of solvent at room temperature.

To ensure the preparation of a product that comprises substantially phase pure Form II, the eplerenone starting material generally is a high purity eplerenone. The eplerenone starting material preferably is at least 65% pure, more preferably at least 90% pure, still more preferably at least 98% pure, and still more preferably at least 99% pure.

After the eplerenone starting material has completely solubilized in the solvent, the solution typically is cooled slowly to crystallize the solvated crystalline form of eplerenone. For most of the solvents described herein, for example, the solution is cooled at a rate slower than about 1.0°C/minute, preferably at a rate of about 0.2°C/minute or slower, and more preferably at a rate between about 5°C/minute and about 0.1°C/minute.

The endpoint temperature at which the Form II crystals are harvested will depend upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, the endpoint temperature typically is less than about 25°C, preferably less than about 5°C, and more preferably less than about -5°C.

Alternatively, other techniques may be used to prepare the Form II crystals. Examples of such techniques include, but are not limited to, (i) dissolving the eplerenone starting material in one solvent and adding a co-solvent to aid in the crystallization of Form II eplerenone, (ii) vapor diffusion growth of Form II eplerenone, (iii) isolation of Form II eplerenone by evaporation, such as rotary evaporation, and (iv) slurry conversion.

The crystals of the solvated crystalline form prepared as described above can be separated from the solvent by any suitable conventional means such as by filtration or centrifugation.

In addition, Form II eplerenone also can be prepared by digesting (as described below) a slurry of high purity eplerenone in methyl ethyl ketone and filtering the digested eplerenone at the boiling point of the slurry.

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6. Preparation of Form I Directly From Solution

It is hypothesized that if the crystallization is performed above the enantiotropic transition temperature (T_t) for Form I and Form II, particularly if Form I growth promoters or Form II growth inhibitors are present or the solvent is seeded with phase pure Form I crystals, Form I should crystallize directly from solution since Form I is more stable at these higher temperatures. The solvent system used preferably comprises a high boiling solvent such as nitrobenzene. Suitable Form I growth promoters would include, but would not be limited to, the diepoxide and the 11,12-olefin.

7. Digestion of Eplerenone With A Solvent

The solvated crystalline forms, Form I and Form II of eplerenone also can be prepared by digestion of an eplerenone starting material in a suitable solvent or mixture of solvents. In the digestion process, a slurry of eplerenone is heated at the boiling point of the solvent or mixture of solvents. For example, an amount of eplerenone starting material is combined with a volume of solvent or mixture of solvents, heated to reflux, and the distillate is removed while an additional amount of the solvent is added simultaneously with the removal of the distillate. Alternatively, the distillate can be condensed and recycled without the addition of more solvent during the digestion process. Typically, once the original volume of solvent has been removed or condensed and recycled, the slurry is cooled and solvated crystals form. The solvated crystals can be separated from the solvent by any suitable conventional means such as by filtration or centrifugation. Desolvation of the

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solvate as previously described yields either Form I or Form II eplerenone depending upon the presence or absence of the selected impurities in the solvated crystals.

A suitable solvent or mixture of solvents generally comprises one or more of the solvents previously disclosed herein. The solvent may be selected, for example, from the group consisting of methyl ethyl ketone and ethanol.

The amount of eplerenone starting material added to the solvent used in the digestion process generally is sufficient to maintain a slurry (i.e., the eplerenone in the solvent or mixture of solvents is not completely solubilized) at the boiling point of the solvent or mixture of solvents. Illustrative values include, but are not limited to, about one gram of eplerenone per four mL methyl ethyl ketone and about one gram of eplerenone per eight mL ethanol.

The solution generally is cooled slowly once solvent turnover is complete to crystallize the solvated crystalline form of eplerenone. For the solvents tested, for example, the solution is cooled at a rate slower than about 20°C/minute, preferably about 10°C/minute or slower, more preferably about 5°C/minute or slower, and still more preferably about 1°C/minute or slower.

The endpoint temperature at which the solvated crystalline form is harvested will depend upon the solubility curve of the solvent or mixture of solvents. For most of the solvents described herein, for example, the endpoint temperature typically is less than about 25°C, preferably less than about 5°C, and more preferably less than about -5°C.

If a product comprising primarily or exclusively Form II is desired, a high purity eplerenone starting material

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typically is digested. The high purity eplerenone starting material preferably is at least 98% pure, more preferably at least 99% pure, and still more preferably at least 99.5% pure. The digested eplerenone product prepared in this manner generally comprises at least 10% Form II, preferably at least 50% Form II, more preferably at least 75% Form II, more preferably at least 90% Form II, still more preferably at least about 95% Form II, and still more preferably substantially phase pure Form II.

If a product comprising primarily or exclusively Form I is desired, a low purity eplerenone starting material typically is digested. The low purity eplerenone starting material generally contains only as much Form I growth promoter and/or Form II growth inhibitor as is needed to yield Form I. Preferably, the low purity eplerenone starting material is at least 65% pure, more preferably at least 75% pure, and still more preferably at least 80% pure. The digested eplerenone product prepared in this manner generally comprises at least 10% Form I, preferably at least 50% Form I, more preferably at least 75% Form I, more preferably at least 90% Form I, still more preferably at least about 95% Form I, and still more preferably substantially phase pure Form I.

8. Preparation of Amorphous Eplerenone

Amorphous eplerenone can be prepared in small quantities by suitable comminution of solid eplerenone, such as by crushing, grinding and/or micronizing. Phase pure amorphous eplerenone can be prepared, for example, by lyophilizing a solution of eplerenone, particularly an aqueous solution of

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eplerenone. These processes are illustrated in Examples 13 and 14 below.

Additional Processing Considerations

1. Thermodynamic Stability Considerations

Form II is more thermodynamically stable than Form I at ambient temperatures. As described in Example 5 below, when an organic slurry containing equivalent amounts of Form I and Form II was allowed to stand overnight at room temperature and the residual solids were then collected and analyzed by X-ray powder diffraction, the analytical results indicated that the eplerenone had completely converted to Form II. The differential scanning calorimetry ("DSC") data (discussed previously) indicate that Form I is more thermodynamically stable than Form II at higher temperatures since it has the higher melting/decomposition temperature. Taken together, the slurry conversion and DSC data indicate that Form I and Form II are related enantiotropically, i.e., a change in the stability relationship between the two polymorphs occurs around a transition temperature (T_t), with Form II being the more stable at lower temperatures. Fig. 12 shows the Gibbs free energy-temperature relationship generally observed for enantiotropically related polymorphs such as Form I and Form II, wherein I and II refer to the crystal forms, T_t refers to the transition temperature, T_m refers to the melting points of Form I and Form II, and L represents the liquid or melt state.

Accordingly, processing temperatures preferably are maintained below the transition temperature during the preparation of compositions comprising Form II. For example, drying temperatures employed for desolvation typically are

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less than about 150°C, preferably less than about 125°C, more preferably less than about 115°C, more preferably less than about 110°C, and still more preferably between about 80°C to about 110°C. In addition, cooling (such as by use of liquid nitrogen) may be necessary during particle size reduction process steps to maintain the temperature of the Form II crystals below the transition temperature.

2. Intrinsic Micronizing Considerations

The method used to prepare crystalline eplerenone may affect the properties of the resulting crystal. For example, Form II prepared by desolvation of a solvate exhibits a higher incidence of surface defects, pores, cracks and fractures within the crystal lattice than Form II prepared by direct crystallization from solution. This "intrinsic micronizing" of the desolvated crystal results in an increase in both the available surface area of the crystal and the dissolution rate of the crystal. Dissolution times, therefore, can be shortened by selection of Form II crystals prepared by desolvation, lengthened by selection of Form II crystals prepared by direct crystallization, or otherwise adjusted by selection of an appropriate combination of Form II crystals prepared by desolvation and Form II crystals prepared by direct crystallization.

This "intrinsic micronizing" also may effectively reduce or eliminate the need for crystal particle size reduction during processing steps where Form II crystals prepared by desolvation are used in the preparation of the pharmaceutical composition. One disadvantage to using such Form II crystals, however, is the need for a desolvation step that is

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not required for Form II crystals prepared by direct crystallization.

Product-By-Process Solid State Forms

The present invention also comprises each of the solid state eplerenone forms prepared in accordance with the processes disclosed in this application. In particular, the invention comprises solvated eplerenone, Form I eplerenone, Form II eplerenone, and combinations thereof, prepared as set forth in this application.

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Combinations of Solid State Forms

Also embraced within this invention are combinations comprising a first solid state form of eplerenone and a second solid state form of eplerenone, wherein the first and second solid state forms of eplerenone are selected from Form I, Form II, solvated eplerenone and amorphous eplerenone. In general, the weight ratio of said first solid state form to said second solid state form preferably is at least about 1:9, more preferably is at least about 1:1, more preferably at least about 2:1, more preferably at least about 5:1, and still more preferably at least about 9:1.

In one embodiment, for example, the first solid state form is Form I and the second solid state form is Form II.

In another embodiment, for example, the first solid state form is Form II and the second solid state form is Form I.

Pharmaceutical Compositions

Also embraced within this invention is a class of pharmaceutical compositions comprising a first solid state

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form of eplerenone and a second solid state form of eplerenone in association with one or more pharmaceutically-acceptable carriers and/or diluents and/or adjuvants (collectively referred to herein as "carrier" materials) and, if desired, other active ingredients. The first solid state form of eplerenone and the second solid state form of eplerenone are selected from Form I, Form II, solvated eplerenone and amorphous eplerenone. In general, the weight ratio of said first solid state form to said second solid state form preferably is at least about 1:9, more preferably is at least about 1:1, more preferably at least about 2:1, more preferably at least about 5:1, and still more preferably at least about 9:1.

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In one embodiment of the invention, the entire amount of eplerenone contained in the composition is present as phase pure Form II.

In another embodiment of the invention, the entire amount of eplerenone contained in the composition is present as phase pure Form I.

In still another embodiment of the invention, the entire amount of eplerenone contained in the composition is present as phase pure solvated crystalline eplerenone.

In still another embodiment of the invention, the entire amount of eplerenone contained in the composition is present as amorphous eplerenone.

In still another embodiment of the invention, the composition comprises both Form I and Form II. The ratio of the amount of Form II to Form I in the composition generally is between about 1:20 to about 20:1. In other embodiments, for example, this ratio is between about 10:1 to about 1:10;

about 5:1 to about 1:5; about 2:1 to about 1:2; or is about 1:1.

In still another embodiment of the invention, the composition comprises a first solid state form of eplerenone and a second solid state form of eplerenone, wherein the first and second solid state forms of eplerenone are selected from Form I, Form II, solvated eplerenone and amorphous eplerenone. In general, the weight ratio of said first solid state form to said second solid state form preferably is at least about 1:9, preferably about 1:1, more preferably at least about 2:1, more preferably at least about 5:1, and still more preferably at least about 9:1.

In each of the above combination and pharmaceutical composition embodiments, the D_{90} particle size of the eplerenone used as a starting material in the pharmaceutical composition generally is between about 25 microns to about 400 microns, preferably less than about 200 microns, more preferably less than about 150 microns, still more preferably less than about 100 microns, and still more preferably less than about 90 microns. In one embodiment, the D_{90} particle size is between about 40 microns to about 100 microns. In another embodiment, the D_{90} particle size is between about 30 microns to about 50 microns. In another embodiment, the D_{90} particle size is between about 50 microns to about 150 microns. In another embodiment, the D_{90} particle size is between about 75 microns to about 125 microns.

These solid state forms of eplerenone of the present invention may be administered by any suitable route, preferably in the form of a pharmaceutical composition adapted to such a route, and in a dose effective for the treatment intended. The solid state forms of eplerenone and

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compositions thereof may, for example, be administered orally, intravascularly, intraperitoneally, subcutaneously, intramuscularly or topically.

The amount of eplerenone that is administered and the dosage regimen for treating a condition or disorder with the solid state forms of eplerenone and/or compositions of this invention depends on a variety of factors, including the age, weight, sex and medical condition of the subject, the severity of the disease, the route and frequency of administration, and the particular compound employed, and thus may vary widely. The pharmaceutical compositions may contain eplerenone in the range of about 0.1 to 2000 mg, preferably in the range of about 0.5 to 500 mg and most preferably between about 1 and 200 mg. A daily dose of about 0.01 to 100 mg/kg body weight, preferably between about 0.5 and about 20 mg/kg body weight and most preferably between about 0.1 to 10 mg/kg body weight, may be appropriate. The daily dose can be administered in one to four doses per day.

For oral administration, the pharmaceutical composition may be in the form of, for example, a tablet, capsule, or suspension. The pharmaceutical composition is preferably made in the form of a dosage unit containing a particular amount of eplerenone. Examples of such dosage units are tablets or capsules. The eplerenone may also be administered by injection of a suspension wherein, for example, saline, dextrose or water may be used as a suitable carrier.

Other formulations may be in the form of a topical ointment or cream, or a suppository, containing eplerenone in a total amount of, for example, 0.075 to 30% w/w, preferably 0.2 to 20% w/w and most preferably 0.4 to 15% w/w. When formulated in an ointment, the eplerenone may be employed

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with either paraffinic or a water-miscible ointment base. Alternatively, the eplerenone may be formulated in a cream with an oil-in-water cream base. If desired, the aqueous phase of the cream base may include, for example at least 30% w/w of a polyhydric alcohol such as propylene glycol, butane-1,3-diol, mannitol, sorbitol, glycerol, polyethylene glycol and mixtures thereof. The topical formulation may desirably include a compound which enhances absorption or penetration of the eplerenone through the skin or other affected areas. Examples of such dermal penetration enhancers include dimethylsulfoxide and related analogs.

The novel solid state forms of eplerenone also can be administered by a transdermal device. Preferably, topical administration will be accomplished using a patch either of the reservoir and porous membrane type or of a solid matrix variety. In either case, the eplerenone is delivered continuously from the reservoir or microcapsules through a membrane into the eplerenone permeable adhesive, which is in contact with the skin or mucosa of the recipient. If the eplerenone is absorbed through the skin, a controlled and predetermined flow of the eplerenone is administered to the recipient. In the case of microcapsules, the encapsulating agent may also function as the membrane.

The oily phase of the emulsions of this invention may be constituted from known ingredients in a known manner. While the phase may comprise merely an emulsifier, it may comprise a mixture of at least one emulsifier with a fat or an oil or with both a fat and an oil. Preferably, a hydrophilic emulsifier is included together with a lipophilic emulsifier which acts as a stabilizer. It is also preferred to include both an oil and a fat. Together, the emulsifier(s) with or

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without stabilizer(s) make-up the so-called emulsifying wax, and the wax together with the oil and fat make up the so-called emulsifying ointment base which forms the oily dispersed phase of the cream formulations. Emulsifiers and emulsion stabilizers suitable for use in the formulation of the present invention include Tween 60, Span 80, cetostearyl alcohol, myristyl alcohol, glyceryl monostearate, and sodium lauryl sulfate, among others.

The choice of suitable oils or fats for the formulation is based on achieving the desired cosmetic properties, since the solubility of the eplerenone in most oils likely to be used in pharmaceutical emulsion formulations is very low. Thus, the cream should preferably be a non-greasy, non-staining and washable product with suitable consistency to avoid leakage from tubes or other containers. Straight or branched chain, mono- or dibasic alkyl esters such as di-isoadipate, isocetyl stearate, propylene glycol diester of coconut fatty acids, isopropyl myristate, decyl oleate, isopropyl palmitate, butyl stearate, 2-ethylhexyl palmitate or a blend of branched chain esters may be used. These may be used alone or in combination depending on the properties required. Alternatively, high melting point lipids such as white soft paraffin and/or liquid paraffin or other mineral oils can be used.

Formulations suitable for topical administration to the eye also include eye drops wherein the eplerenone is suspended in suitable carrier, especially an aqueous carrier for the eplerenone. The eplerenone is preferably present in such formulations in a concentration of 0.5 to 20%, advantageously 0.5 to 10% and particularly about 1.5% w/w.

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For therapeutic purposes, the novel solid state forms of eplerenone ordinarily are combined with one or more adjuvants appropriate to the indicated route of administration. If administered per os, the compound may be admixed with lactose, sucrose, starch powder, cellulose esters of alkanoic acids, cellulose alkyl esters, talc, stearic acid, magnesium stearate, magnesium oxide, sodium and calcium salts of phosphoric and sulfuric acids, gelatin, acacia gum, sodium alginate, polyvinylpyrrolidone, and/or polyvinyl alcohol, and then tableted or encapsulated for convenient administration. Such capsules or tablets may contain an immediate release formulation, or may contain a controlled-release formulation such as a dispersion of the eplerenone in hydroxypropylmethyl cellulose. Formulations for parenteral administration may be in the form of aqueous or non-aqueous isotonic sterile injection suspensions. These suspensions may be prepared from sterile powders or granules having one or more of the carriers or diluents mentioned for use in the formulations for oral administration. Other adjuvants and modes of administration are well and widely known in the pharmaceutical art.

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Methods of Treatment or Prophylaxis

The present invention also comprises methods for the treatment and/or prophylaxis of an aldosterone-mediated condition and/or disorder such as hypertension in a subject, the methods comprising treating the subject having or susceptible to such condition or disorder with a therapeutically-effective amount of Form I, Form II, solvated crystalline form, or an amorphous form of eplerenone, or combinations thereof. These solid state forms of eplerenone

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would be useful for the treatment and/or prophylaxis of a condition and/or disorder in a subject where administration of an aldosterone-antagonist is indicated including, but not limited to, the treatment of hyperaldosteronism such as hypertension, heart failure including cardiac insufficiency, cirrhosis of the liver, excess collagen/fibrosis, benign prostate hypertrophy and depression.

Besides being useful for human treatment, these solid state forms of eplerenone are also useful for veterinary treatment of companion animals, exotic animals and farm animals, including mammals, rodents, and the like. More preferred animals include horses, dogs, and cats.

These solid state forms of eplerenone also may be used (i) in combination therapies partially or completely in place of other conventional aldosterone receptor antagonists, or (ii) in combination therapies with other active drugs. The phrase "combination therapy" is intended to embrace the administration of each agent in a sequential manner in a regimen that will provide beneficial effects of the drug combination, and is intended as well to embrace co-administration of these agents in a substantially simultaneous manner, such as in a single capsule or injection having a fixed ratio of these active agents or in multiple, separate capsules or injections for each agent. Non-limiting examples of combination therapy include the treatment of cardiovascular diseases using a combination of an aldosterone receptor antagonist and an angiotensin II receptor antagonist as described in WO96/24373; the treatment of congestive heart failure using a combination of an aldosterone receptor antagonist and an angiotensin II antagonist as described in WO96/40257; and the treatment of heart failure using a

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combination of an aldosterone receptor antagonist, an ACE inhibitor and a diuretic as described in WO96/24372.

Working Examples

The following examples contain detailed descriptions of the methods of preparation of the various solid state forms of eplerenone described in this application. These detailed descriptions fall within the scope, and serve to exemplify the invention. These detailed descriptions are presented for illustrative purposes only and are not intended as a restriction on the scope of the invention. All parts are by weight and temperatures are in degrees Centigrade unless otherwise indicated. The eplerenone starting material used in each of the following examples was prepared in accordance with scheme 1 set forth in Ng et al., WO98/25948.

Example 1: Preparation of (a) methyl ethyl ketone solvate from high purity eplerenone starting material and (b) Form II crystalline eplerenone from resulting solvate

A. Preparation of Methyl Ethyl Ketone Solvate:

High purity eplerenone (437 mg; greater than 99% purity with less than 0.2% diepoxide and 11,12 epoxide present) was dissolved in 10 mL of methyl ethyl ketone by heating to boiling on a hot plate with magnetic stirring at 900 rpm. The resulting solution was allowed to cool to room temperature with continuous magnetic stirring. Once at room temperature, the solution was transferred to a 1°C bath with maintenance of the stirring for one hour. After one hour, the solid methyl ethyl ketone solvate was collected by vacuum filtration.

B. Preparation of Form II crystalline eplerenone:

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The solid methyl ethyl ketone solvate prepared in Step A above was dried in an oven at 100°C for four hours at ambient pressure. The dried solid was determined to be pure Form II by DSC and XPRD analysis.

Example 2: Preparation of additional solvates from high purity eplerenone starting material

Additional solvated crystalline forms were prepared by replacing methyl ethyl ketone with one of the following solvents: n-propanol, 2-pentanone, acetic acid, acetone, butyl acetate, chloroform, ethanol, isobutanol, isobutyl acetate, isopropanol, methyl acetate, ethyl propionate, n-butanol, n-octanol, propyl acetate, propylene glycol, t-butanol, tetrahydrofuran, and toluene and carrying out the crystallization substantially as described above in Step A of Example 1.

Example 3: Preparation of Methyl Ethyl Ketone Solvate by Vapor Diffusion Growth

Eplerenone (400 mg; greater than 99.9% purity) was dissolved in 20 mL of methyl ethyl ketone by warming on a hot plate to form a stock solution. An 8 mL amount of the stock solution was transferred to a first 20 mL scintillation vial and diluted to 10 mL with methyl ethyl ketone (80%). A 10 mL amount of the stock solution was transferred to a second 20 mL scintillation vial and diluted to 10 mL with methyl ethyl ketone (40%). The final 2 mL of the stock solution was diluted to 10 mL with methyl ethyl ketone (20%). The four vials containing the dilutions were transferred to a dessicator jar containing a small amount of hexane as an anti-solvent. The dessicator jar was sealed and the hexane

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vapor allowed to diffuse into the methyl ethyl ketone solutions. Methyl ethyl ketone solvate crystals grew in the 80% dilution sample by the next day.

Example 4: Preparation of Methyl Ethyl Ketone Solvate by Rotary Evaporation

About 400 mg of eplerenone (greater than 99.9% purity) is weighed into a 250 mL round bottom flask. Solvent (150 mL) is added to the flask and, if necessary, the solution is heated gently until the solid is dissolved. The resulting clear solution is placed on a Buchi rotary evaporator with a bath temperature of about 85°C and the solvent is removed under vacuum. Solvent removal is stopped when approximately 10 mL of solvent remain in the round bottom flask. The resulting solids are analyzed by appropriate method (XPRD, DSC, TGA, microscopy, etc.) for determination of form.

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Example 5: Slurry Conversion

Approximately 150 mg of Form II eplerenone and 150 mg of Form I eplerenone were added to 5 mL of ethyl acetate. The resulting slurry was allowed to stir at 300 rpm (magnetic stirring) overnight. The next day a sample of the solid was collected by filtration. Analysis of the sample by XPRD indicated that the sample was entirely composed of Form II eplerenone.

Example 6: Preparation of (a) solvate from low purity eplerenone starting material and (b) Form I crystalline eplerenone from resulting solvate

Samples containing varying amounts of the impurity 7-methyl hydrogen 4α,5α:9α,11α-diepoxy-17-hydroxy-3-oxo-17α-

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pregnane-7α,21-dicarboxylate, γ-lactone (the "diepoxide") or the impurity 7-methyl hydrogen 11α,12α-epoxy-17-hydroxy-3-oxo-17α-pregn-4-ene-7α,21-dicarboxylate, γ-lactone (the "11,12-epoxide") were prepared by adding the desired amount of the impurity to a 7 mL scintillation vial together with an amount of eplerenone sufficient to provide a total sample mass of 100 mg. The weight percent of the diepoxide or 11,12-epoxide in each sample is given in Tables X-6A and X-6B, respectively. A micro-flea magnetic stirrer was added to each scintillation vial along with 1 mL of methyl ethyl ketone. The vials were loosely capped and the solid dissolved by heating to reflux on a hot plate with magnetic stirring. Once the solids were dissolved, the solutions were allowed to cool to room temperature on the hot plate. Magnetic stirring was maintained during the cooling period. After the solutions reached room temperature, the solids were collected by vacuum filtration and immediately analyzed by X-ray powder diffraction (XPRD). The solids were then placed in a 100°C oven and dried for one hour at ambient pressure. The dried solids were analyzed by XPRD for Form I content by monitoring the area of the Form I diffraction peak at about 12.1 degrees two theta. All XPRD diffraction patterns were recorded using an Inel Multipurpose Diffractometer.

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TABLE X-6A

Weight Percent Diepoxide	Weight Eplerenone (mg)	Weight Diepoxide (mg)
0%	100.44	--
1%	99.08	1.24
2%	98.09	2.24
3%	97.08	3.04
5%	95.09	5.04

TABLE X-6B

Weight Percent 11,12-Epoxide	Weight Eplerenone (mg)	Weight 11,12-Epoxide (mg)
0%	101.38	0
1%	99.23	1.10
5%	94.97	5.36
10%	90.13	10.86

A. Diepoxide Results

Fig. 13 shows the X-ray powder diffraction patterns for the wet cake (methyl ethyl ketone solvate) obtained from the (a) 0%, (b) 1%, (c) 3%, and (d) 5% diepoxide-doped methyl

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ethyl ketone crystallizations. The peak intensities have been normalized for ease of comparison. No peaks characteristic of Form I or the diepoxide are present in the diffraction patterns. The patterns are characteristic of the methyl ethyl ketone solvate of eplerenone.

Fig. 14 shows the X-ray powder diffraction patterns for the dried solids obtained from the (a) 0%, (b) 1%, (c) 3%, and (d) 5% diepoxide-doped methyl ethyl ketone crystallizations. The peak intensities have been normalized for ease of comparison. No Form I was detected for the dried samples corresponding to the methyl ethyl ketone crystallizations performed at doping levels of 0 and 1%. Form I was detected in the dried samples corresponding to the methyl ethyl ketone crystallizations performed at doping levels of 3 and 5%. The area for the Form I diffraction peak at about 12.1 degrees two theta and the estimated Form I content for each sample are given in Table X-6C below.

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TABLE X-6C

Weight Percent of Diepoxide in Starting Eplerenone Mixture	Weight Percent of Diepoxide in Resulting Crystals (HPLC)	Form I Peak Area 12° Two Theta Peak	Estimated Weight Percent of Form I
0%	---	None Detected	None Detected
1%	0.29%	None Detected	None Detected
3%	0.58%	1168	10%
5%	1.05%	4175	30%

The results reported in Table X-6C confirm that the presence of the diepoxide affects the formation of Form I during the desolvation. These results indicate that the

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diepoxide is effective in inducing the formation of Form I eplerenone when it is incorporated into and/or adsorbed onto the methyl ethyl ketone solvate crystals.

The 3% diepoxide doping experiment was repeated to analyze the impact of the route of preparation on the amount of Form I formed during the desolvation. In this experiment, the methyl ethyl ketone solvate obtained from the doped crystallization was divided into two portions. The first portion was left untreated while the second portion was lightly ground in a mortar and pestle to induce a higher level of crystal defects. The two portions were both dried at 100 °C for one hour at ambient pressure. The dried solids were analyzed by XPRD. The XPRD patterns are given in Fig. 15 for the dried solids from the methyl ethyl ketone crystallization with 3% doping of diepoxide (a) without grinding of the solvate prior to drying, and (b) with grinding of the solvate prior to drying. The XPRD patterns indicated a greater amount of Form I in the ground sample relative to the unground sample. These results suggest that the conditions under which the methyl ethyl ketone solvate is isolated and handled can affect the crystal form that results from the desolvation.

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B. 11,12-Epoxide Results

Fig. 16 shows the X-ray powder diffraction patterns for the wet cake (methyl ethyl ketone solvate) obtained from the (a) 0%, (b) 1%, (c) 5%, and (d) 10% 11,12-epoxide-doped methyl ethyl ketone crystallizations. The peak intensities have been normalized for ease of comparison. No peaks characteristic of Form I or the 11,12-epoxide are present in the diffraction

patterns. The patterns are characteristic of the methyl ethyl ketone solvate of eplerenone.

Fig. 17 shows the X-ray powder diffraction patterns for the dried solids obtained from the (a) 0%, (b) 1%, (c) 5%, and (d) 10% 11,12-epoxide-doped methyl ethyl ketone crystallizations. The peak intensities have been normalized for ease of comparison. No Form I was detected for the dried samples corresponding to the methyl ethyl ketone crystallizations performed at doping levels of 0, 1% and 5%. Form I was detected in the dried samples corresponding to the methyl ethyl ketone crystallization performed at a doping level of 10%. The area for the Form I diffraction peak at 12.1 degrees two theta and estimated Form I content for each sample are given in Table X-6D.

TABLE X-6D

Weight Percent 11,12-Epoxide in Starting Eplerenone Mixture	Weight Percent 11,12-Epoxide in Resulting Crystals (HPLC)	Form I Peak Area 12° Two Theta Peak	Estimated Weight Percent of Form I
0%	Not Available	None Detected	None Detected
1%	Not Available	None Detected	None Detected
5%	Not Available	None Detected	None Detected
10%	Not Available	1541	10-15%

The results reported in Table X-6D confirm that the presence of the 11,12-epoxide impacts the formation of Form I during the desolvation. The percentage of impurity in the methyl ethyl ketone crystallization required to induce the formation of Form I eplerenone appears to be greater for the 11,12-epoxide than for the diepoxide.

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Example 7: Effect of Crystallization and Drying on Final Crystal Form

The following four experiments analyzing the effect of crystallization and drying on the final crystal form were conducted: (i) methyl ethyl ketone crystallization of eplerenone (2^3+3 statistical design of experiment), (ii) crystallization of poor quality mother liquor residue, (iii) crystallization of high purity eplerenone with form I seeding, and (iv) crystallization of low purity eplerenone with form II seeding. Variables in the design of the experiments included cooling rate, starting material purity level, and end point temperature of crystallization. For purposes of this Example, high purity eplerenone was defined as ultra-pure milled eplerenone (HPLC analysis showed this material to be 100.8% pure) and low purity eplerenone was defined as 89% pure eplerenone. To prepare the low purity eplerenone, stripped-down mother liquors from the process for the preparation of eplerenone were analyzed and blended to yield a material that was 61.1% eplerenone, 12.8% diepoxide and 7.6% 11,12-epoxide. This material was then blended with a sufficient amount of high purity eplerenone to yield the 89% eplerenone.

A. Methyl Ethyl Ketone Crystallization

In the methyl ethyl ketone crystallization experiment, all runs were performed using 60 g of high purity eplerenone. High endpoint was defined as 45°C and low endpoint was defined as 5°C. High cooling rate was defined as 3°C/minute cooling and low cooling rate was defined as 0.1°C/minute cooling. Center points were 1.5°C/minute cooling, 94.5% pure eplerenone, and a 25°C endpoint.

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After a background reading was taken with the FTIR, 250 mL of methyl ethyl ketone was charged to a 1L Mettler RC-1, MP10 reactor and stirred at 100 rpm. After several scans, eplerenone was charged to the reactor followed by an additional 470 mL of methyl ethyl ketone. Agitation was increased to 500 rpm to suspend solids and the batch temperature was increased to 80°C. The batch temperature was held at 80°C to ensure dissolution of the eplerenone. Black or white specks generally were visible in the resulting transparent solution. The batch temperature was then ramp cooled at the desired rate to the desired endpoint, where it was maintained for one hour before being pulled into a transfer flask and filtered. The vacuum was reactor, transfer flask and cake were then washed with 120 mL methyl ethyl ketone. Once the wash was pulled through the cake, the stopped. About 10 grams of each wet cake were dried in a vacuum oven under nominal conditions of 75°C with a light nitrogen bleed. For the "high, high, high" and "low, low, low" experiments described below, fluid bed drying was operated under high and low conditions. High fluid bed drying was defined as 100°C with a blower setting of "4" while low fluid bed drying was defined as 40°C with a blower setting of "1".

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B. Crystallization of Poor Quality Mother Liquor Residue

In the crystallization of poor quality mother liquor residue experiment, 60 g of the 61.1% pure material and 720 mL methyl ethyl ketone were charged directly to a 1L Mettler RC-1, MP10 reactor. The 61.1% pure material was not blended with high purity eplerenone prior to being charged to the reactor. The resulting mixture was heated to 80°C and was an opaque

slurry at that temperature. The crystallization continued and the mixture was filtered at 45°C under fast cooling conditions.

C. Form I Seeding

In the Form I seeding experiment, 60 g of pure (100.8%) eplerenone and 720 mL of methyl ethyl ketone were charged to a 1L Mettler RC-1, MP10 reactor. The mixture was heated to 80°C and then cooled to 25°C at a rate of 1.5° C/minute. When the solution had cooled to 62°C, it was seeded with 3 g of phase pure Form I crystals to initiate crystallization. The Form I seed crystals were prepared by the digestion process described in Example 9 below.

D. Form II Seeding

In the Form II seeding experiment, 66.6 g of 89.3% eplerenone (prepared by mixing 48.3 g of 100% eplerenone with 18.3 g of 61.1% eplerenone) and 720 mL of methyl ethyl ketone were charged to a 1L Mettler RC-1, MP10 reactor. The mixture was heated to 80°C and then cooled to 25°C at a rate of 1.5° C/minute. When the solution had cooled to 63°C, it was seeded with 3 g of phase pure Form II crystals to initiate crystallization. The Form II seed crystals were prepared by the crystallization and desolvation process described in Example 1 above.

Results from the experiments are reported in Table X-7A. In the n+1 crystallization experiment, Form I was detected only in the experiments employing low purity eplerenone where the product contained the diepoxide. Elevated levels of the diepoxide in the final product were also observed with higher cooling rates.

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The crystallization of poor quality mother liquor residue experiment yielded poor quality material that appeared to be a mixture of the diepoxide and Form I when analyzed by X-ray powder diffraction.

The Form I seeding experiment (where high purity eplerenone was seeded with Form I) yielded a product that was 77% Form I based on X-ray powder diffraction analysis, but entirely Form I based on DSC. The X-ray powder diffraction model, however, had not been tested for linearity beyond about 15% Form I. This experiment was the only one of the four experiments of this Example where Form I was created in the absence of the diepoxide.

The Form II seeding experiment (where low purity eplerenone was seeded with Form II) yielded a product that was entirely Form II.

The data obtained for the high fluid bed drying of eplerenone appeared to correspond to the data obtained for the vacuum oven drying. The low fluid bed dryings yielded results that differed from those of the vacuum oven dryings.

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TABLE X 7A

Cooling Rate[1]	Cooling Endpoint	Impurity Level[2]	Nucleation Temperature (°C)	Weight Percent 11 12 Epoxide[4]	Weight Percent Diepoxide	Assay For Desolvated Crystal	Percent Yield	Weight Percent Form I (XPRD)
+	+		57 0	ND	ND	100 3	66 1	ND
+	~		54 9	ND	ND	100 3	98 1	ND
	+		60 9	ND	ND	100 3		ND
			63 4	ND	ND	100 5	79 3	ND
+	+	++	N/A	4 8	36 6	43 3	27	100[5]
+	+	+	52 2	0 49	0 88	98	62	29
+		+	53 3	0 56	1 0	98 1	87	9
0	0	0	59 0	0 18	0 36	99 4	75	5
	+	+	63 3	0 20	0 44	99 4	36	31
		+	61 4	0 18	0 40	99 5	87	ND
0	0	0	60 6	0 18	0 36	99 5	79 2	ND
0	0	0	55 9	0 38	0 80	98 6	80 5	<3%
0	0	100 8% eplerenone seeded with Form I		0 03	ND	100 4	82 2	77/100[6]
0	0	89 3% eplerenone seeded with Form II		0 33	0 50	97 5	80 2	ND

[1]Cooling Rate (+) = 3 C/min ; (0) = 1 5 °C/min ; and ( ) = 0 1 °C/min

[2]Cooling Endpoint (+) = 45 C; (0) = 25 °C; and ( ) = 5°C

Impurity Level: ; (+) = 89 3% purity eplerenone starting material; (++) = 61 1% purity eplerenone starting material; (0) = 100 8% purity eplerenone starting material and ( ) = 94 5% purity eplerenone starting material

[4]Weight percent after drying solvate in a vacuum oven at 75 C

[5]Appears to be mixture of Form I and diepoxide when analyzed by XPRD

[6]Appears to be 77% Form I when analyzed by XPRD and 100% Form I when analyzed by DSC

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A. Material Purity

A cube plot of product purity, starting material purity, cooling rate and endpoint temperature based on the data reported in Table X-7A is shown in Fig. 18. The cube plot suggests that the use of a higher purity material at the start of crystallization will yield a higher purity product. The endpoint temperature of crystallization does not appear to greatly affect the product purity. The cooling rate, however, appears to have an effect with slightly less pure product resulting from a faster cooling rate. In fact, the level of diepoxide generally was higher with faster cooling rates.

Fig. 19 shows a half normal plot that was prepared using the results of cube plot to determine which variables, if any, had a statistically significant effect on the product purity. Starting material purity had the greatest statistically significant effect on product purity, although cooling rate and the interaction between cooling rate and starting material purity were also seen as statistically significant effects.

Fig. 20 is an interaction graph based on these results and showing the interaction between starting material purity and cooling rate on product purity. With the high purity eplerenone (100.8% eplerenone starting material) the cooling rate appears to have little or no effect on final purity. With the low purity eplerenone (89.3% eplerenone starting material), however, the product purity decreases as cooling rate increases. This result suggests that more impurities crystallize out in eplerenone crystallizations conducted at higher cooling rates.

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B. Form I Content

A cube plot of Form I weight fraction, starting material product purity, cooling rate and endpoint temperature based on the data reported in Table X-7A is shown in Fig. 21. The cube plot suggests that the use of a higher purity eplerenone at the start of crystallization will yield a lower amount of Form I. The endpoint temperature of crystallization also appears to have an effect on the form of the final product. The cooling rate does not appear to greatly affect the formation of Form I although some Form I may result from faster cooling at the low endpoint temperature in the presence of impurities.

Fig. 22 shows a half normal plot that was prepared using the results of the cube plot to determine which variables, if any, had a statistically significant effect on the amount of Form I in the final material. Starting material purity, endpoint temperature of the crystallization and the interaction between the two variables were seen as statistically significant effects.

Fig. 23 is an interaction graph based on these results and showing the interaction between starting material purity and endpoint temperature on final Form I content. With the high purity eplerenone (100.8% eplerenone starting material), endpoint temperature appears to have little effect on Form I content. No Form I resulted in either case with pure eplerenone. With low purity eplerenone (89.3% eplerenone starting material), however, Form I was present in both cases, with significantly more Form I at higher endpoint temperatures.

Table X-7B reports the weight fraction of Form I measured in materials dried using either a fluid bed (LAB-LINE/P.R.L.

Hi-Speed Fluid Bed Dryer, Lab-Line Instruments, Inc.) or a vacuum oven (Baxter Scientific Products Vacuum Drying Oven, Model DP-32). Similar Form I content was observed for comparable materials dried in either the high fluid bed or the vacuum oven. A difference was observed, however, for comparable materials dried in the low fluid bed relative to the vacuum oven.

TABLE X-7B

Cooling Rate	End Point	Impurity Level	Drying Type	Weight Percent Form I
High	High	High	Vacuum Oven	29%
High	High	High	High Fluid Bed	25%
High	High	High	Low Fluid Bed	4.7%
Low	Low	Low	Vacuum Oven	ND
Low	Low	Low	High Fluid Bed	ND
Low	Low	Low	Low Fluid Bed	5.5%

Example 8: Crystallization of a Mixture of Form I and Form II From Methyl Ethyl Ketone To Prepare a Solvate, and (b) Desolvation of the Solvate to Prepare Form II

Form I eplerenone (10 g) was combined with 80 mL of methyl ethyl ketone. The mixture was heated to reflux (79°C) and stirred at this temperature for about 30 minutes. The resulting slurry was then cooled with a stepwise, holdpoint protocol by maintaining the slurry at 65°C, 50°C, 35°C and 25°C for about 90 minutes at each temperature. The slurry was filtered and rinsed with about 20 mL methyl ethyl ketone. The isolated solid was initially dried on the filter and then in a vacuum oven at 40-50°C. The drying was completed in the vacuum oven

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at 90-100°C. The desolvated solid was obtained with an 82% recovery. XPRD, MIR and DSC confirmed that the solid had a Form II crystalline structure.

Example 9: Digestion of Low Purity Eplerenone Starting Material With a Solvent to Prepare Form I

A. Digestion With Ethanol Solvent:

Low purity eplerenone (24.6 g; 64% by weight assay via HPLC) was combined with 126 mL of ethanol 3A. The slurry was heated to reflux and the distillate removed. An additional 126 mL of ethanol 3A was simultaneously added as 126 ml of solvent was removed via atmospheric distillation. Upon completion of the solvent turnover, the mixture was cooled to 25 °C and stirred for one hour. The solid was filtered and rinsed with ethanol 3A. The solid was air-dried to give the ethanol solvate. The solvate was further dried in a vacuum oven at 90-100°C for six hours to obtain 14.9 g of Form I eplerenone.

B. Digestion With Methyl Ethyl Ketone Solvent

In an alternative digestion process, 1 gram of low purity eplerenone (about 65% pure) was digested in 4 mL of methyl ethyl ketone for two hours. After the two hours, the mixture was allowed to cool to room temperature. Once cooled, the solid was collected by vacuum filtration and determined to be the methyl ethyl ketone solvate by XPRD analysis. The solid was dried at 100°C for 30 to 60 minutes. The dried solids were determined to be pure Form I by XPRD.

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Example 10: Digestion of High Purity Eplerenone Starting Material With a Solvent to Prepare Form II

A. Digestion With Ethanol Solvent:

High purity eplerenone (1 gram) was digested in 8 mL of ethanol for approximately two hours. The solution was then allowed to cool to room temperature and the solids were collected by vacuum filtration. Analysis of the solids by XPRD immediately after filtration indicated that the solids were a solvate (presumably the ethanol solvate). The solids were subsequently dried at 100°C at atmospheric pressure for 30 minutes. The dried solid was analyzed by XPRD and determined to be predominately Form II (no Form I detected).

B. Digestion with Methyl Ethyl Ketone Solvent:

High purity eplerenone (1 gram) was digested in 4 mL of methyl ethyl ketone for two hours. After the two hours, the solution was allowed to cool to room temperature and the solids collected by vacuum filtration. The solid was immediately analyzed by XPRD and determined to be a solvate of eplerenone (presumably the methyl ethyl ketone solvate). The solvate was subsequently dried at 100°C at ambient pressure for 30 to 60 minutes. The dried solids were analyzed by XPRD and determined to be primarily Form II with no diffraction peaks for Form I present.

Example 11: Crystallization of Form II Directly From Solution

Procedure A: Eplerenone (2.5 g) was dissolved in ethyl acetate by heating to 75°C. Once the eplerenone dissolved,

the solution was held at 75°C for 30 minutes to ensure complete dissolution. The solution was then cooled at 1 °C/min to 13°C. Once at 13°C, the slurry was allowed to stir for two hours at 750 rpm with an overhead stirrer. The crystals were collected by vacuum filtration and dried in a vacuum oven at 40°C for one hour. The XPRD pattern and DSC thermogram of the solid were characteristic of Form II eplerenone. Thermal gravimetric analysis (TGA) of the solid indicated no weight loss from the solid up to 200°C.

Procedure B: In an alternative procedure, 2 g of eplerenone was dissolved in 350 mL of 15/85% acetonitrile/water by heating on a hot plate with magnetic stirring. Once the eplerenone was dissolved, the solution was allowed to cool to room temperature overnight with magnetic stirring. The resulting solid was collected by vacuum filtration. The crystals were birefringent and had a triangular, plate-like crystal habit. The solid had an XPRD and DSC characteristic of Form II eplerenone. TGA indicated no weight loss up to 200°C.

Procedure C: In an alternative procedure, 640 mg of eplerenone was placed in a 50 mL flask with 20 mL of ethyl benzene. The resulting slurry was heated to 116°C and became a clear solution. The clear solution was cooled to 25°C over 30 minutes. Nucleation began at 84°C during the cooling period. The resulting solids were filtered from the solution and air-dried to give 530 mg of solids (83% recovery). Hot-stage microscopy and XPRD confirmed that the solids were Form II crystals.

Procedure D: In an alternative procedure, 1.55 g of eplerenone was added to 2.0 mL of nitrobenzene and heated to 200°C. The resulting slurry was stirred overnight at 200°C. The solution was allowed to cool to room temperature (natural air convection) the following day and the solid was isolated. The solid was determined to be Form II eplerenone by XPRD and polarized light microscopy.

Procedure E: In an alternative procedure, 5.0 g of eplerenone (purity greater than 99%) was added to 82 g of methanol (104 mL). Under stirring action (210 rpm), the solution was heated to 60°C and held at that temperature for 20 minutes to ensure complete dissolution. The solution was then cooled to -5°C at a rate of 0.16°C/minute under stirring. The crystals were collected by filtration and dried in a vacuum oven at 40°C for 20 hours. The dried solids were determined to be pure Form II eplerenone by DSC and XPRD analysis.

Procedure F: In an alternative procedure, 6.0 g of eplerenone (ethanol solvate containing 9% ethanol and having a corrected purity of 95.2%) was added to 82 g of methanol (104 mL). Under stirring action (210 rpm), the solution was heated to 60°C and held at that temperature for 20 minutes to ensure complete dissolution. The solution was then cooled to 50°C at a rate of 0.14°C/minute and then held at that temperature for about 2.5 hours. The solution was then cooled to -5°C at a rate of 0.13°C/minute under stirring. The crystals were collected by filtration and dried in a vacuum

oven at 40°C for 16 hours. The dried solids were determined to be pure Form II eplerenone by DSC and XPRD analysis.

Example 12: Crystallization of Form I Directly From Solution

150.5 mg of the diepoxide and 2.85 g of eplerenone were added to 1.5 mL of nitrobenzene. The mixture was magnetically stirred at 200°C for several hours. The slurry was then allowed to cool to room temperature by natural air convection. The sample was dried and analyzed by polarized light microscopy and XPRD. The XPRD indicated that the sample was a mixture of Form I and Form II. The crystals were translucent by microscopy, indicating that desolvation (and conversion to either Form I or Form II) did not occur.

Example 13: Preparation of Amorphous Eplerenone By Comminution

Approximately one-half of a steel Wig-L-Bug container was filled with about 60 g of eplerenone (greater than 99.9% purity). A steel ball and cap were placed on the sample container and agitated for 30 seconds by the Wig-L-Bug apparatus. The eplerenone was scraped off the surface of the Wig-L-Bug container and the container agitated for an additional 30 seconds. The resulting solid was analyzed by XPRD and DSC and determined to be a mixture of amorphous eplerenone and Form II crystalline eplerenone.

Example 14: Preparation of Amorphous By Lyophilization

Approximately 100 mg of crude eplerenone was weighed into a beaker containing 400 mL of water. The solution was heated slightly for five minutes, and then sonicated and heated with stirring for an additional five minutes.

Approximately 350 mL of the eplerenone solution was filtered into a 1000 mL round bottom flask containing 50 mL of HPLC water. The solution was flashed frozen in a dry ice/acetone bath over a time period of one to two minutes. The flask was attached to a Labconco Freezone 4.5 freeze dryer and dried overnight. The solids in the flask were transferred to a small brown bottle. A small aliquot was observed under polarized light microscopy at 10X, 1.25X optivar in cargille oil (1.404) and observed to be at least 95% amorphous eplerenone. Figures 24 and 25 show the XPRD pattern and DSC thermogram obtained for the amorphous eplerenone. The peak observed at 39 degrees two theta in Figure 24 is attributable to the aluminum sample container.

Example 15: Measurement of Form II Solubility

The aqueous solubility of Form II eplerenone was measured at pH 7 (100 mM phosphate buffer) at 5, 25, and 40°C. Approximately 30 mg of Form II eplerenone was mixed with approximately 10 mL of buffer to form a slurry of eplerenone at both 5 and 25 °C. Approximately 40 mg of Form II eplerenone was mixed with approximately 10 mL of buffer to form a slurry of eplerenone at 40 °C. Samples were prepared in duplicate for each condition. The slurries were allowed to equilibrate in appropriate temperature water shaker baths and the solutions were analyzed for eplerenone content by ultraviolet visible analysis (245 nm) at the 1, 5, 12, 19, 27, and 36 day time intervals. Data from each condition was appropriately averaged to determine the solubility of eplerenone at each condition and is reported in Table X-15. The residual solids from each time point were analyzed by DSC and TGA at the end of the 36 day equilibration and were

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determined to be Form II.

TABLE X-15

Temperature (°C)	Form II Solubility
5	0.24 mg/mL
25	0.29 mg/mL
40	0.39 mg/mL

Example 16: Measurement of Intrinsic Dissolution Rates

Intrinsic dissolution rates were measured for the following four eplerenone polymorph samples: (i) Form II eplerenone prepared by direct crystallization from acetonitrile using water as an anti-solvent in the same manner as Example 12, Procedure B, (ii) Form I eplerenone prepared by digestion in ethanol in the same manner as Example 10, Procedure A, (iii) a mixture of Form I (5 weight percent) and Form II (95 weight percent), and (iv) Form II eplerenone that was micronized to provide the following particle size distribution: 10% of the particles under 9 microns, 50% of the particles under 22 microns, and 90% of the particles under 41 microns.

Eplerenone (150 mg) was weighed and placed into a VanKel intrinsic dissolution cavity. The powder was compressed at 1200 psi using a Carver press. The sample was then mounted onto the intrinsic dissolution apparatus. The dissolution media used was 1% SDS in HPLC grade water. All experiments were conducted at 37°C for two hours. Before the start of the experiment, 500 mL of the dissolution medium was equilibrated at 37°C for 30 minutes in the dissolution bathing chamber. An initial sample was taken from each dissolution vessel which served as time zero for the experiment. The eplerenone

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tablets were then lowered into the dissolution medium. Samples were drawn at determined intervals for the determination of rate of dissolution. Care was taken to avoid air bubbles forming at the surface of the tablet. The samples were analyzed by UV absorbance detection at 243 nm. Intrinsic dissolution rates were calculated from the slope of the linear portion of the concentration versus time profiles corrected for volume and normalized for the surface area of the dissolution tablet (0.5 cm^2).

Fig. 26 reports the intrinsic dissolution rates measured for the four samples. These studies appear to indicate that Form I eplerenone has faster intrinsic dissolution rate than Form II eplerenone. X-Ray powder diffraction measurements comparing compressed against uncompressed eplerenone confirmed that the polymorphs did not interconvert upon compression or during the course of the dissolution studies.

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Example 17: Eplerenone Polymorph Composition

Tablets containing 25 mg, 50 mg, 100 mg and 200 mg doses of Form II eplerenone are prepared and have the following composition:

Ingredient	Weight % of Tablet
Form II Eplerenone	29.41
Form I Eplerenone	Not Detected
Lactose Monohydrate (#310, NF)	42.00
Microcrystalline Cellulose (NF, Avicel PH101)	18.09
Croscarmellose Sodium (NF, Ac-Di-Sol)	5.00
Hydroxypropyl Methylcellulose (#2910, USP, Pharmacoat 603)	3.00
Sodium Lauryl Sulfate (NF)	1.00
Talc (USP)	1.00
Magnesium Stearate (NF)	0.5
Total	100.00

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Example 18: Eplerenone Polymorph Composition

Capsules (hard gelatin capsule, #0) are prepared containing a 100 mg dose of eplerenone and have the following composition:

Ingredient	Amount (mg)
Form II Eplerenone	90.0
Form I Eplerenone	10.0
Lactose, Hydrous, NF	231.4
Microcrystalline Cellulose, NF	45.4
Talc, USP	10.0
Croscarmellose Sodium, NF	8.0
Sodium Lauryl Sulfate, NF	2.0
Colloidal Silicon Dioxide, NF	2.0
Magnesium Stearate, NF	1.2
Total Capsule Fill Weight	400.0

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Example 19: Eplerenone Polymorph Composition

Capsules (hard gelatin capsule, size #0) are prepared containing a 200 mg dose of eplerenone and have the following composition:

Ingredient	Amount (mg)
Form II Eplerenone	190.0
Form I Eplerenone	10.0
Lactose, Hydrous, NF	147.8
Microcrystalline Cellulose, NF	29.0
Talc, USP	10.0
Croscarmellose Sodium, NF	8.0
Sodium Lauryl Sulfate, NF	2.0
Colloidal Silicon Dioxide, NF	2.0
Magnesium Stearate, NF	1.2
Total Capsule Fill Weight	400.0

Example 20: Preparation of Milled Eplerenone

Dried methyl ethyl ketone solvate is first delumped by passing the solvate through a 20 mesh screen on a Fitzmill. The delumped solid is then pin milled using an Alpine Hosakawa stud disk pin mill operating under liquid nitrogen cooling at a feed rate of approximately 250 kilograms/hour. Pin milling produces milled eplerenone with a D_{90} size of approximately 65-100 microns.

Example 21: Effect of Eplerenone Particle Size (Dog Study)

The effect of the particle size of Form II eplerenone on eplerenone plasma concentrations and relative bioavailability was studied in a dog model. Four heathy female beagle dogs

weighing between 8 to 12 kg were intragastrically administered one immediate release capsule containing the formulation described in Table X-21A below followed by about 10 mL of water.

Table X-21A

INGREDIENT	WEIGHT % OF TABLET	Amount (mg)
Eplerenone	50.00	200.00
Lactose, Fast-Flo, Hydrous	36.95	147.80
Microcrystalline Cellulose (Avicel PH102)	7.25	29.00
Sodium Lauryl Sulfate	0.50	2.00
Croscarmellose Sodium	2.00	8.00
Talc	2.50	10.00
Colloidal Silicon Dioxide	0.50	2.00
Magnesium Stearate	0.30	1.20
Total	100.00	400.00
Capsules, Size #0, White Opaque	1 Capsule	

The dogs were fasted for 15 to 20 hours prior to administration of the capsule and were not fed again until at least 4 hours after dose administration. Blood samples (approximately 3 mL) were collected by venipuncture in chilled tubes containing heparin at 0, 0.5, 1, 2, 3, 4, 6, 8 and 24 hours after dose administration. The blood samples were immediately placed on ice. Separation of plasma from the blood samples was complete after about 15 minutes of centrifugation. The resulting plasma samples were frozen at about -20°C and stored until analyzed. Analysis was performed using an LC/MS/MS procedure.

This experiment was conducted using the same four dogs for three formulations that were identical except for the

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particle size of the eplerenone starting material. The three formulations tested used eplerenone starting materials having a D_{90} particle size (that is, at least 90% of the particles were) less than about 212 microns, less than about 86 microns, and less than about 36 microns, respectively. A minimum of a five day wash-out period was allowed between administration of each of the formulations. Reducing the D_{90} particle size of the eplerenone starting material from about 212 microns to above 86 microns increased relative bioavailability by almost 100%. The mean results are reported in Tables X-21B and X-21C below.

Table X-21B

Time (hours)	Blood Serum Eplerenone Concentration (μg/mL)		
	D_{90} = 212 microns	D_{90} = 86 microns	D_{90} = 36 microns
0	0	0	0
0.5	1.83	3.65	1.99
1.0	2.40	6.18	5.86
2.0	3.77	6.89	6.77
3.0	2.85	5.70	6.60
4.0	2.61	4.39	5.56
6.0	1.63	3.11	3.31
8.0	1.10	1.90	2.09
24.0	0.0252	0.032	0.0706

Table X-21C

Pharmacokinetic Parameter	Pharmacokinetic Parameter Value		
	D_{90} = 212 microns	D_{90} = 86 microns	D_{90} = 36 microns
C_{max} (μg/mL)	3.98	7.02	7.39
T_{max} (hours)	1.50	1.75	2.25
AUC ((μg/mL)hr)	26.6	49.2	53.1
Relative Bioavailability (%)	53.25	100	107.9

Example 22: Effect of Eplerenone Particle Size (Human Study)

The effect of the particle size of a Form II eplerenone starting material used in a pharmaceutical composition on eplerenone plasma concentrations and relative bioavailability was studied in a human model. Subjects received single doses of Form II eplerenone medication on Days 1, 8, 15, 22 and 29 according to a randomization schedule. All medication was administered with 180 mL of water at 0800 hours. Blood samples for eplerenone pharmacokinetic analyses were collected at -0.5 (predose), 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 16, 24, 36 and 48 hours postdose. Urine samples were collected and pooled between the hours of 0-24 and 24-48 hour postdose.

Plasma and urinary concentrations of eplerenone were determined using a validated high performance liquid chromatography method with MS/MS Detection. Pharmacokinetic data obtained is reported in Table X-23A. The particle size distributions of the eplerenone used in the preparation of the medications was determined in the dry powder state using laser light scattering.

Table X-23A

Pharmacokinetic Parameter	Pharmacokinetic Parameter Value		
	100 mg Capsule A (D_{50}=40 microns)	100 mg Tablet (D_{50}=82 microns)	100 mg Capsule B (D_{50}=96 microns)
C_{max} (ng/mL)	1747	1704	1669
T_{max} (hours)	1.8	1.8	1.3
AUC ((ng/mL)hr)	11349	11945	11981

The specific composition of each medication is reported in Table X-23B below.

Table X-23B

INGREDIENT	WEIGHT % OF TABLET/CAPSULE		
	Capsule A	Tablet A	Capsule B
Form II Eplerenone	25	30	25
Lactose Monohydrate	–	42	57.86 (fast flo lactose)
Lactose, Hydrous NF	57.8	–	–
Microcrystalline Cellulose (Avicel PH101)	11.4	17.5 (7.5% intragranular plus 10% extragranular)	11.34 (Avicel PH 102)
Croscarmellose Sodium (Ac-Di-Sol)	2	5	2
Hydroxypropyl Methylcellulose (Pharmacoat 603)	–	3	–
Sodium Lauryl Sulfate	0.5	1	0.5
Talc	2.5	1	2.5
Magnesium Stearate	0.3	0.5	0.3
Colloidal Silicon Dioxide	0.5	–	0.5
Total	100	100	100

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1446,1405

All mentioned references are expressly incorporated by reference herein as if fully set forth at length. In addition, U.S. Provisional Application Serial No. 60/111,646 filed December 9, 1998, and the corresponding nonprovisional application, U.S. Application Serial No. 09/_____ filed December 8, 1999, are expressly incorporated by reference herein as if fully set forth at length.

When introducing elements of the present invention or the preferred embodiment(s) thereof, the articles "a", "an", "the" and "said" are intended to mean that there are one or more of the elements. The terms "comprising", "including" and "having" are intended to be inclusive and mean that there may be additional elements other than the listed elements.

Although this invention has been described with respect to specific embodiments, the details of these embodiments are not to be construed as limitations.

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WHAT IS CLAIMED IS

1. A crystalline form of eplerenone comprising a solvent, wherein said form does not comprise methylene chloride, ethyl ether or isopropanol.

2. A form of Claim 1 wherein said form does not comprise methylene chloride, ethyl ether, isopropanol, methyl ethyl ketone or ethanol.

3. A form of Claim 1 wherein said form comprises a solvent selected from the group consisting of methylethyl ketone, 2-pentanone, acetic acid, acetone, butyl acetate, chloroform, ethanol, isobutanol, isobutyl acetate, methyl acetate, ethyl propionate, n-butanol, n-octanol, n-propanol, propyl acetate, propylene glycol, t-butanol, tetrahydrofuran, toluene and t-butyl acetate.

4. A form of Claim 1 wherein said form comprises a solvent selected from the group consisting of 2-pentanone, acetic acid, acetone, butyl acetate, chloroform, ethanol, isobutanol, isobutyl acetate, methyl acetate, ethyl propionate, n-butanol, n-octanol, n-propanol, propyl acetate, propylene glycol, t-butanol, tetrahydrofuran, toluene and t-butyl acetate.

5. A form of Claim 1 wherein said form comprises ethanol.

6. A form of Claim 1 wherein said form comprises methylethyl ketone.

7. A form of Claim 6 having an X-ray powder diffraction pattern with a peak at 13.6 ± 0.2 degrees two theta.

8. A form of Claim 6 having an X-ray powder diffraction pattern with peaks at 7.6 ± 0.2 and 13.6 ± 0.2 degrees two theta.

9. A form of Claim 6 having an X-ray powder diffraction pattern substantially as shown in Figure 1-C.

10. A form of Claim 6 having an orthorhombic crystal system.

11. A form of Claim 6 having a $P2_12_12_1$ space group.

12. A form of Claim 6 having unit cell a, b and c values of about 23.53 Å, about 8.16 Å, and about 13.08 Å, respectively.

13. A form of Claim 6 having unit cell α, β and γ values that are each about 90°.

14. Particles of a form of Claim 1 wherein at least 90% of said particles have a particle size less than about 400 microns.

15. Particles of a form of Claim 1 wherein at least 90% of said particles have a particle size less than about 200 microns.

16. The particles of Claim 14 wherein at least 90% of said particles have a particle size between about 25 microns to about 400 microns.

17. The particles of Claim 14 wherein at least 90% of said particles have a particle size between about 50 microns to about 150 microns.

18. The particles of Claim 14 wherein at least 90% of said particles have a particle size between about 40 microns to about 100 microns.

19. The particles of Claim 14 wherein at least 90% of said particles have a particle size between about 30 microns to about 50 microns.

20. Eplerenone comprising a crystalline form of any one of Claims 1 to 6, wherein said eplerenone has a phase purity of at least about 50%.

21. The eplerenone of Claim 20 wherein said eplerenone has a phase purity of at least about 90%.

22. The eplerenone of Claim 20 wherein said eplerenone is a substantially phase pure form.

23. A pharmaceutical composition comprising a therapeutically-effective amount of the eplerenone of any one of Claims 1 to 22 in association with at least one pharmaceutically-acceptable carrier, adjuvant or diluent.

24. A composition of Claim 23 wherein the only eplerenone crystalline form present in the composition is a form of any one of Claims 1 to 6.

25. A method of treating or preventing an aldosterone-mediated condition or disorder in a subject, said method comprising administering to a subject having or susceptible to said condition or disorder a therapeutically-effective amount of the eplerenone of any one of Claims 1 to 22.

26. The method of Claim 25 for use in treatment of hyperaldosteronism.

27. The method of Claim 25 for use in treatment of hypertension.

28. The method of Claim 25 for use in treatment of heart failure.

29. The method of Claim 25 for use in treatment of cirrhosis of the liver.

30. The method of Claim 25 for use in treatment of a condition selected from the group consisting of excess collagen/fibrosis, benign prostate hypertrophy and depression.

31. A method of preparing eplerenone comprising a crystalline form of any one of Claims 1 to 6, the method comprising crystallizing eplerenone from a pharmaceutically acceptable solvent or mixture of solvents to yield said form.

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32. The method of Claim 31 wherein the solvent or mixture of solvents comprises a solvent selected from the group consisting of methylethyl ketone, 2-pentanone, acetic acid, acetone, butyl acetate, chloroform, ethanol, isobutanol, isobutyl acetate, methyl acetate, ethyl propionate, n-butanol, n-octanol, n-propanol, propyl acetate, propylene glycol, t-butanol, tetrahydrofuran, toluene and t-butyl acetate.

33. The method of Claim 31 wherein the solvent or mixture of solvents comprises a solvent selected from the group consisting of 2-pentanone, acetic acid, acetone, butyl acetate, chloroform, isobutanol, isobutyl acetate, methyl acetate, ethyl propionate, n-butanol, n-octanol, n-propanol, propyl acetate, propylene glycol, t-butanol, tetrahydrofuran, toluene and t-butyl acetate.

34. The method of Claim 31 wherein the solvent or mixture of solvents comprises a solvent selected from the group consisting of methylethyl ketone and ethanol.

35. The method of Claim 31 wherein the eplerenone used in the crystallization has a chemical purity of at least about 99%.

36. The method of Claim 31 wherein the crystallization is carried out in the presence of a Form II growth promoter or Form I growth inhibitor.

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37. The method of Claim 31 wherein the crystallization is carried out in the presence of a Form I growth promoter or Form II growth inhibitor.

38. The method of Claim 31 wherein the crystallization is carried out in the presence of a Form I growth promoter having a single crystal structure substantially identical to the single crystal structure of the form of Claim 1.

39. The method of Claim 31 wherein the crystallization is carried out in the presence of a Form I growth promoter having an X-ray powder diffraction pattern substantially identical to the X-ray powder diffraction pattern of the form of Claim 1.

40. The method of Claim 31 wherein the crystallization is carried out in the presence of a compound selected from the group consisting of 7-methyl hydrogen 4α,5α:9α,11α-diepoxy-17-hydroxy-3-oxo-17α-pregnane-7α,21-dicarboxylate, γ-lactone; 7-methyl hydrogen 11α,12α-epoxy-17-hydroxy-3-oxo-17α-pregn-4-ene-7α,21-dicarboxylate, γ-lactone; and 7-methyl hydrogen 17-hydroxy-3-oxo-17α-pregna-4,9(11)-diene-7α,21-dicarboxylate, γ-lactone.

41. The method of Claim 31 wherein the crystallization is carried out in the presence of 7-methyl hydrogen 4α,5α:9α,11α-diepoxy-17-hydroxy-3-oxo-17α-pregnane-7α,21-dicarboxylate, γ-lactone.

42. The method of Claim 31 wherein the solvent or mixture of solvents is seeded with a nonsolvated crystalline form of eplerenone prior to crystallizing the eplerenone.

1412

43. The method of Claim 31 wherein at least about 50 weight percent of the crystallized eplerenone is said form.

44. The method of Claim 31 wherein at least about 90 weight percent of the crystallized eplerenone is said form.

45. The method of Claim 31 wherein at least about 99 weight percent of the crystallized eplerenone is said form.

46. A method of preparing eplerenone comprising a form of any one of Claims 1 to 6, the method comprising digesting eplerenone in a pharmaceutically acceptable solvent or mixture of solvents to yield said form.

47. The method of Claim 46 wherein the solvent comprises ethanol or methylethyl ketone.

48. The method of Claim 46 wherein at least about 50 weight percent of the eplerenone prepared is said form.

49. The method of Claim 46 wherein at least about 90 weight percent of the eplerenone prepared is said form.

50. The method of Claim 46 wherein at least about 99 weight percent of the eplerenone prepared is said form.

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1413

ABSTRACT OF THE DISCLOSURE

Novel solvated crystalline forms of eplerenone exclusive of methylene chloride, ethyl ether and isopropanol are described. These crystalline forms have unique chemical and physical properties relative to other solid state forms of eplerenone and are characterized by their x-ray powder diffraction pattern, infrared spectrum, nuclear magnetic resonance spectrum, differential scanning calorimetric thermogram, and other physical characterizations.

6680271 086 980 109

Figure 2 66 021-2 869T09

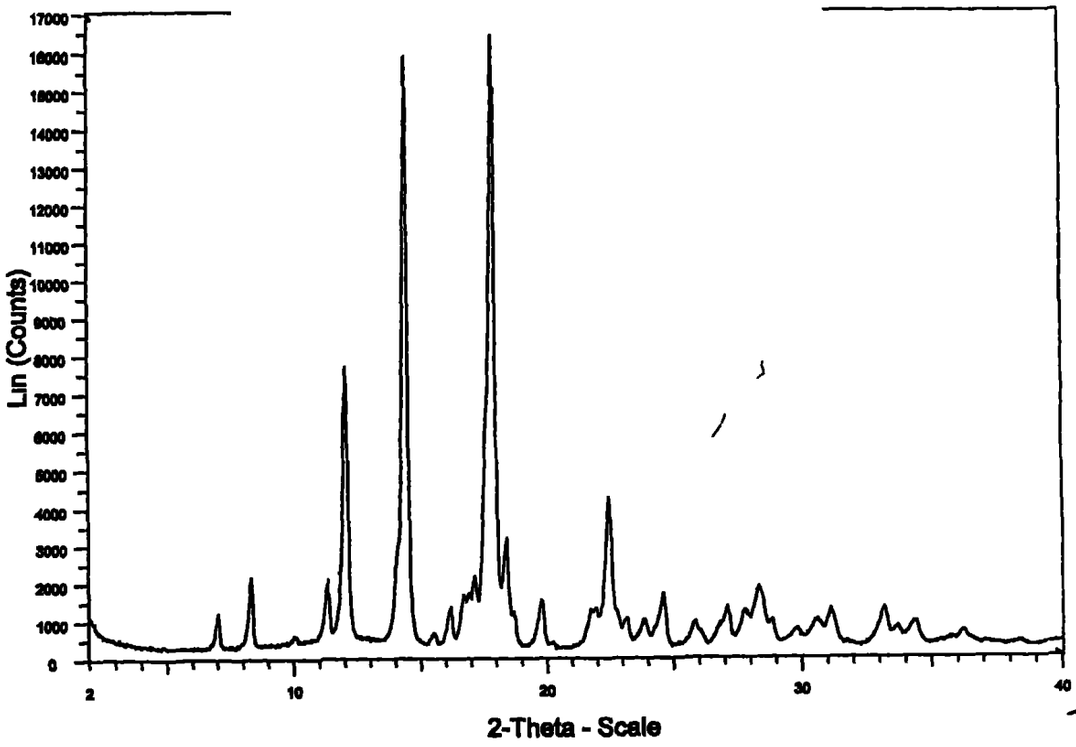
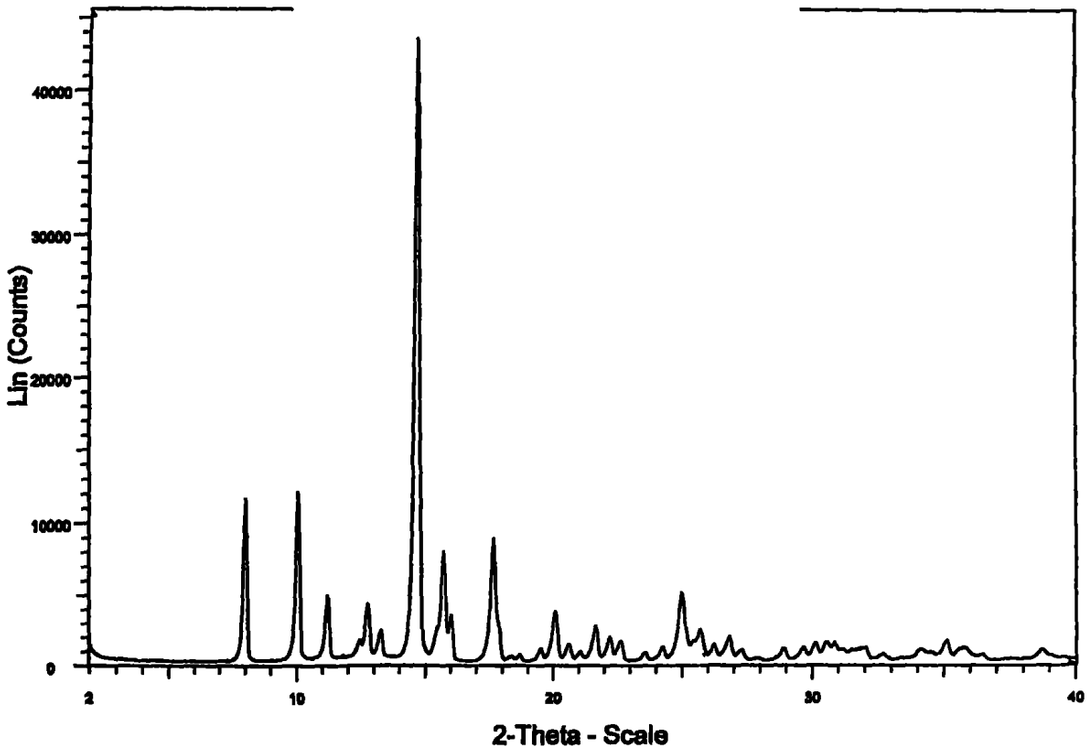


Figure 1a

668021-[illegible] 6 TO



1486

Figure 6C

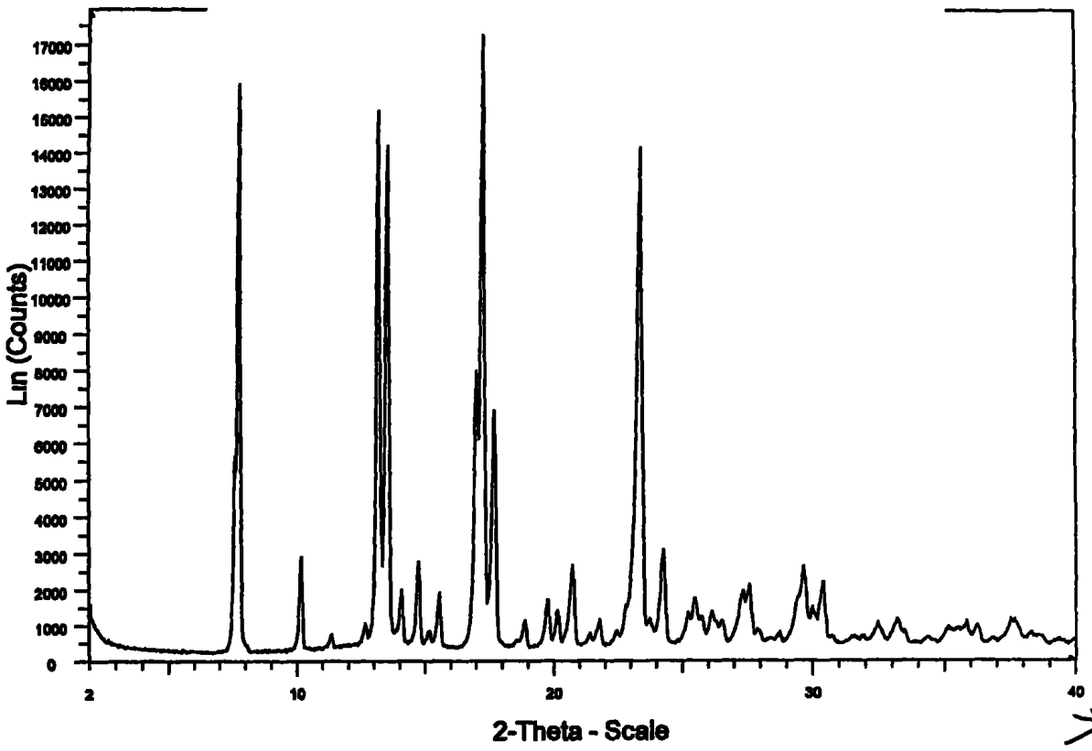

Lin (Counts)
17000
16000
15000
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
2
10
20
30
40
2-Theta - Scale

Figure 1 D

n-propyl alcohol solvate

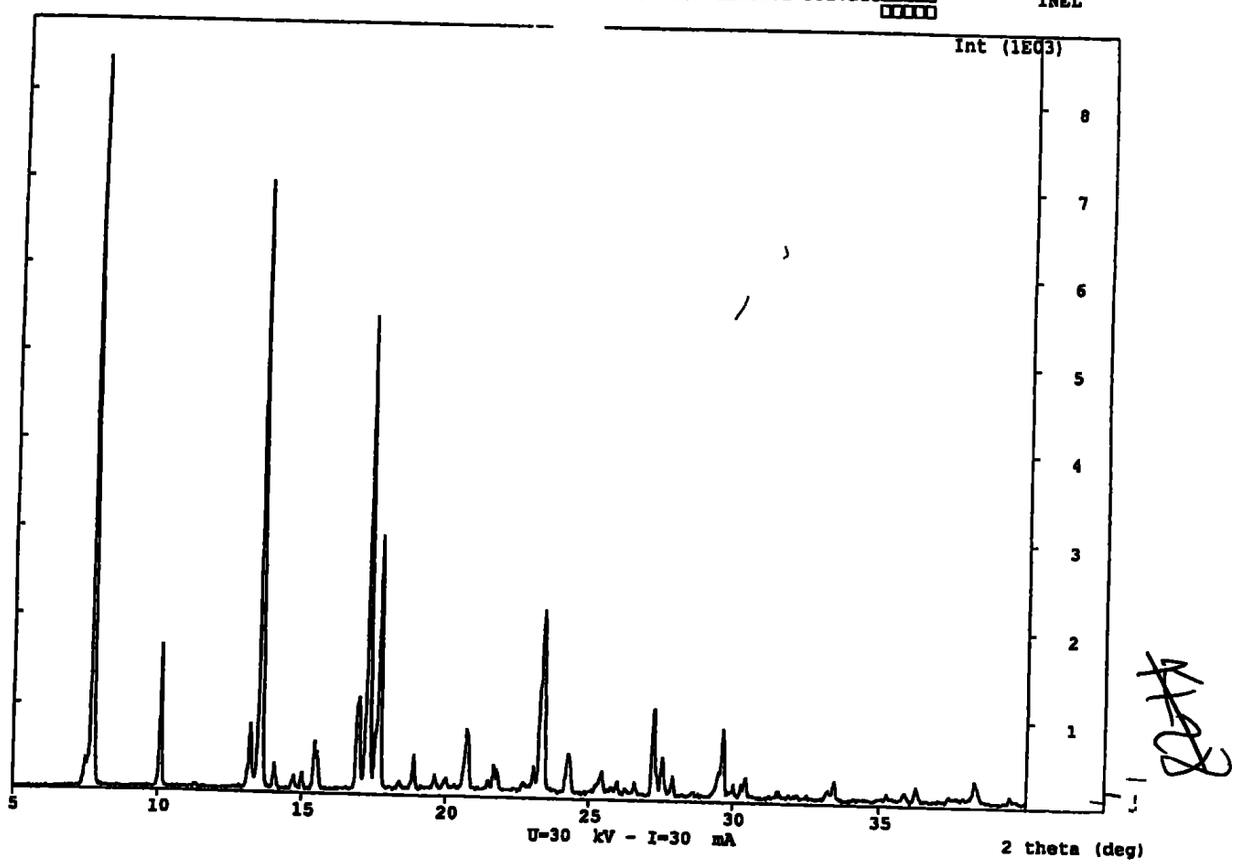
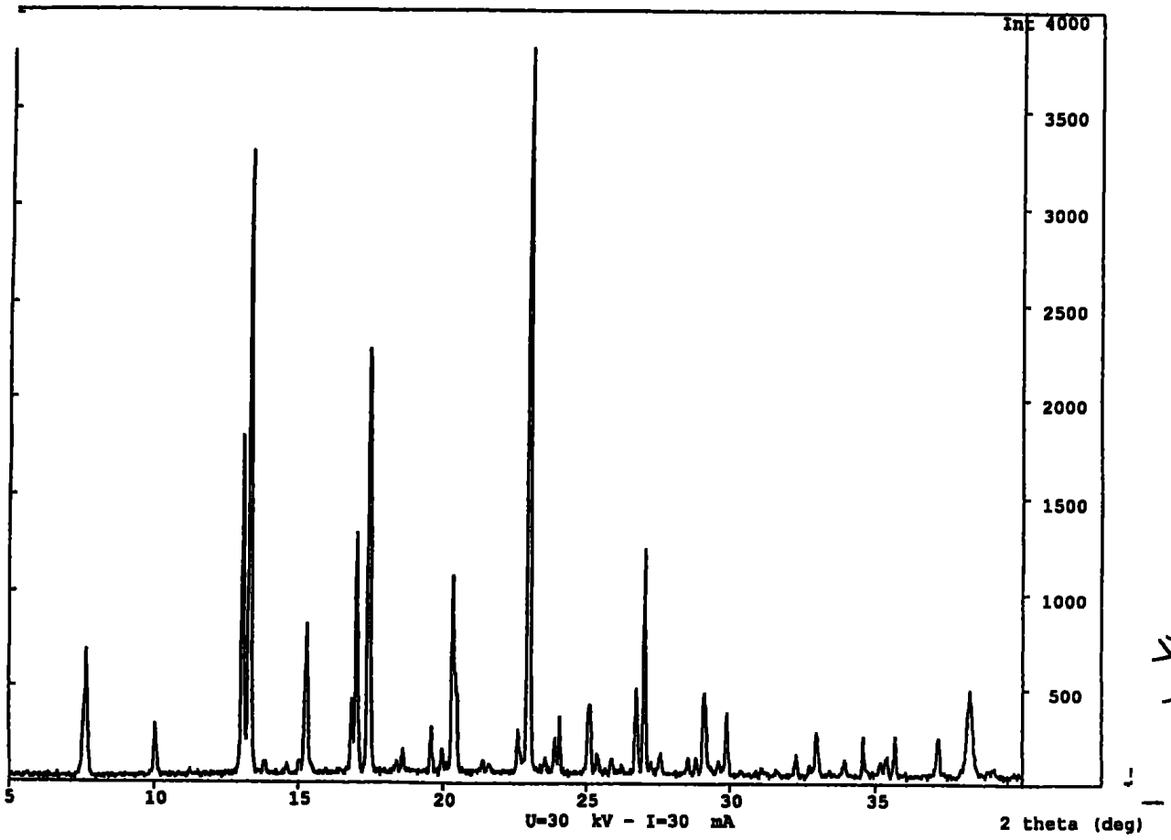


Figure 1-E

[redacted] THF solvate

INEL



1418

1409

Figure 1-B

Ethyl Propionate solvate

INEL

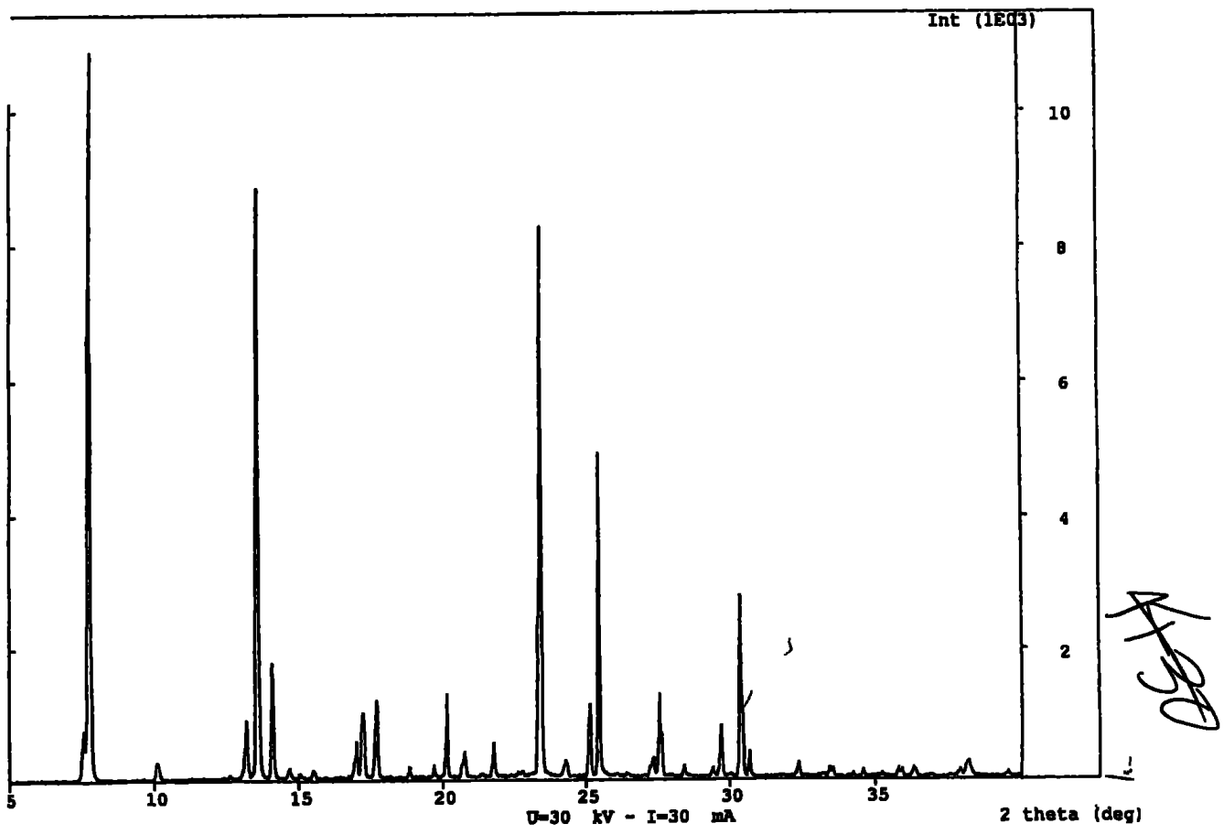
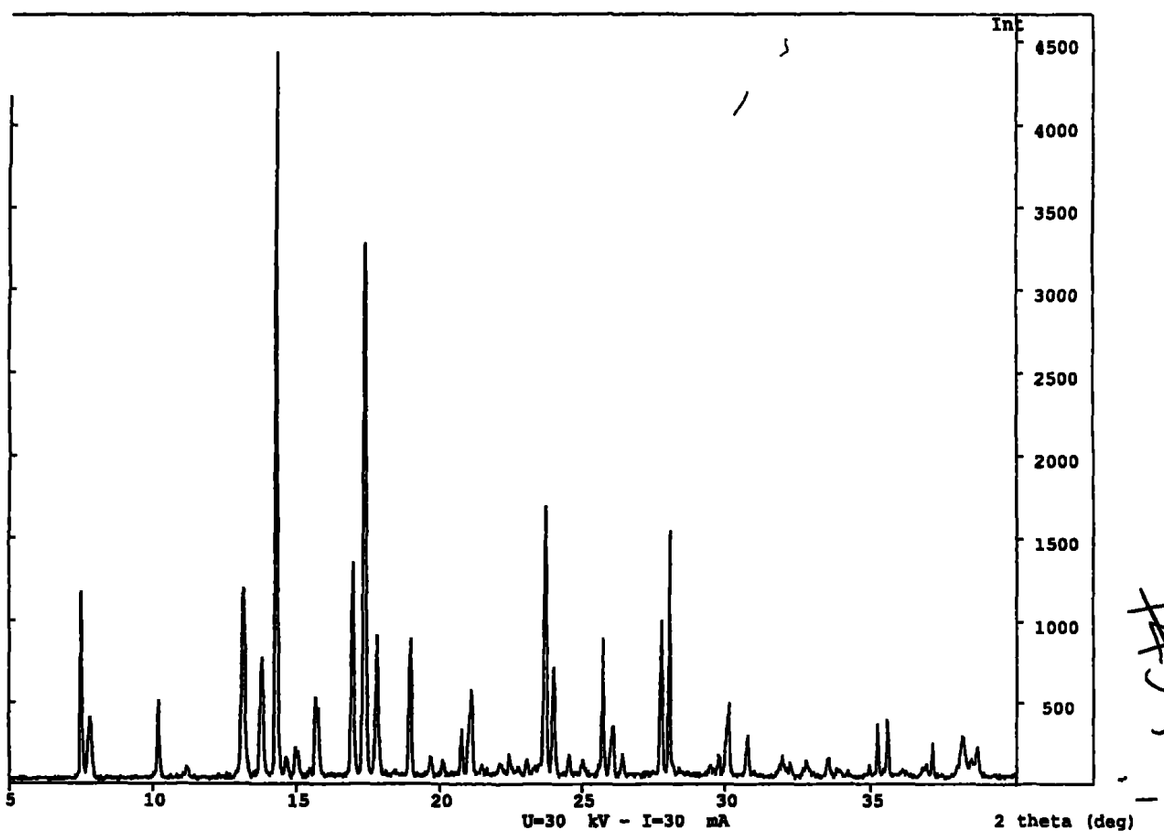


Figure 1-G

1420

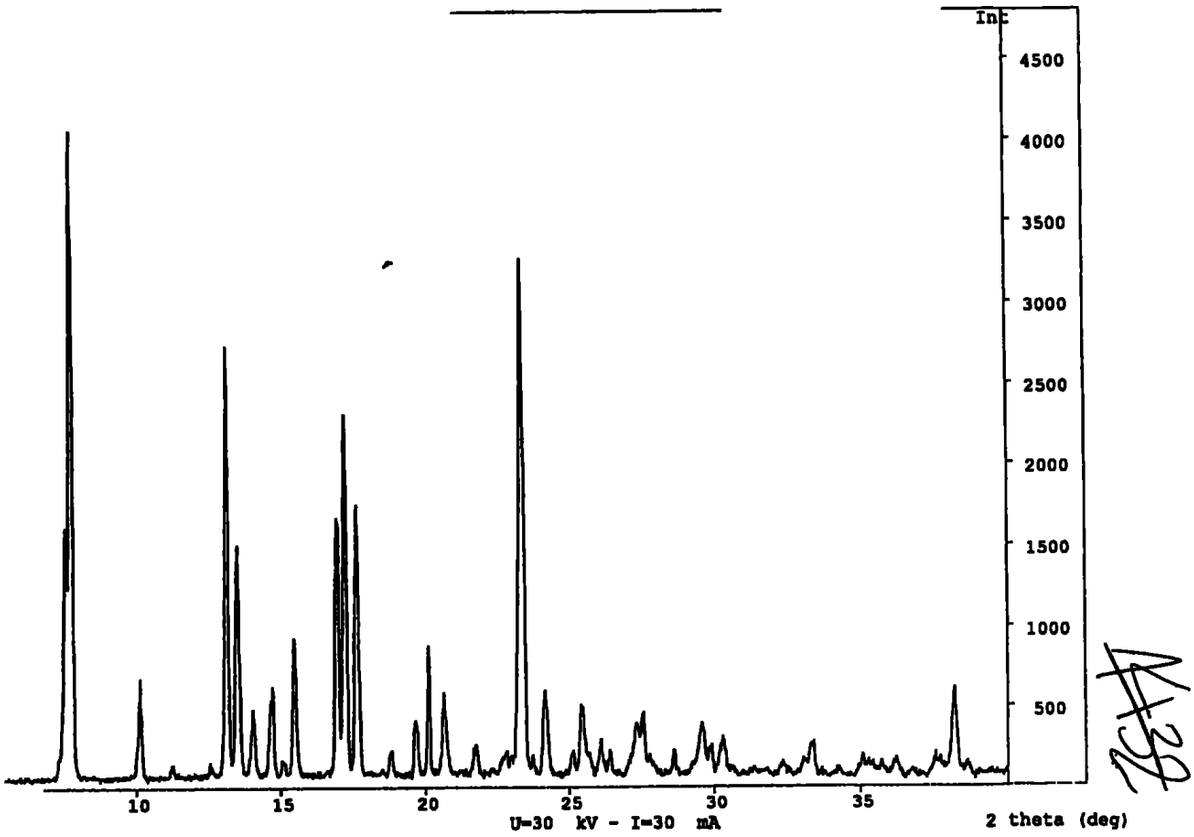


1431

Figure 1-H

668021 [illegible]

INEL 1421



A-30

Figure 1-I

INEL

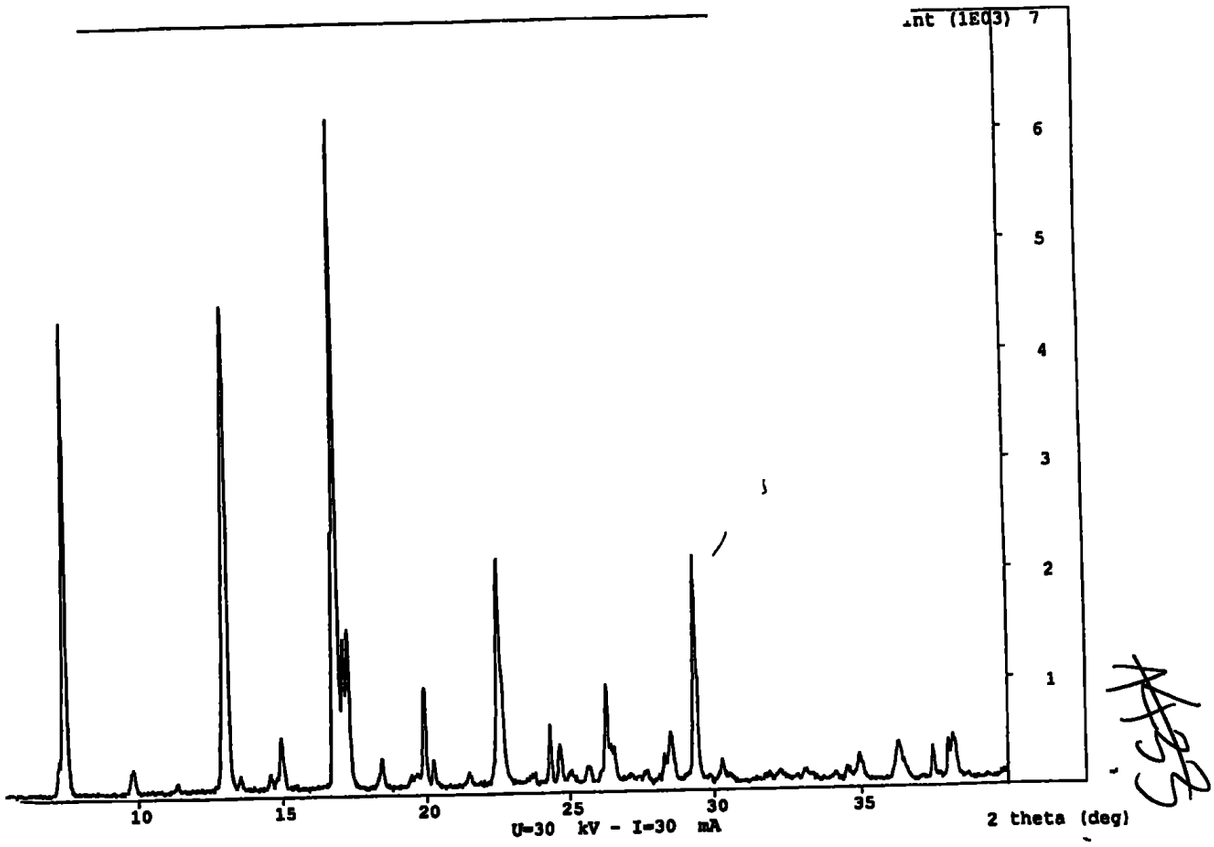
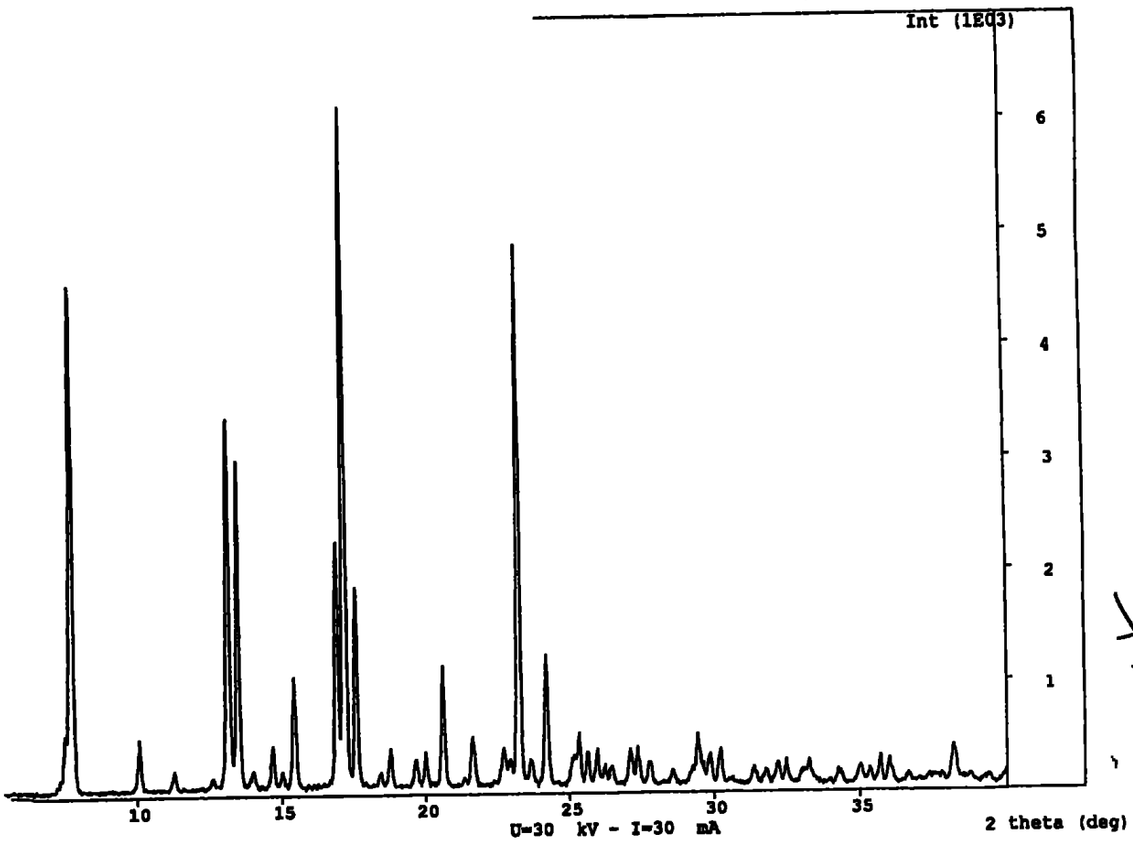


Figure 1-J



1434

Figure 1-K

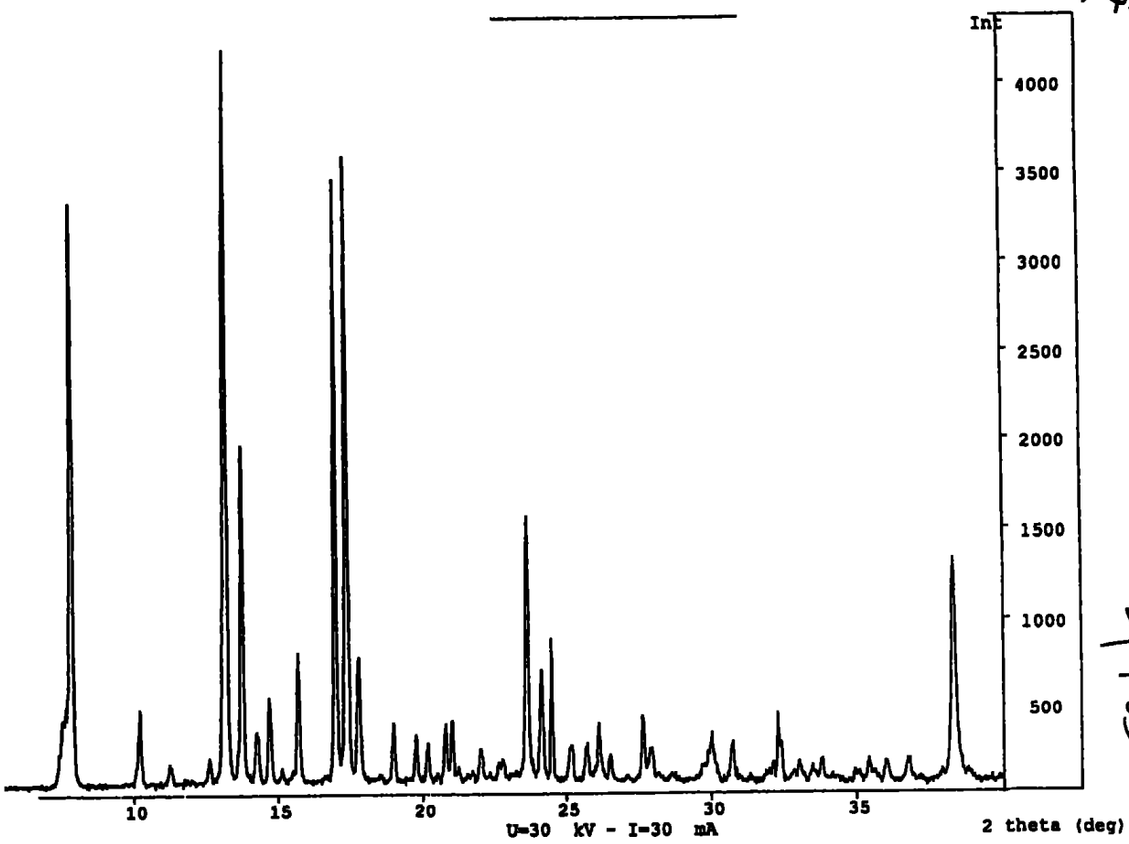
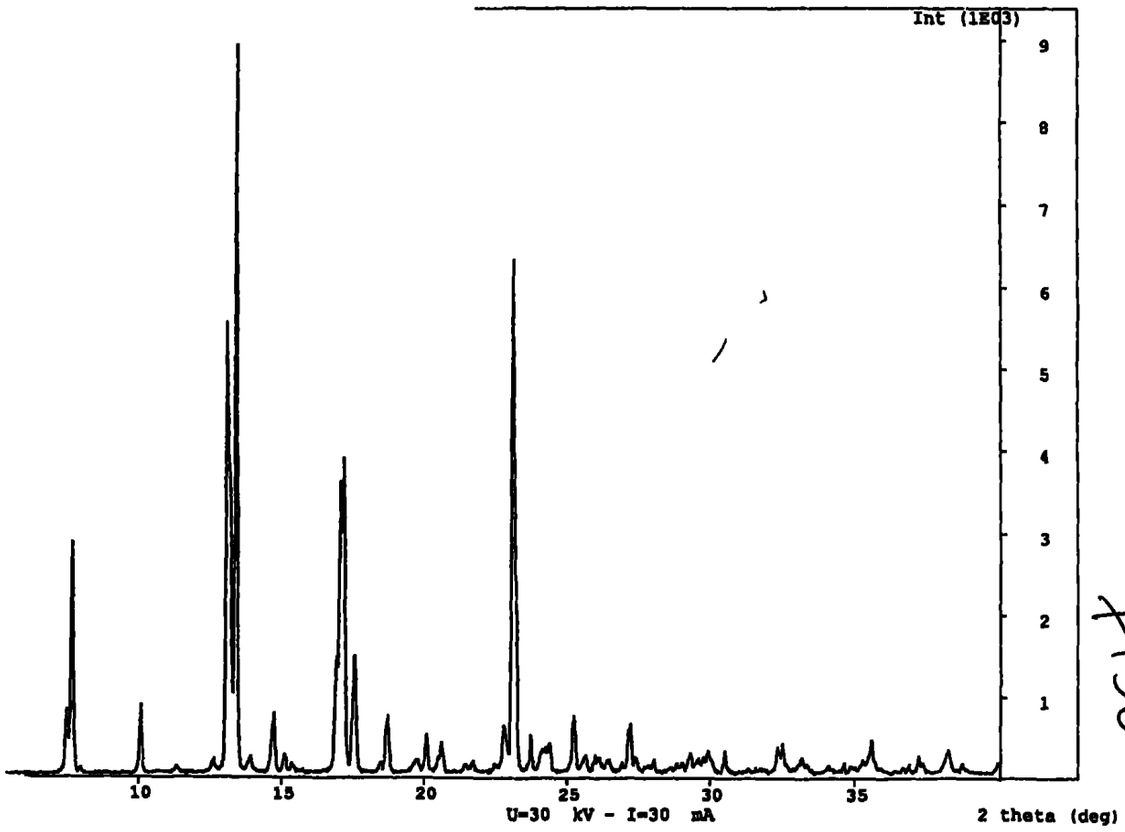

INEL
Int
4000
3500
3000
2500
2000
1500
1000
500
10
15
20
25
30
35
U=30 kV - I=30 mA
2 theta (deg)

Figure 1-L

[redacted]ate

INEL

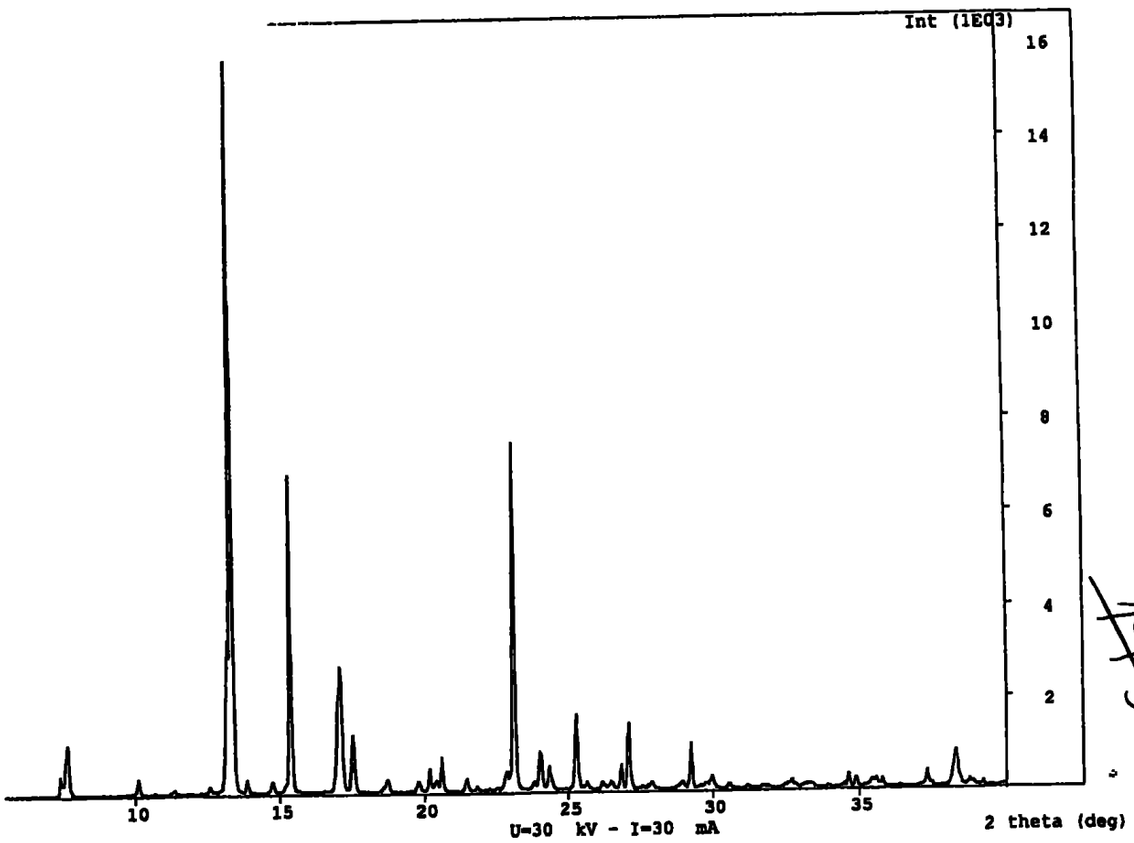
1425



1436

Figure 1-M

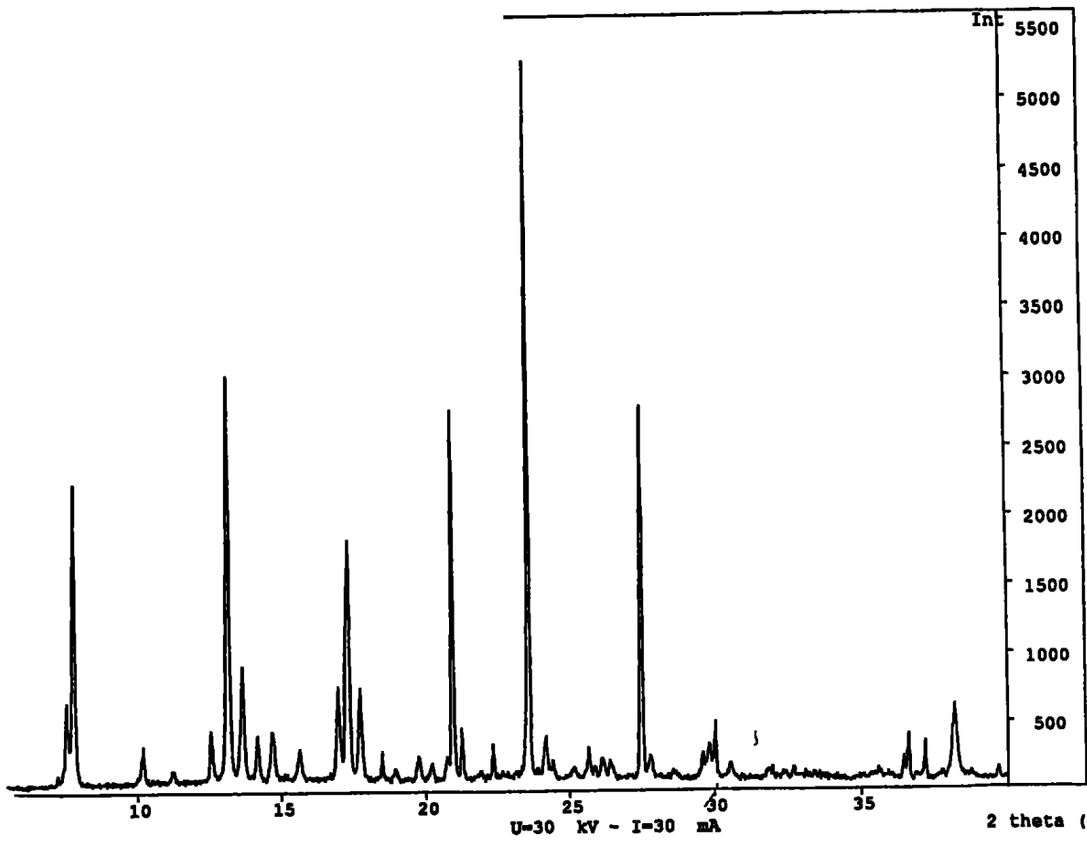
INEL 1426



1439

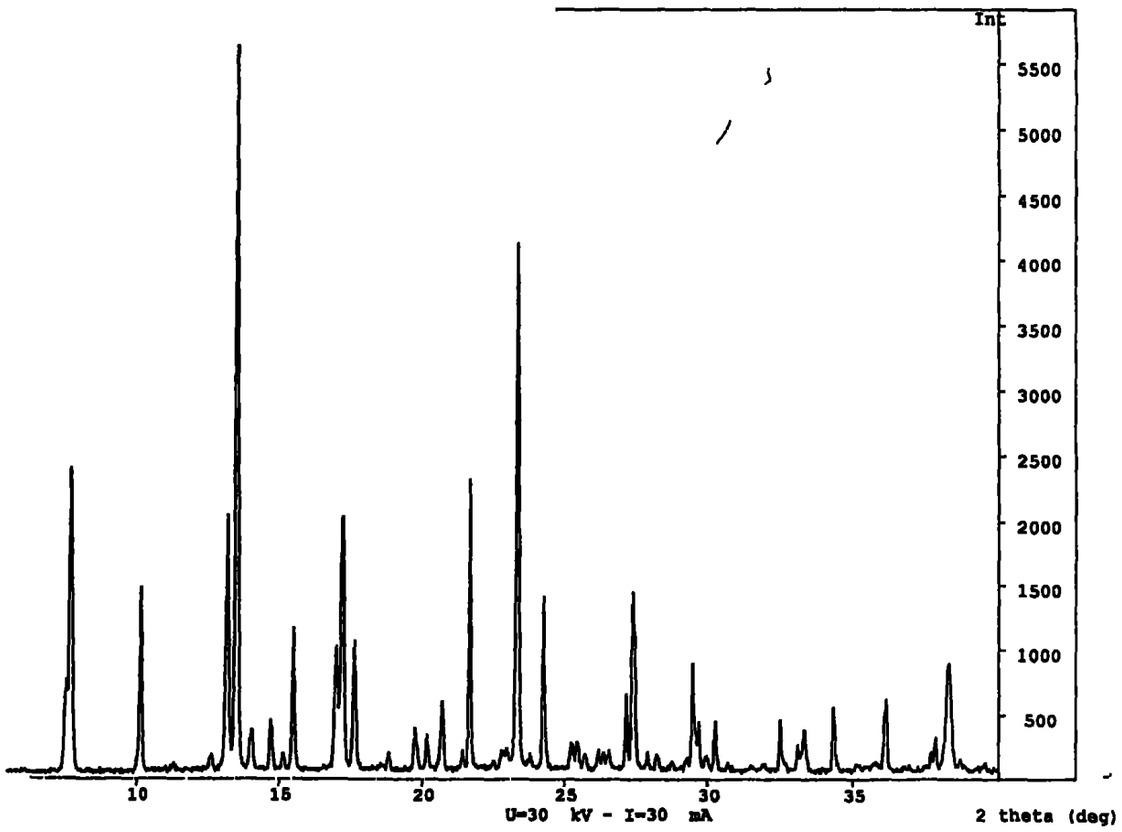
Figure 1-N

INEL 1427



4138

Figure 1-0



INEL 1428

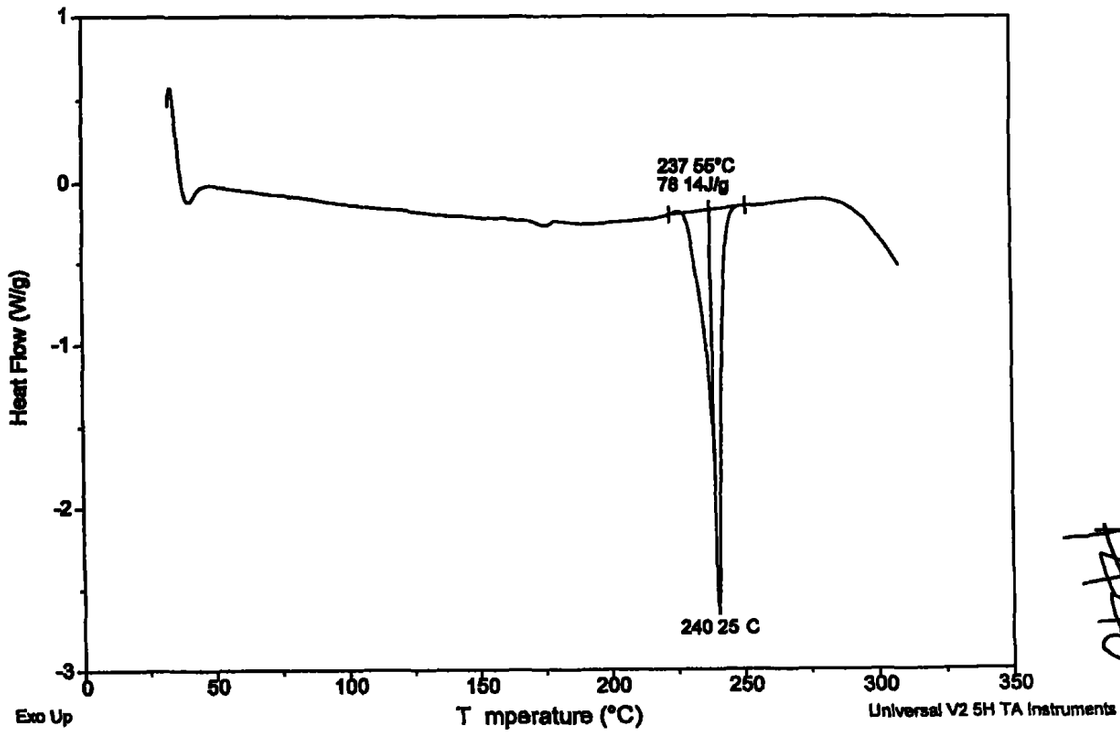
1428

Figure 2-A

668021 [redacted] 69109

1429

Size. 0 6360 mg
Method 10 DEG C/MIN AMB TO 300
Comment SEALED PAN



1440

Figure 2-B

66 021 [redacted]0 6910

1430

Size 1 7840 mg
Method 10 DEG C/MIN AMB TO 300
Comment: SEALED PAN

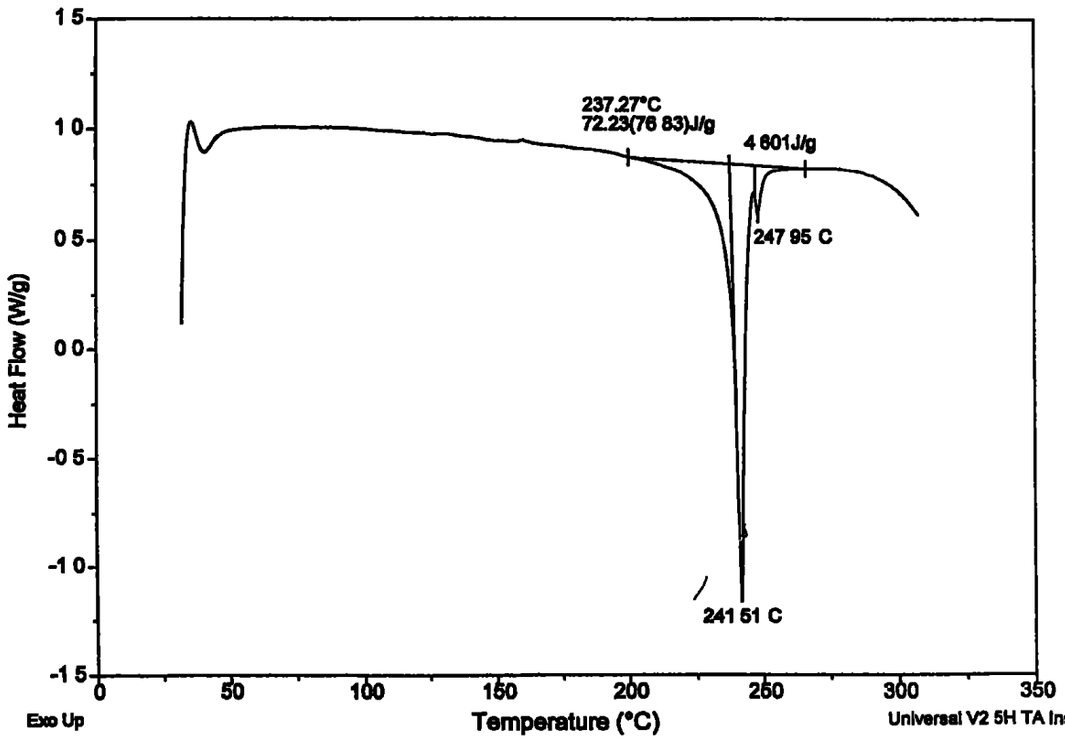
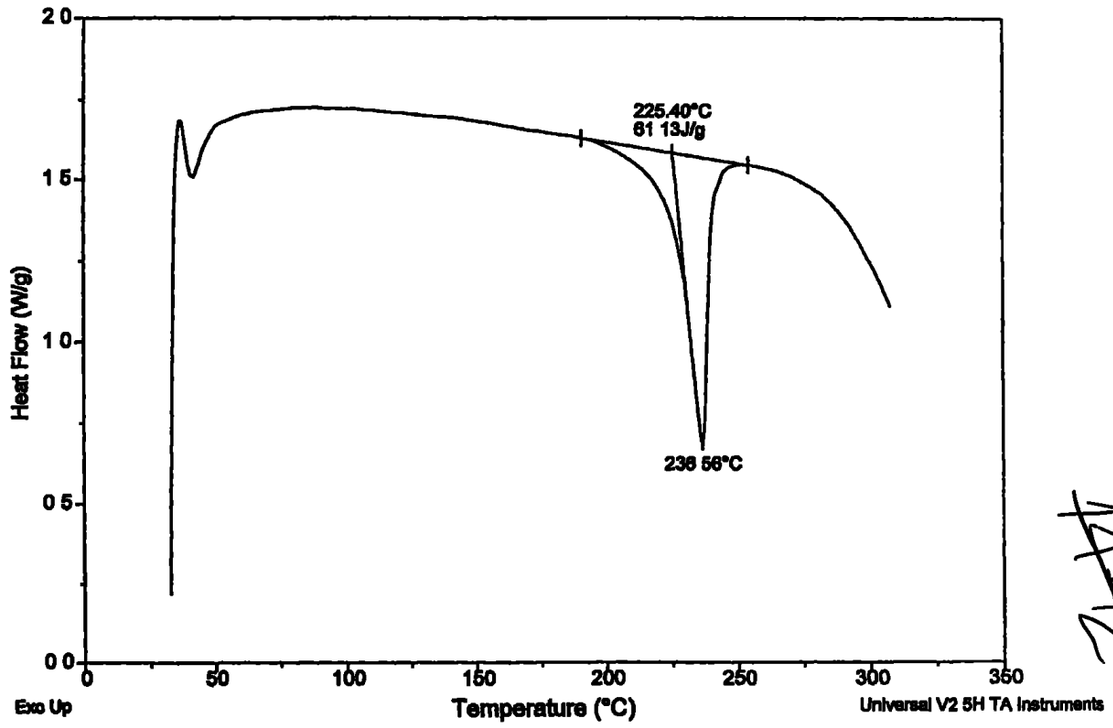


Figure 8-C

08S 2T 20 69T09

Size 1.4230 mg
Method 10 DEG C/MIN AMB TO 300
Comment: SEALED PAN



2774

Figure 2-D

66 021*[illegible]0 69T 9

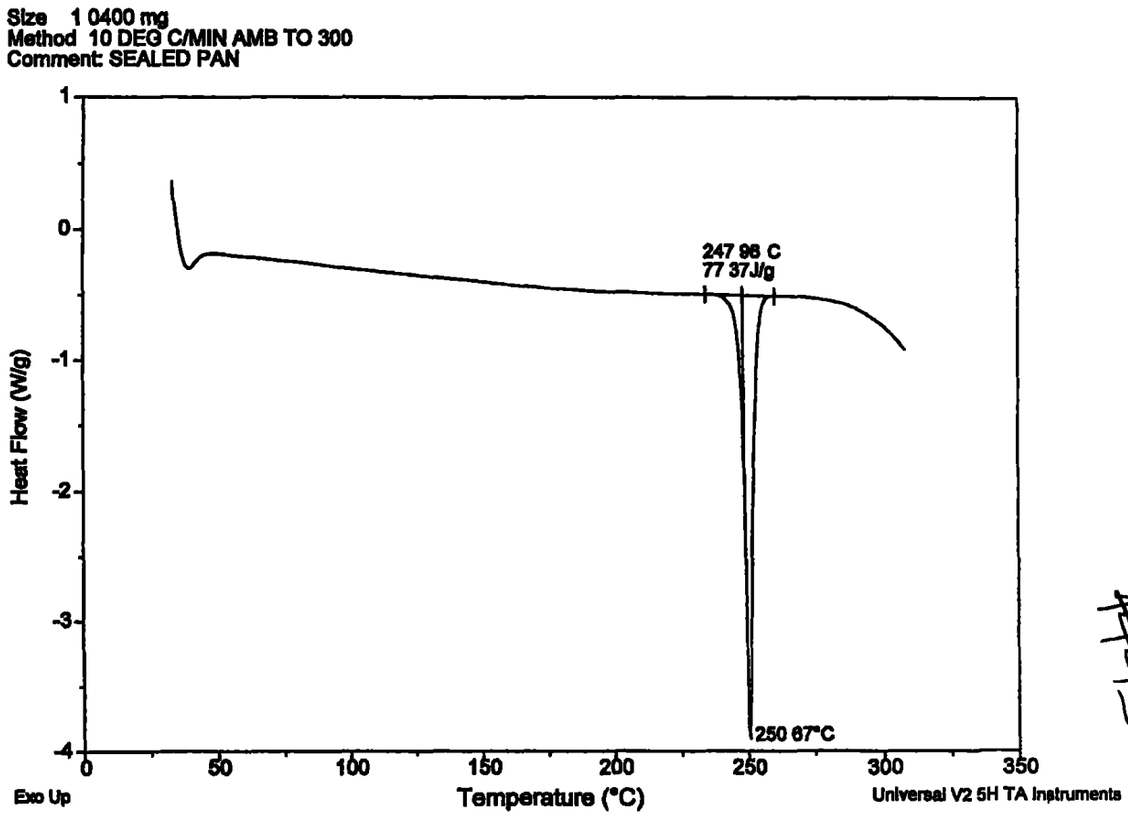


Figure 2-E

668021 0869105

1433

Sample Weight: 16.300 mg
Comment:
N-Propyl Alcohol solvate 1
unwashed exo

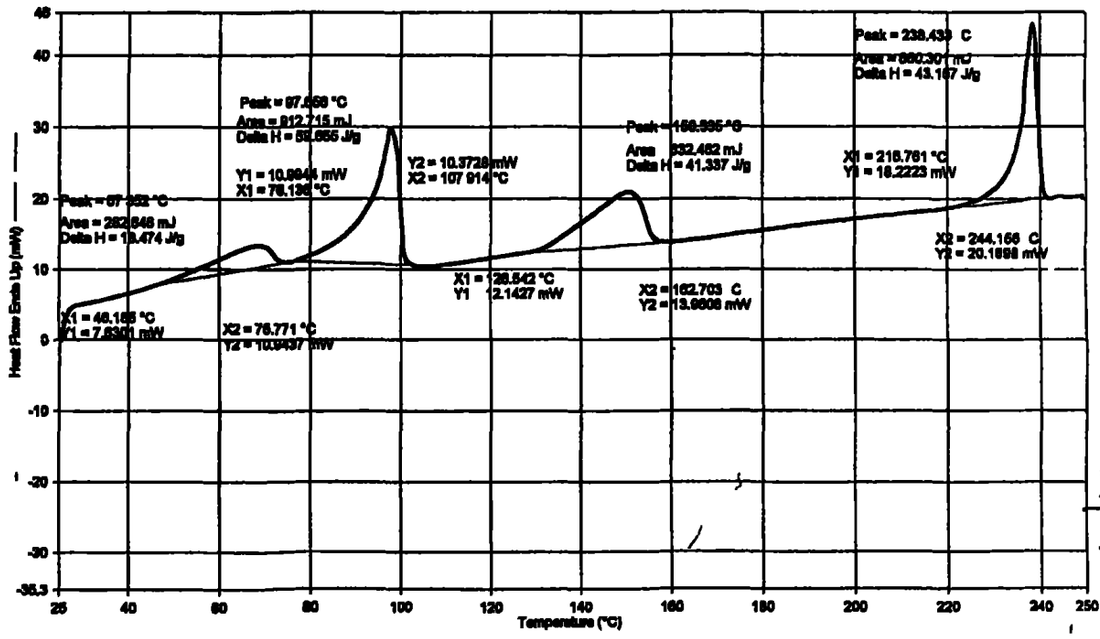


Figure 2-F

[redacted]

1434

Sample Weight: 10.400 mg
Comment: THF solvate 1
unsealed pan

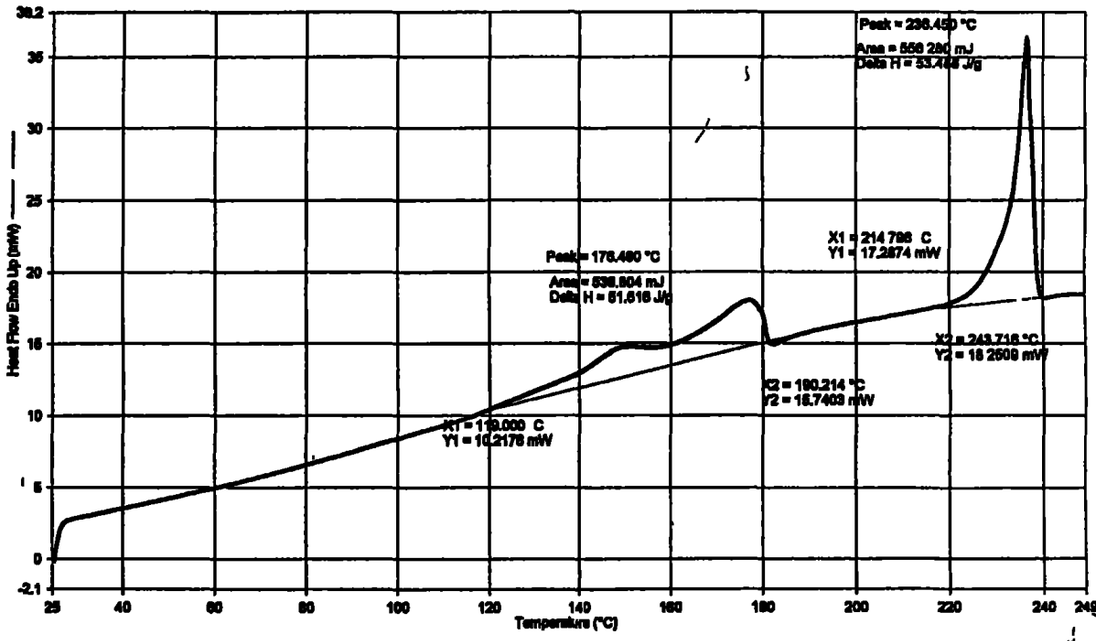


Figure 2C

1435

Sample Weight: 7.200 mg
Comment: Lot 99K020-F3b
Ethyl propionate solvate
unsealed pan

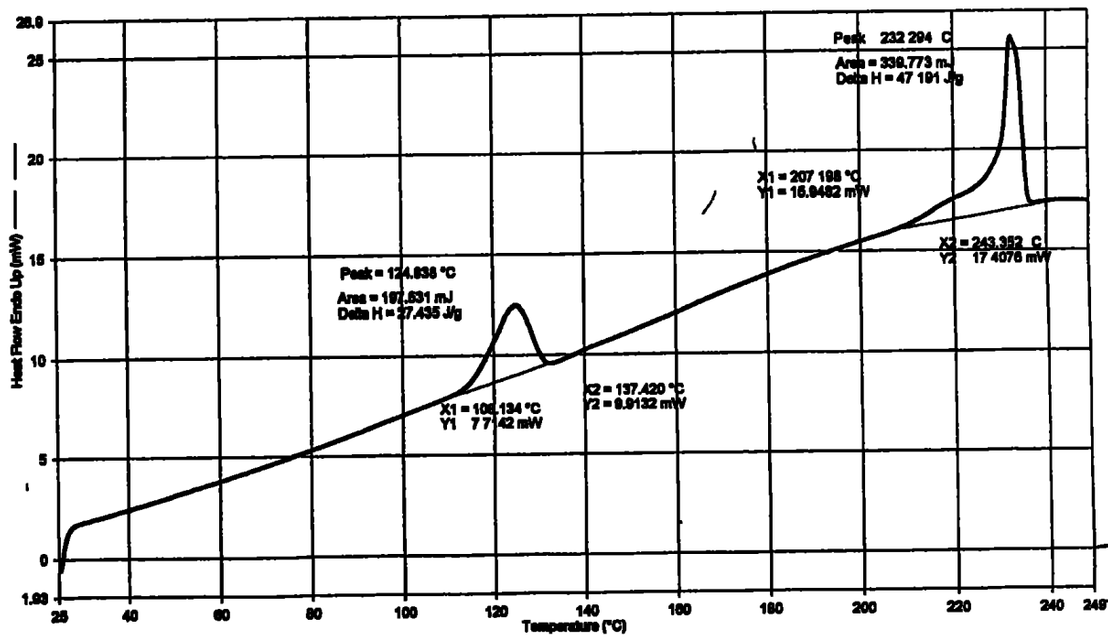


Figure 2-H

668021 [redacted] 0869109

1436

Sample Weight: 8.000 mg
Comment: Acetic ACid solvate 1
unsealed pan

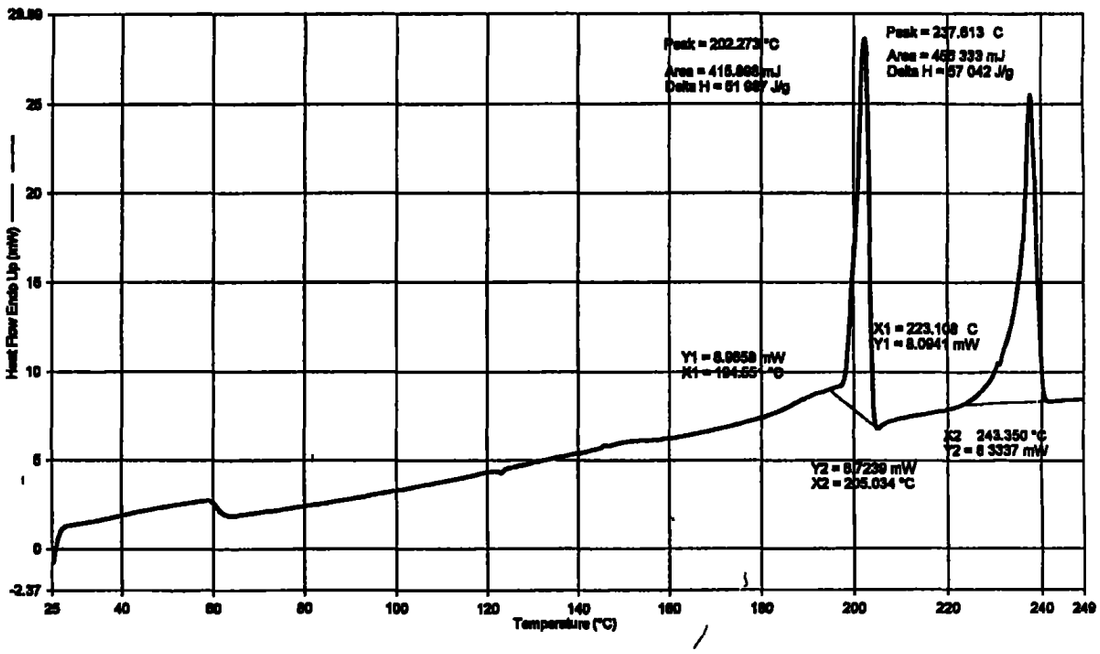


Figure 2-I

1437

Sample Weight: 12.700 mg
Comment: Chloroform solvate 1
unsealed pan

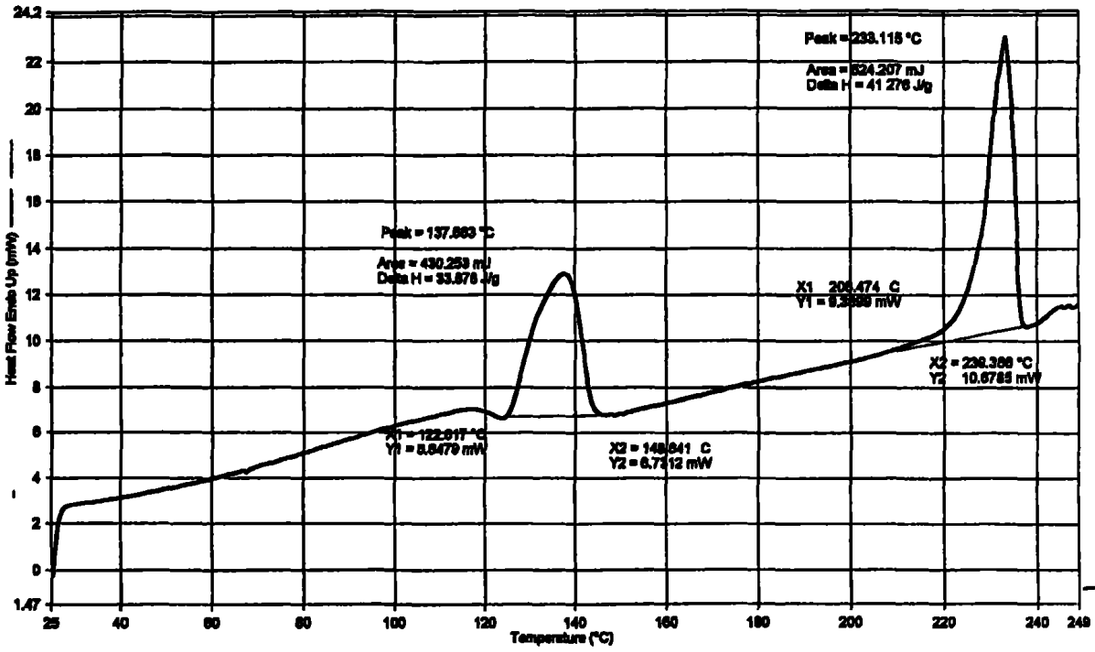


Figure 2-J

Sample Weight: 6.704 mg
Comment: Acetone Solvate

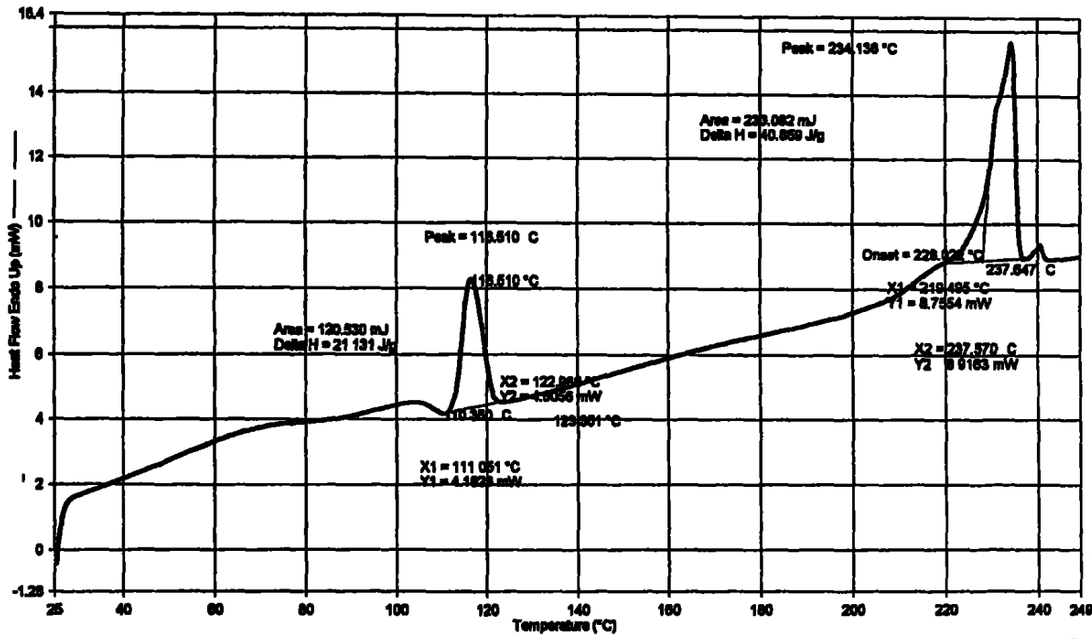
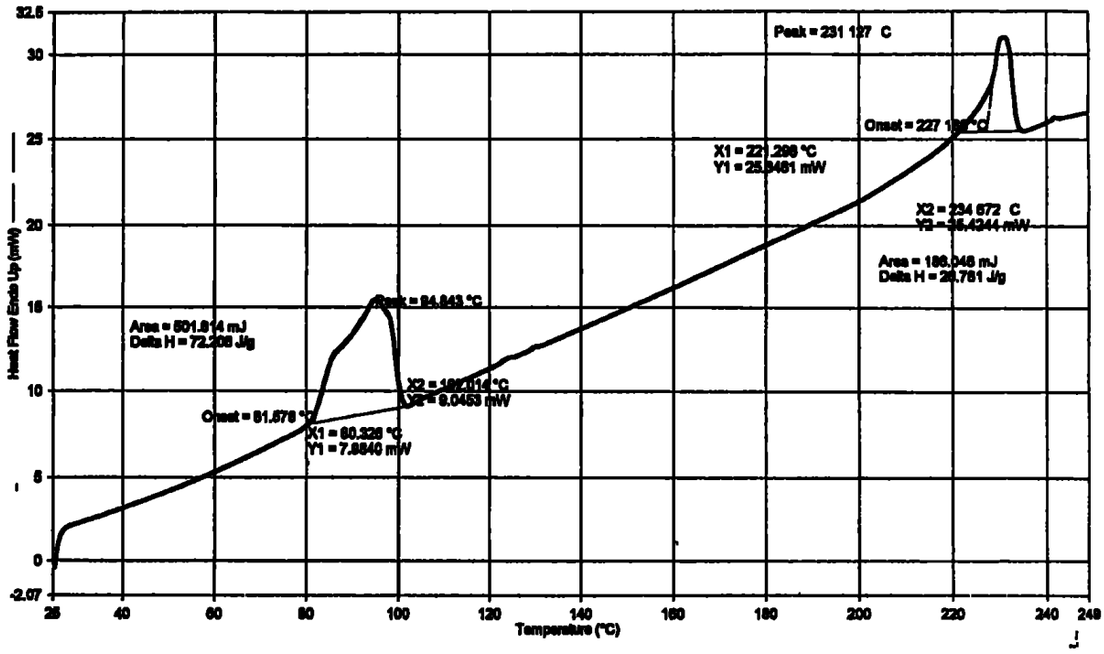


Figure 2-K

668021 [redacted] 0869709

1439

Sample Weight: 6.947 mg
Comment: toluene Solvate



1450

Figure 2-L

Sample Weight: 8.174 mg
Comment: Isopropanol Solvate

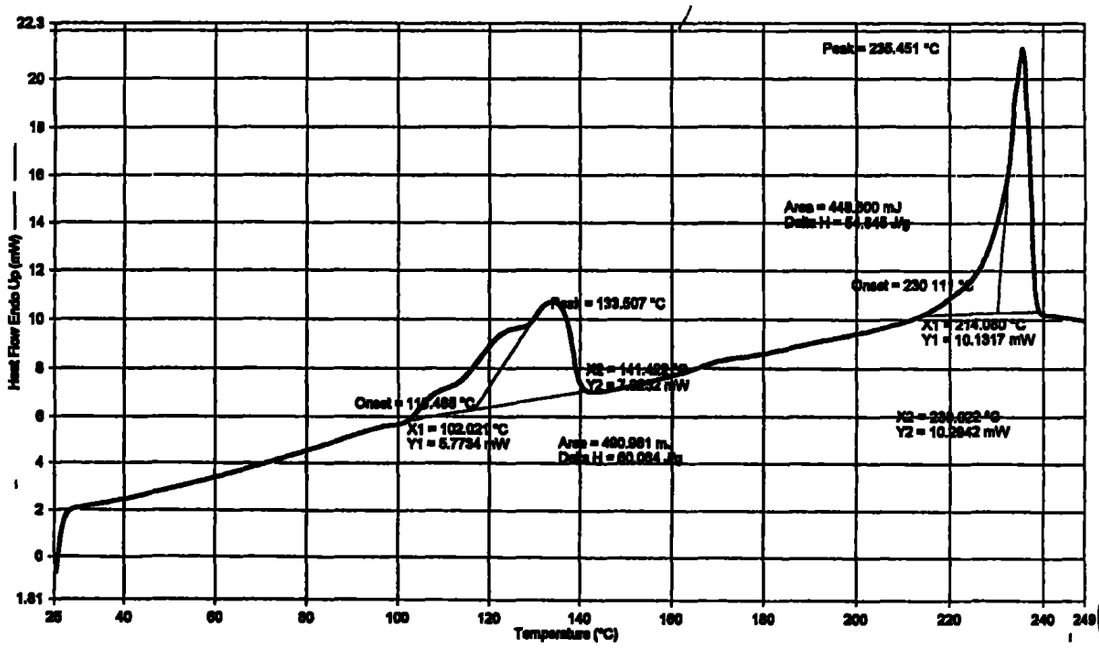


Figure 2 M

1441

Sample Weight: 6.122 mg
Comment:
ethanol solvate
10 °C/min sealed pan

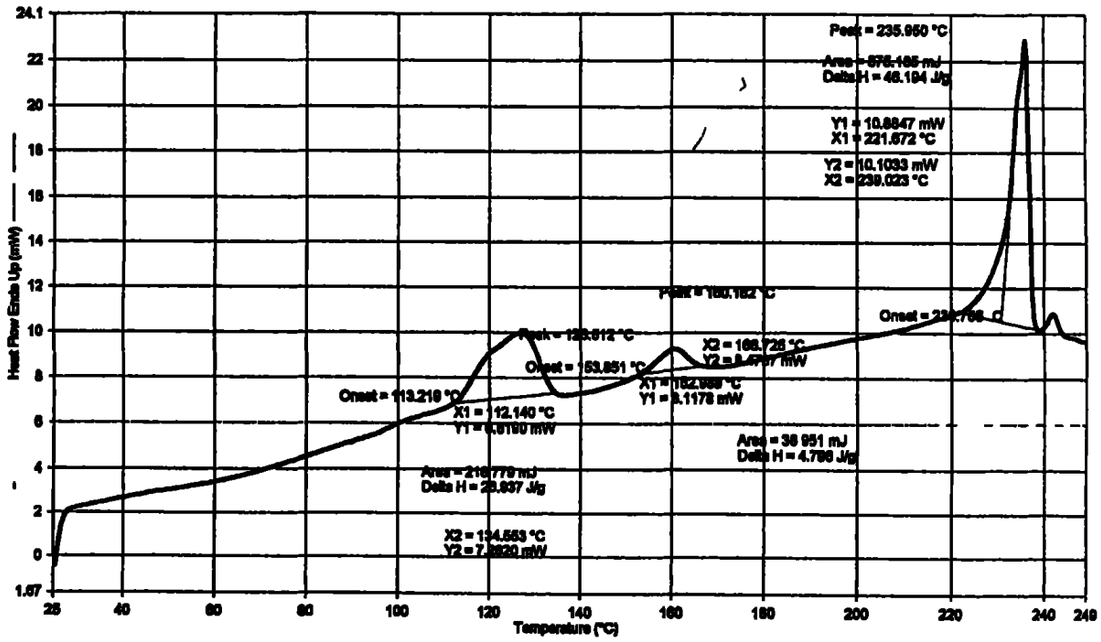
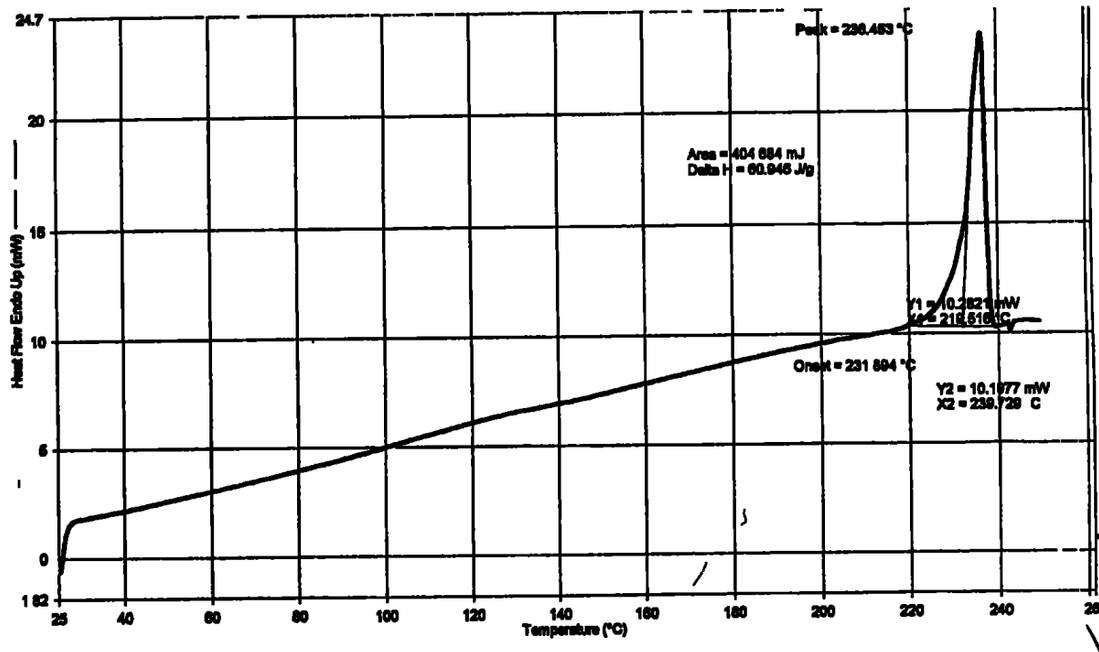


Figure 2-N

[redacted]

1442

Sample Weight: 6.640 mg
Comment: t-butyl acetate solvate
10 °C/min sealed pan



1453

Figure 2-0

66 021 2086910

1443

Sample Weight: 6.900 mg
Comment:
Isobutyl acetate solvate
10 °C/min pierced pan

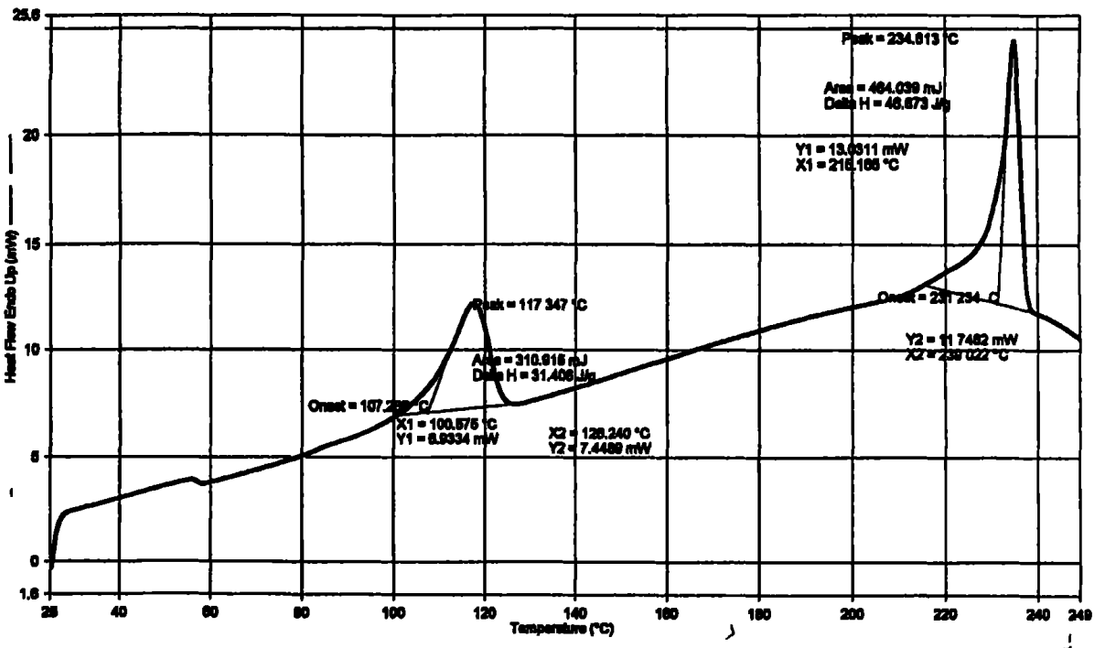


Figure 2-P

1444

Sample weight: 6.000 mg
Comment: butyl acetate solvate
10 °C/min sealed pan

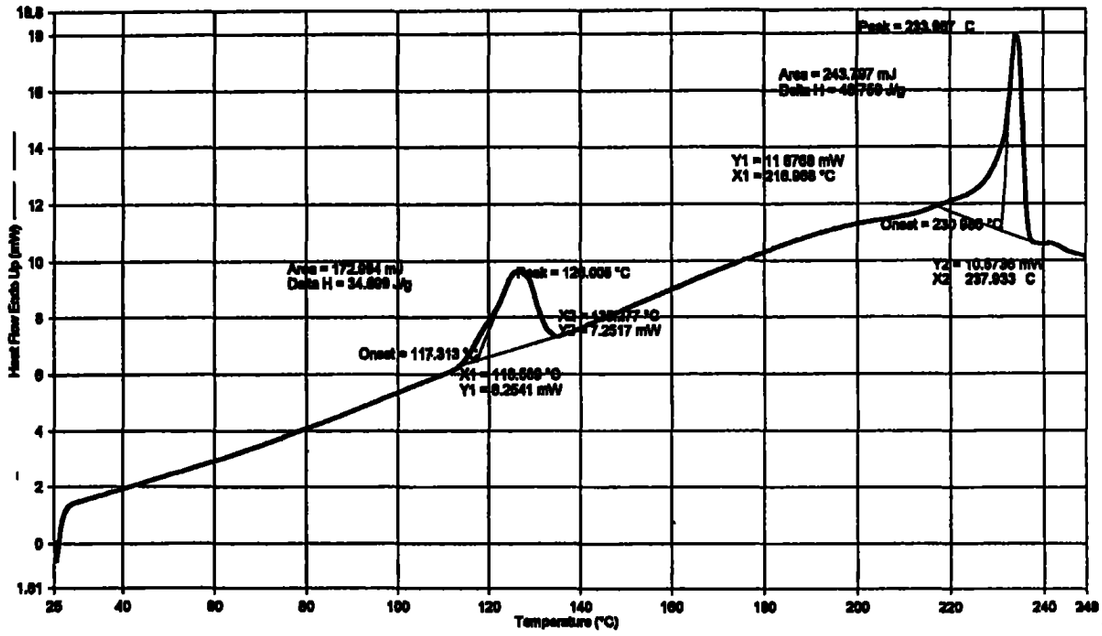


Figure 2-9

00 021 00 69709

1445

Sample Weight: 8.536 mg
Comment:
methyl acetate solvate 1
10 °C/min sealed pan

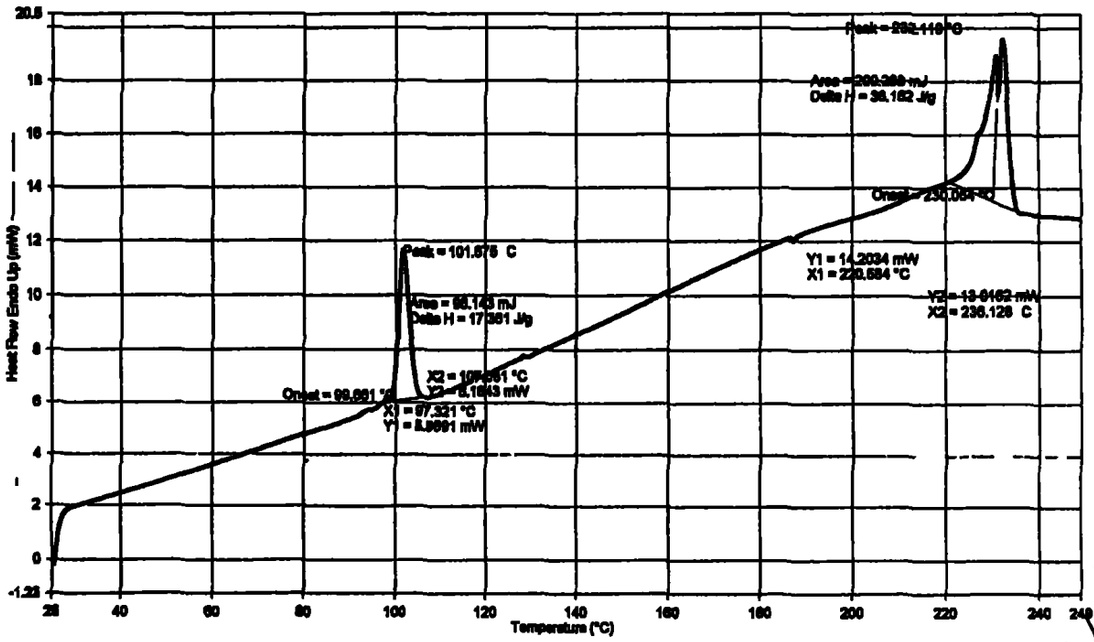


Figure 2-R

1446

Sample Weight: 6.074 mg
Comment: propyl acetate solvate 1
10 °C/min sealed pan

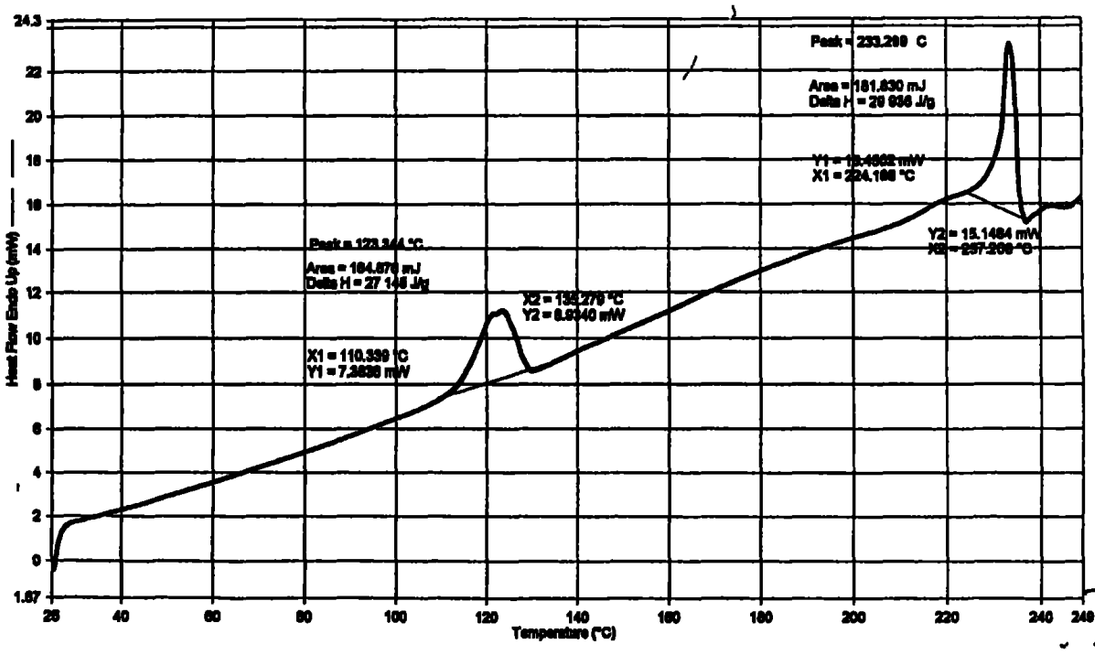


Figure 2-S

[redacted]

n-butanol solvate

1442

Size 0 6230 mg
Method 10 DEG C/MIN AMB TO 300
Comment UNSEALED PAN

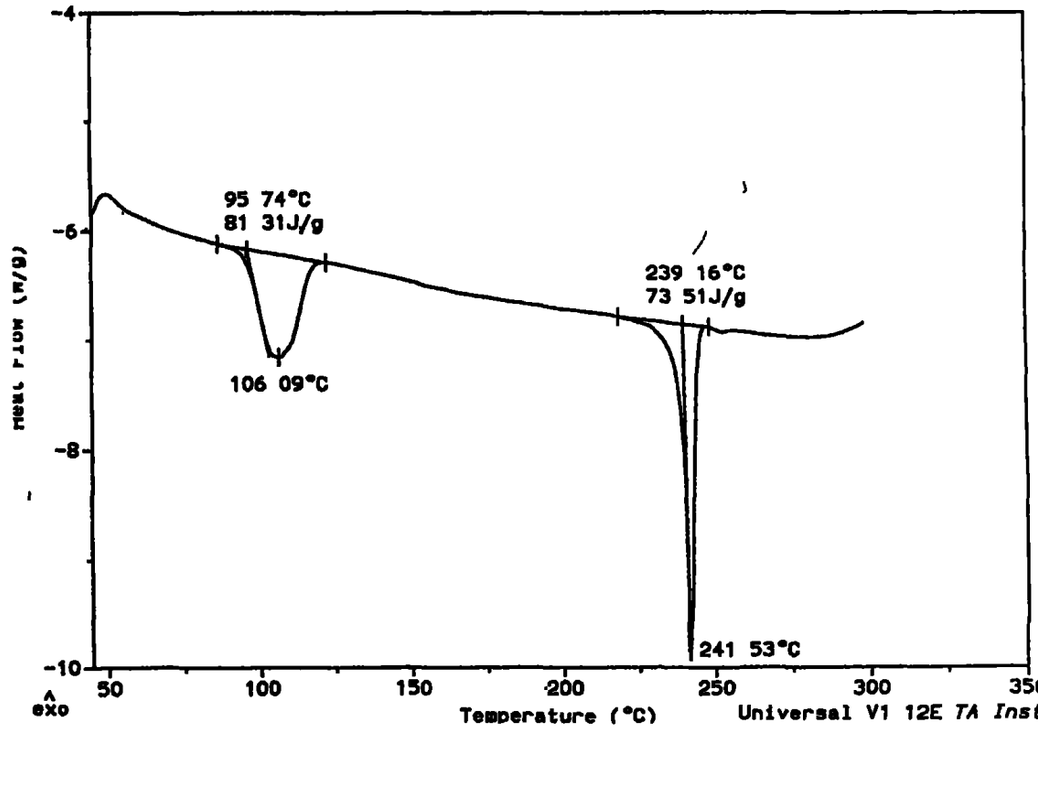


Figure 2-T

66 02T [redacted]8659T09

n-octanol solvate

Size 0 5220 mg
Method 10 DEG C/MIN AMB TO 300
Comment UNSEALED PAN

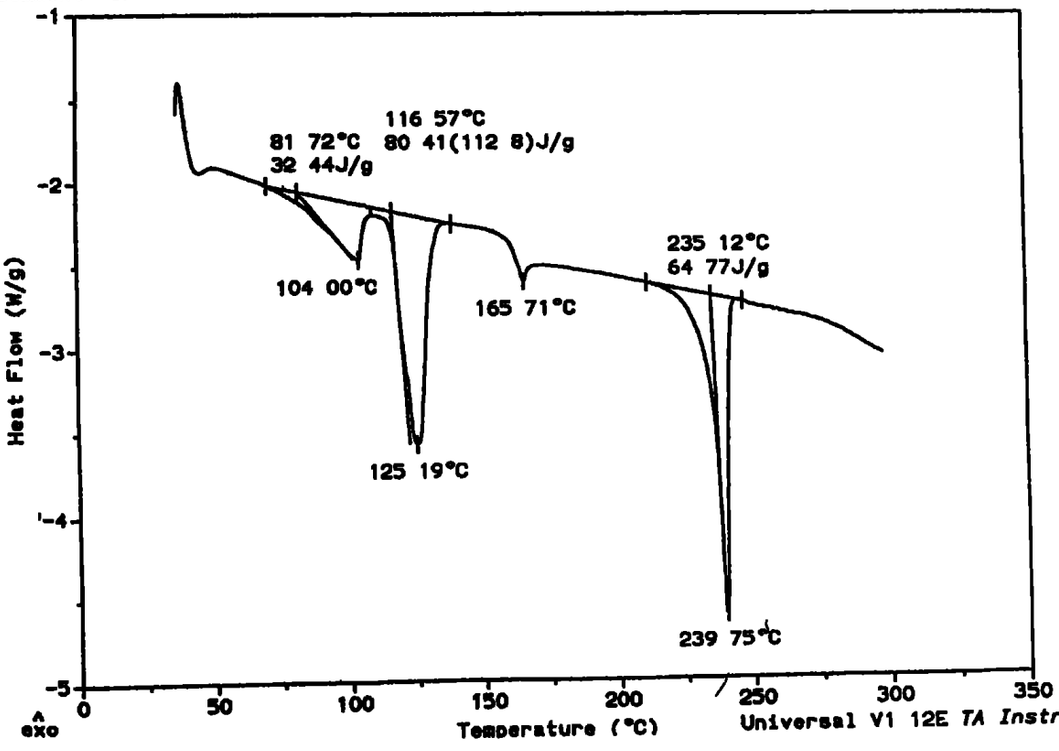
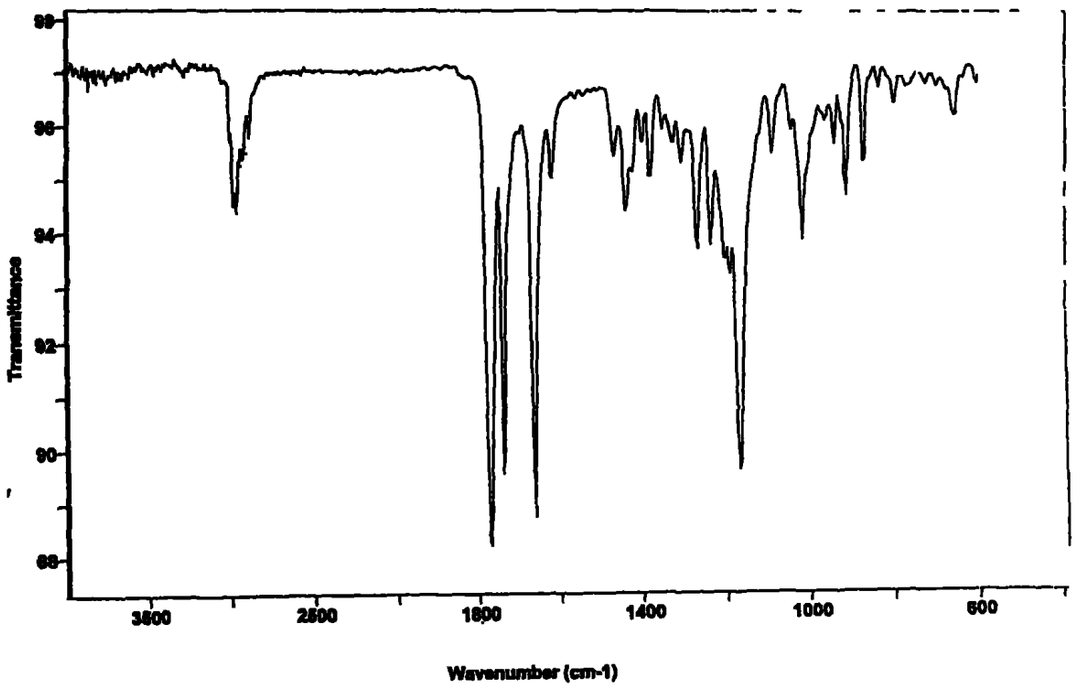


Figure 3-A

66 021' 208b5T0

1449



A60

Figure 2-8

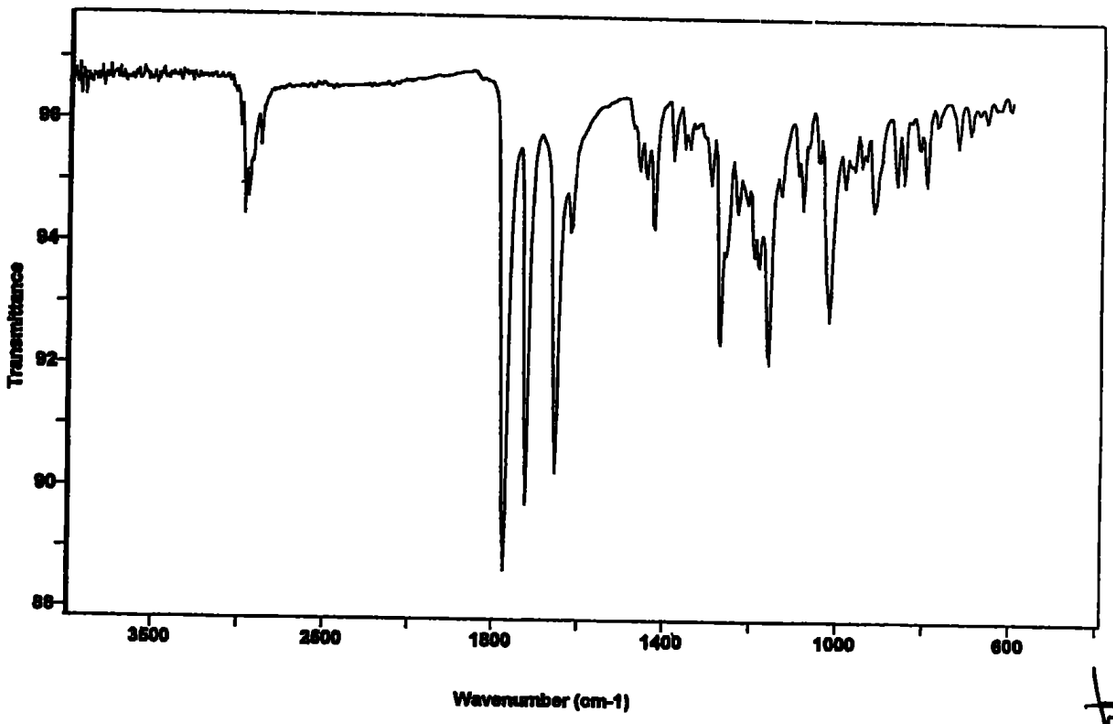

Transmittance
96
94
92
90
88
3500
2500
1800
1400
1000
600
Wavenumber (cm-1)

Figure 1-C

6016980 12089T09

1451

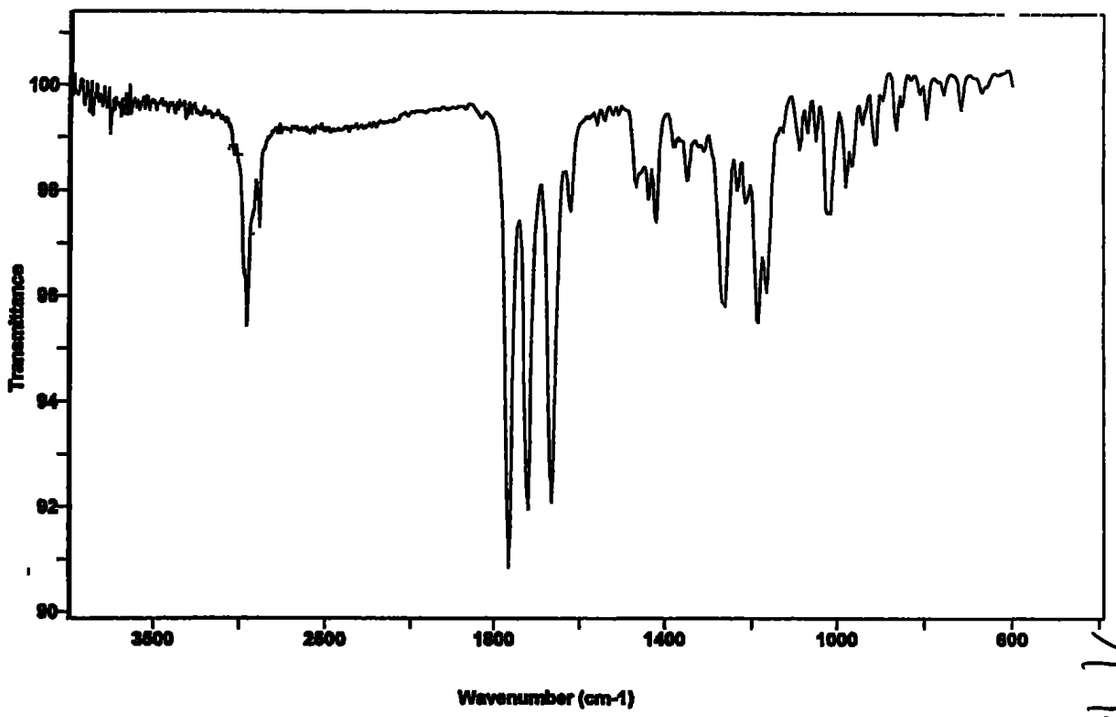
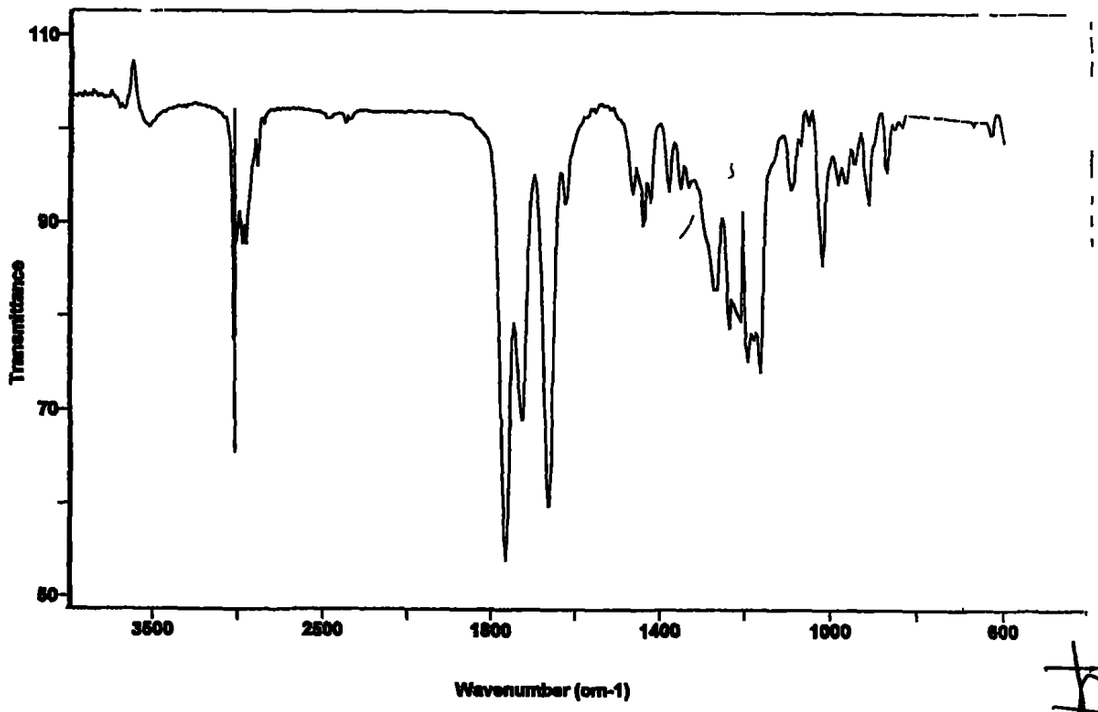


Figure 3-D

6015869709 .120 90

1452



1463

Figure 3 E

n propyl alcohol solvate

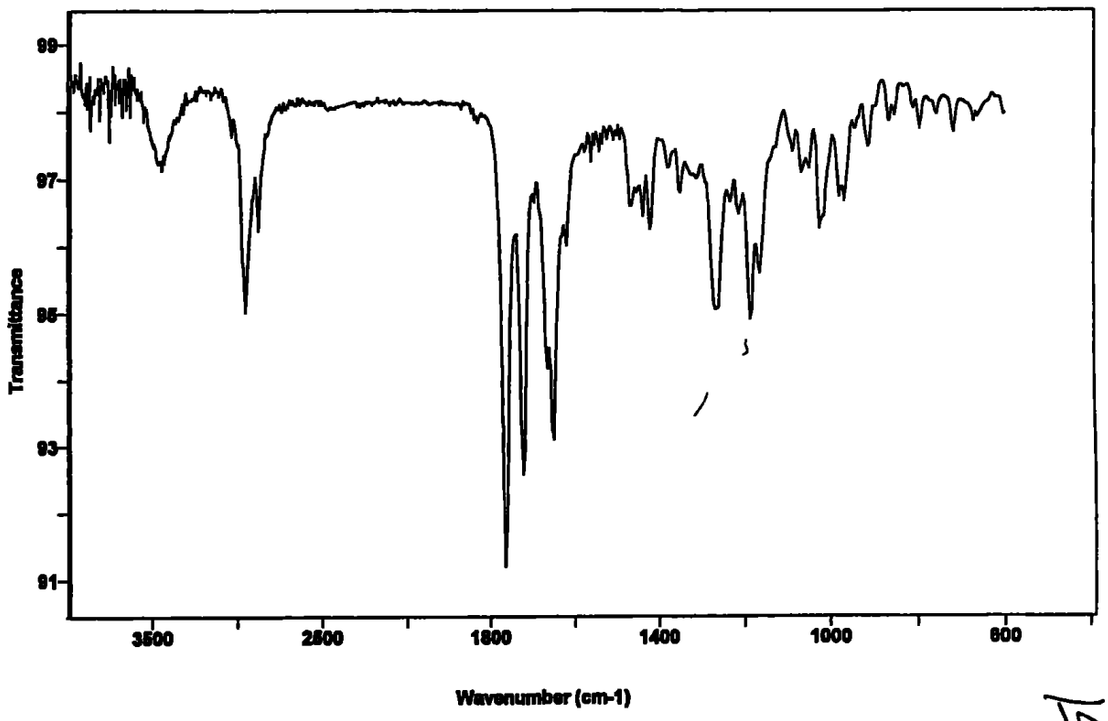


Figure 3-F

tetrahydrofuran solvate

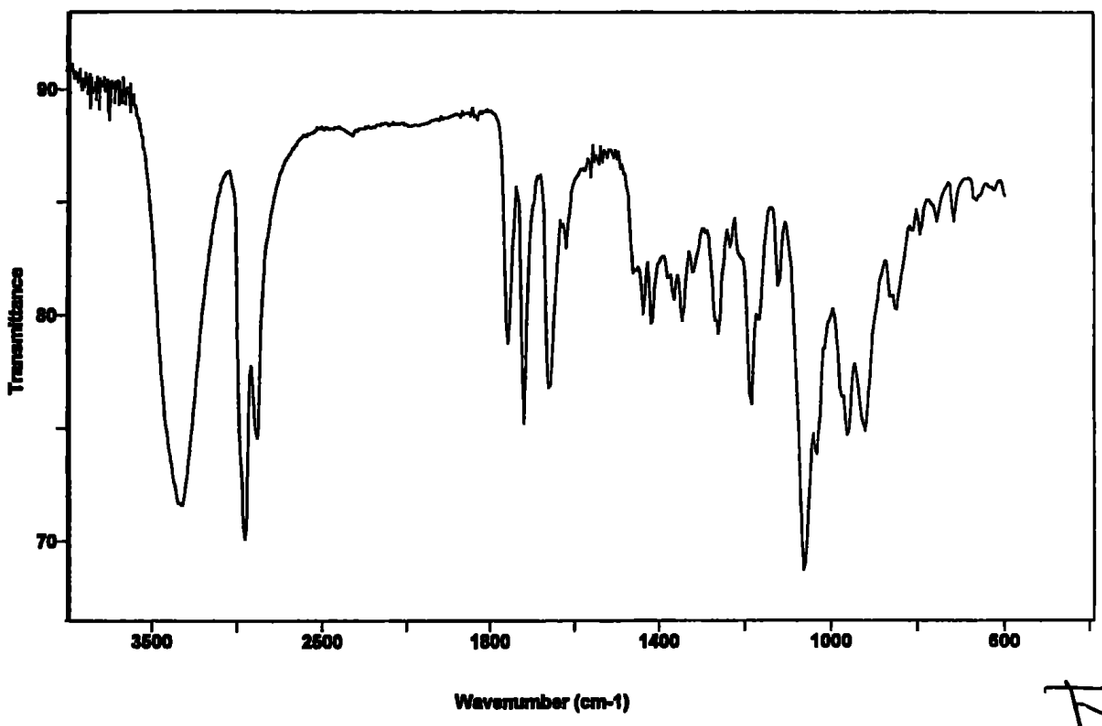


Figure 3-G

ethyl propionate solvate

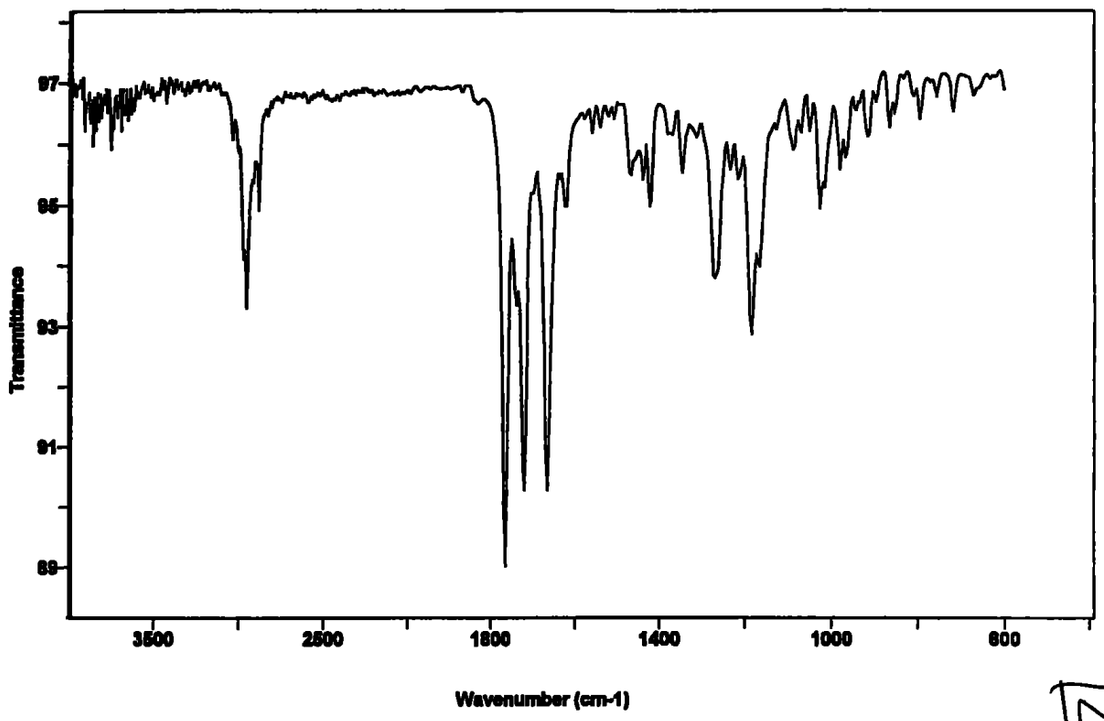


Figure 3-H

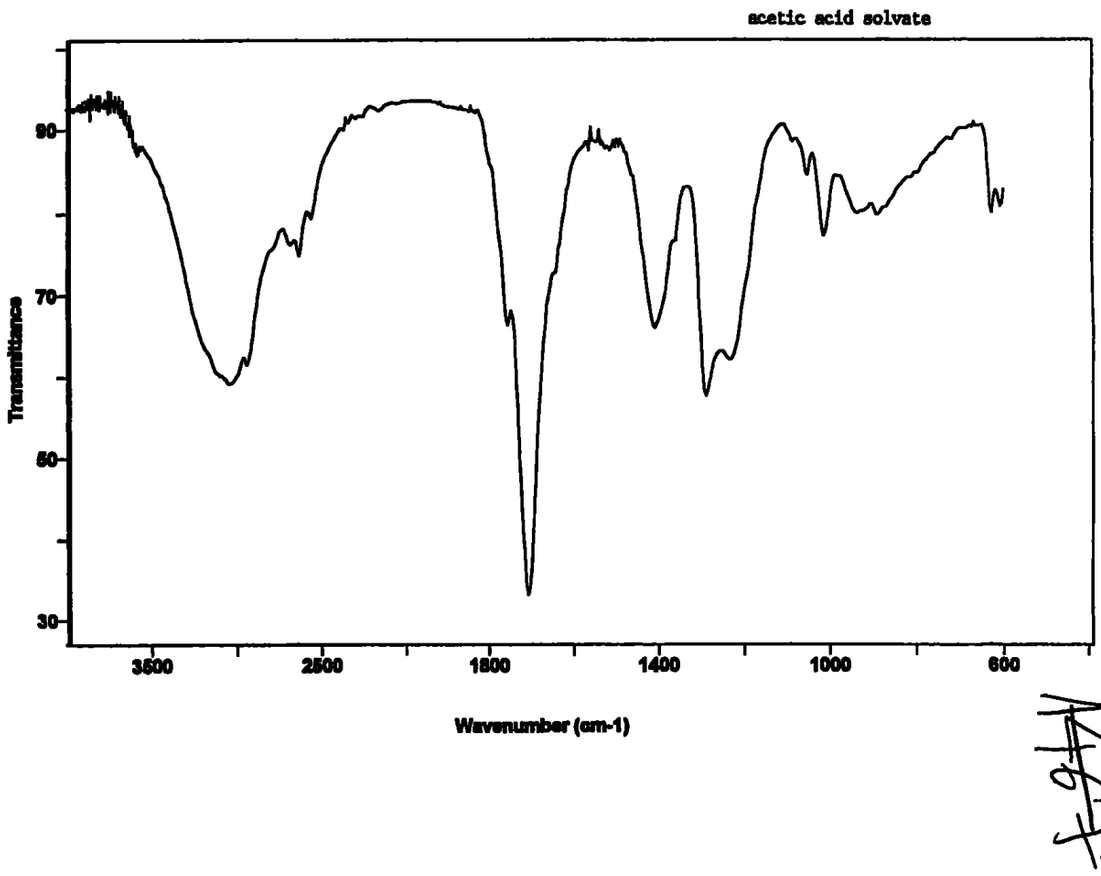
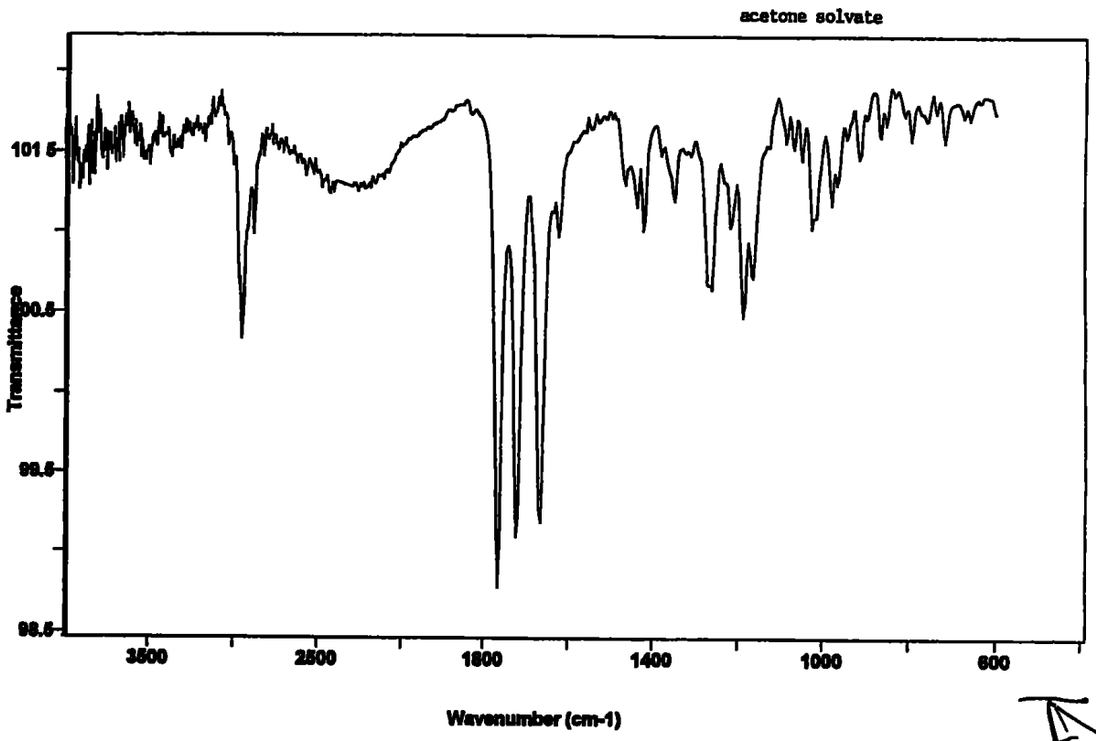

acetic acid solvate
90
70
50
30
Transmittance
3500
2500
1800
1400
1000
600
Wavenumber (cm-1)

Figure 3-I

668021 [illegible]9705

1457



4/6

Figure 3-J

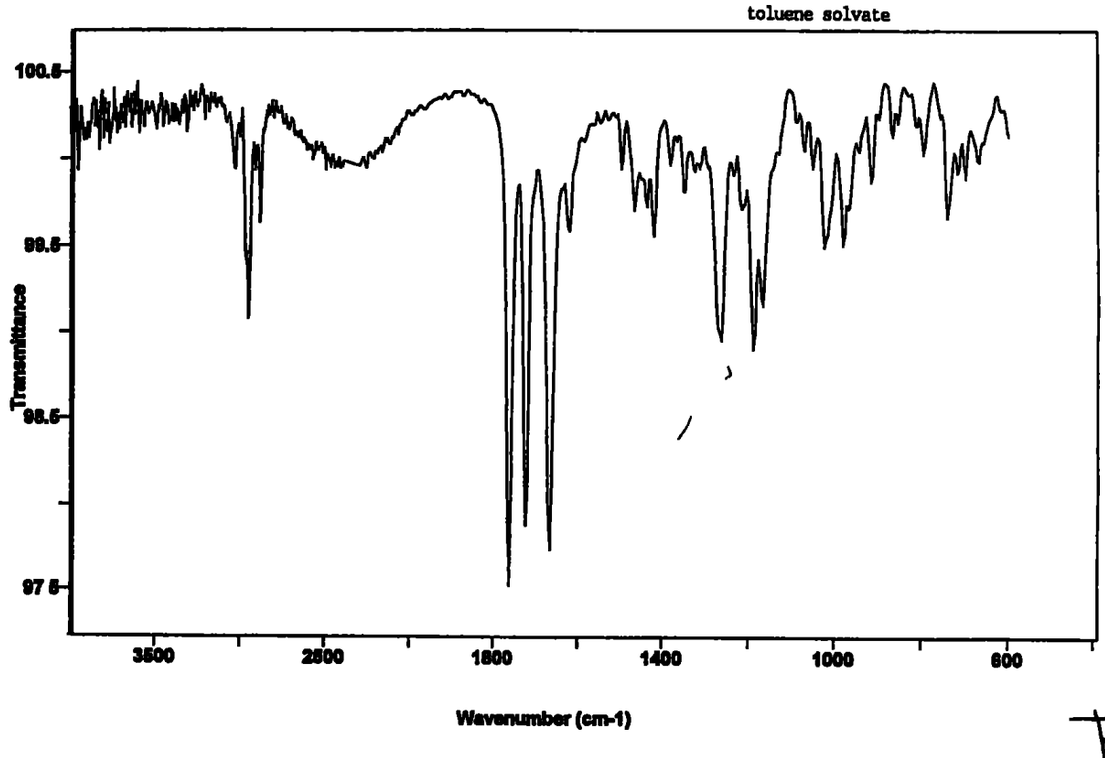

toluene solvate
100.5
99.5
98.5
97.5
Transmittance
3500
2500
1800
1400
1000
800
Wavenumber (cm-1)

Figure 3-K

60 021 0869105

isopropanol solvate

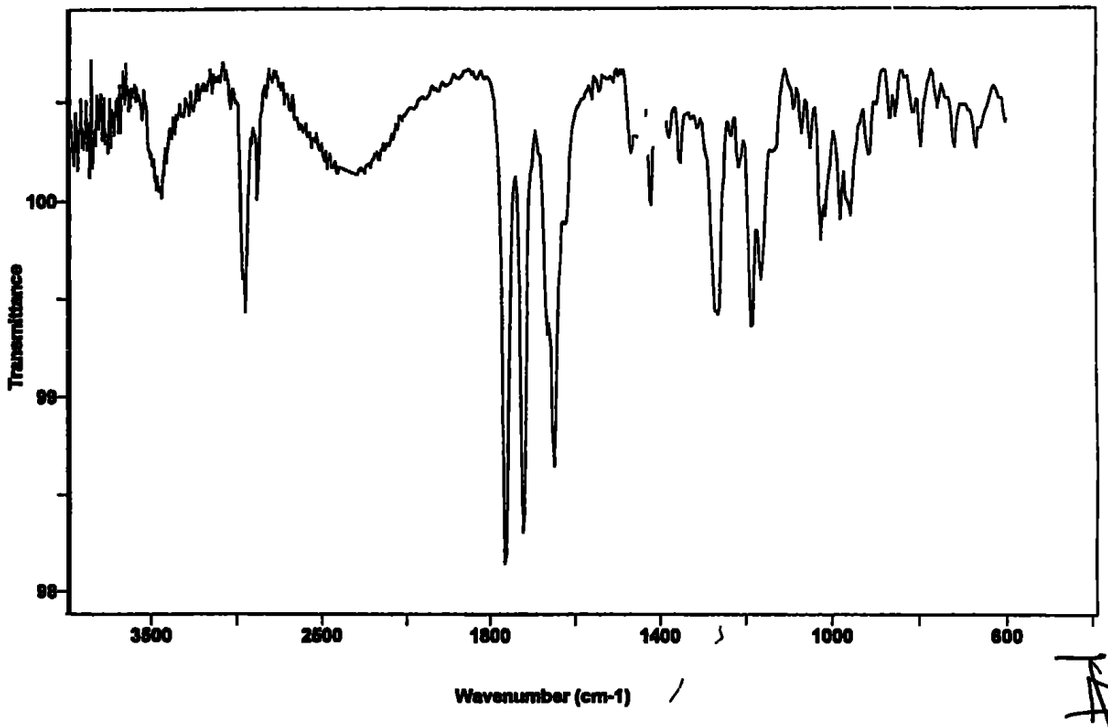
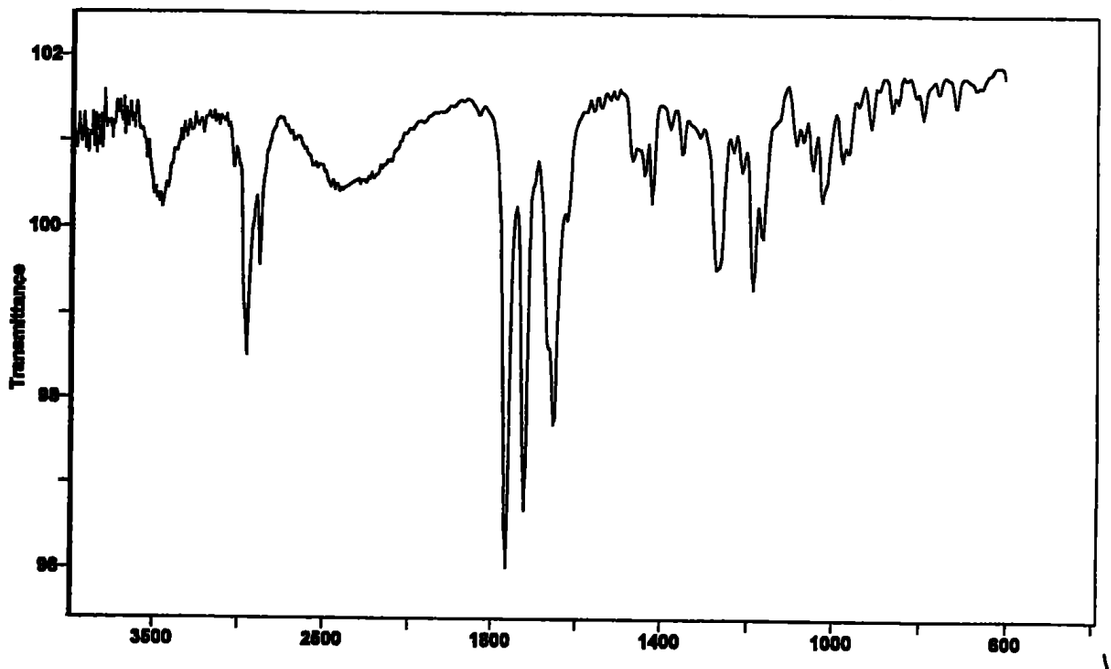


Figure 3-L

66 021 00 69T09

1960

ethanol solvate



File # 1 = P4828A02

Wavenumber (cm-1)

X-Zoom CURSOR

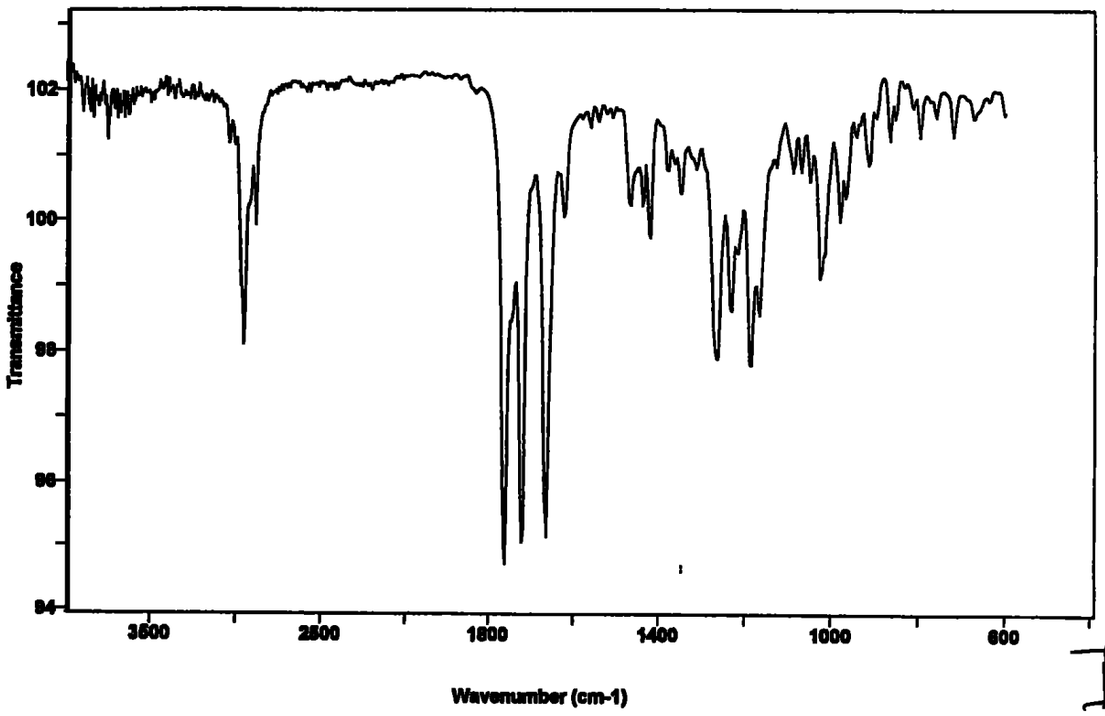
FIG. 3A

Figure 3-M

668021 0869709

1461

isobutyl acetate solvate



1472

butyl acetate solvate

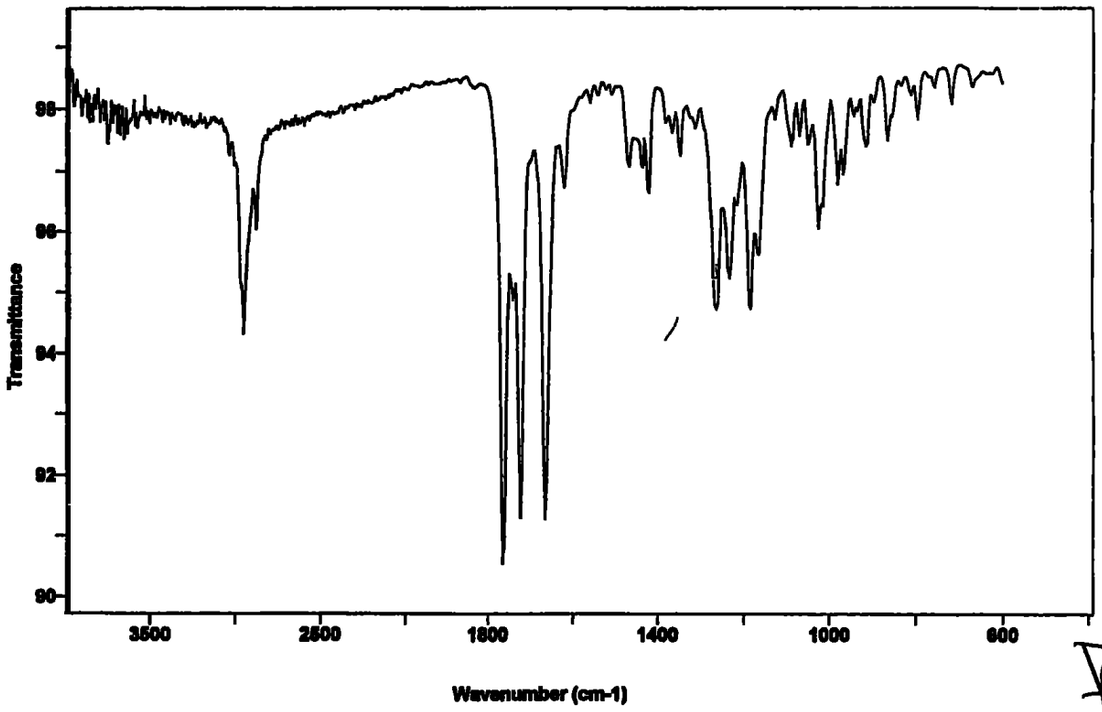


Figure 3-0

bb 027 0869709

propyl acetate solvate

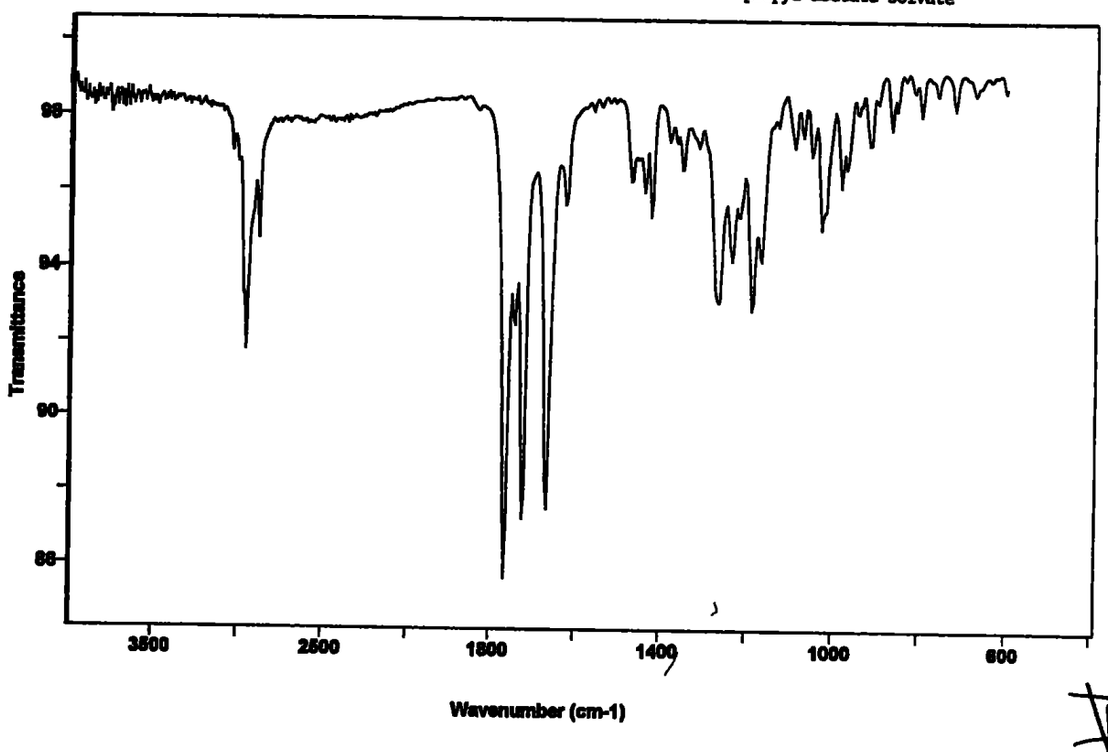


Figure 3-P

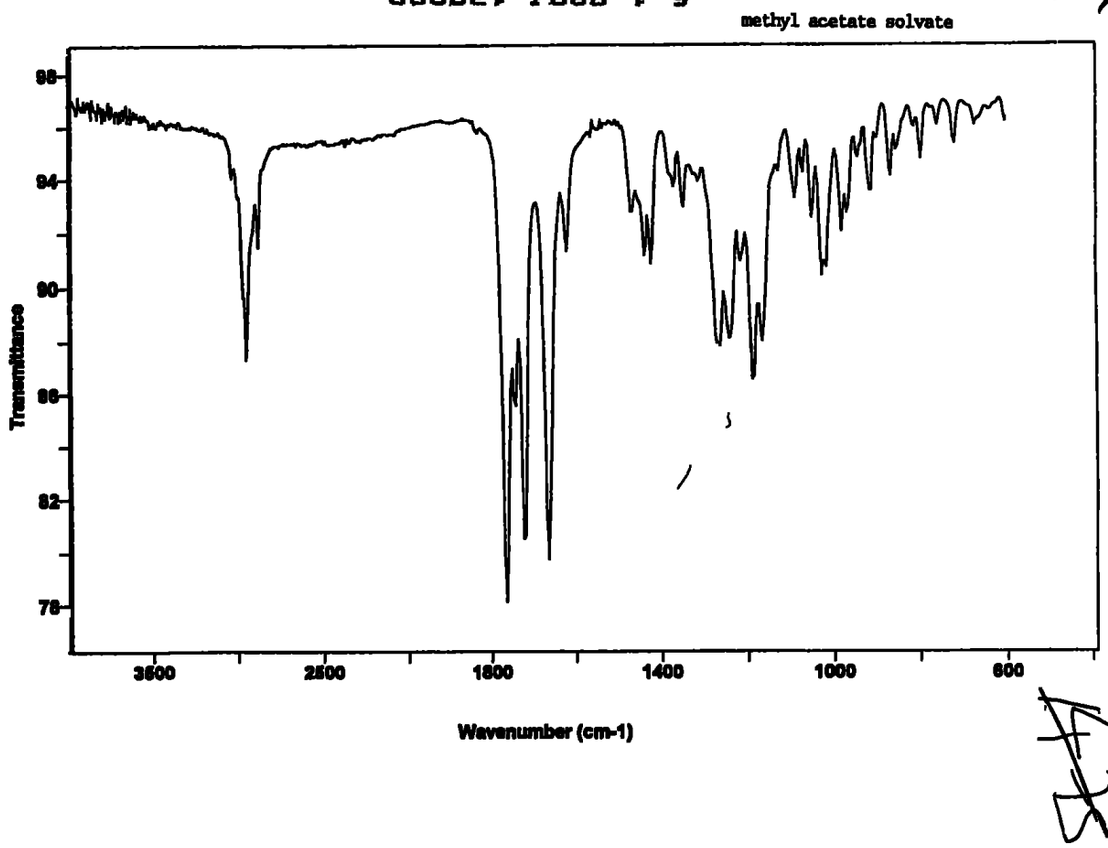

methyl acetate solvate
98
94
90
86
82
78
Transmittance
3500
2500
1800
1400
1000
600
Wavenumber (cm-1)

Figure 3-9

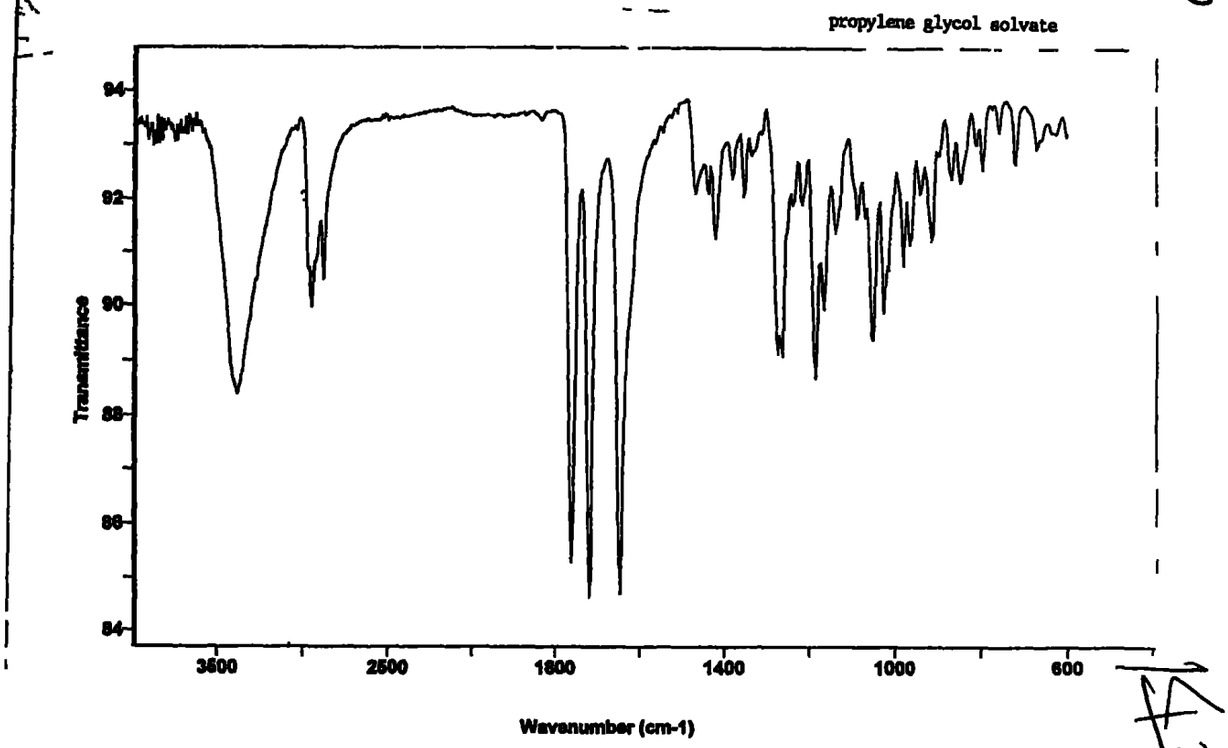

propylene glycol solvate
Transmittance
94
92
90
88
86
84
3500
2500
1800
1400
1000
600
Wavenumber (cm-1)

Figure 3-B

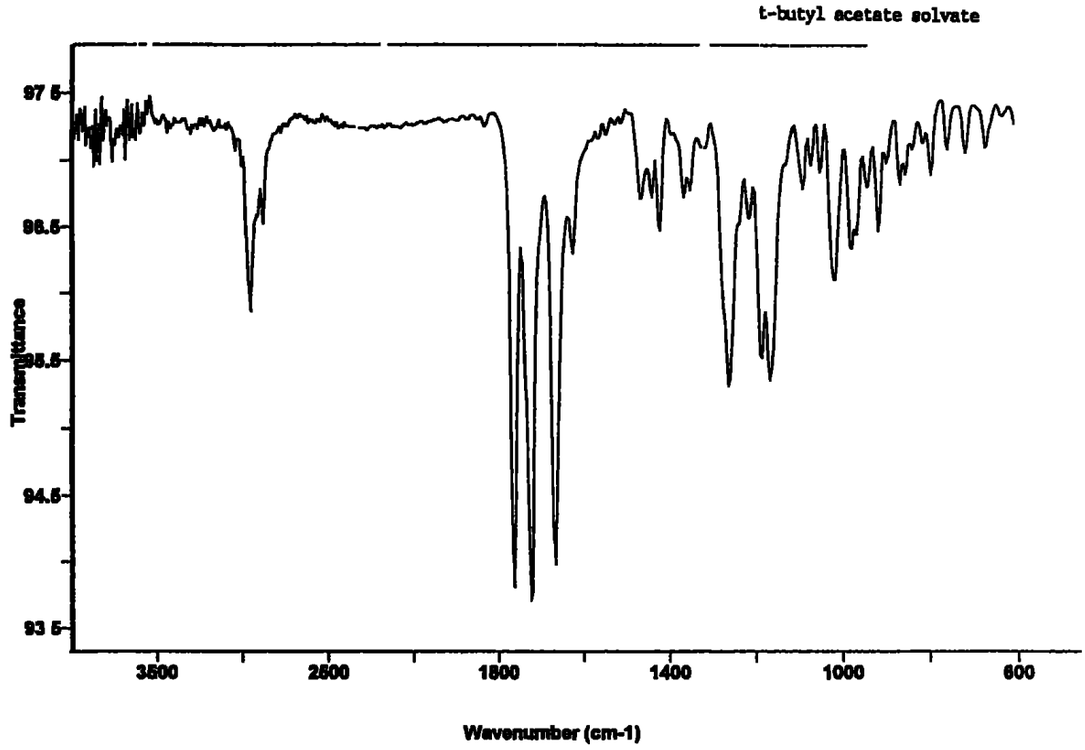

t-butyl acetate solvate
97.5
96.5
95.5
94.5
93.5
Transmittance
3500
2500
1800
1400
1000
600
Wavenumber (cm-1)

Figure 4

66 021 20 69109

1467

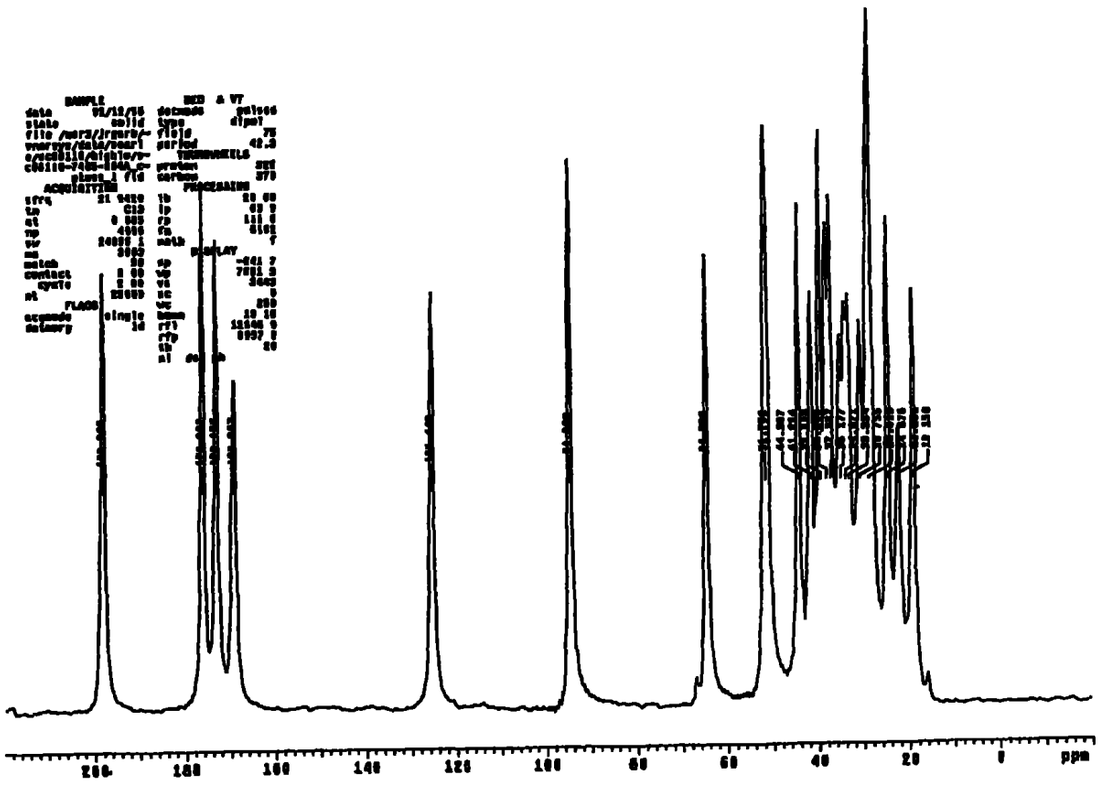


Figure 5

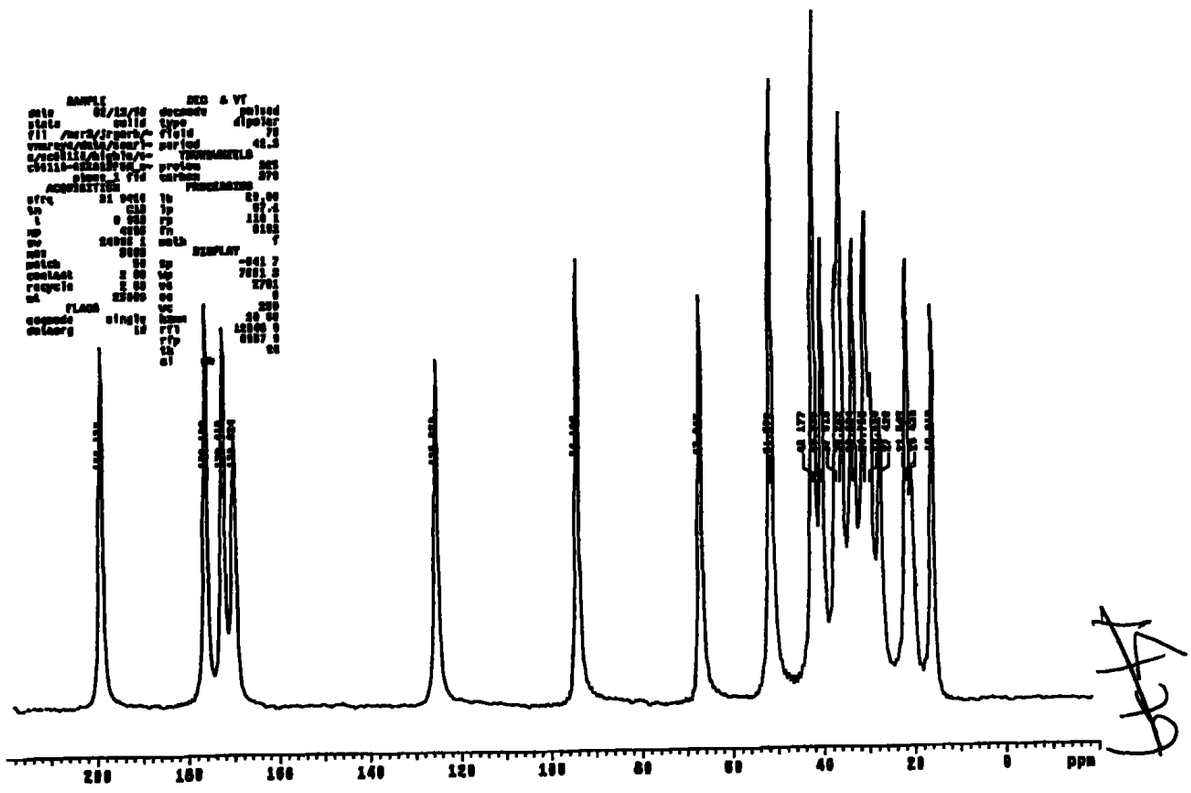


Figure 6-A

1469

Size 3 3010 mg
Method 10 deg ramp to 300 deg

methyl ethyl ketone solvate

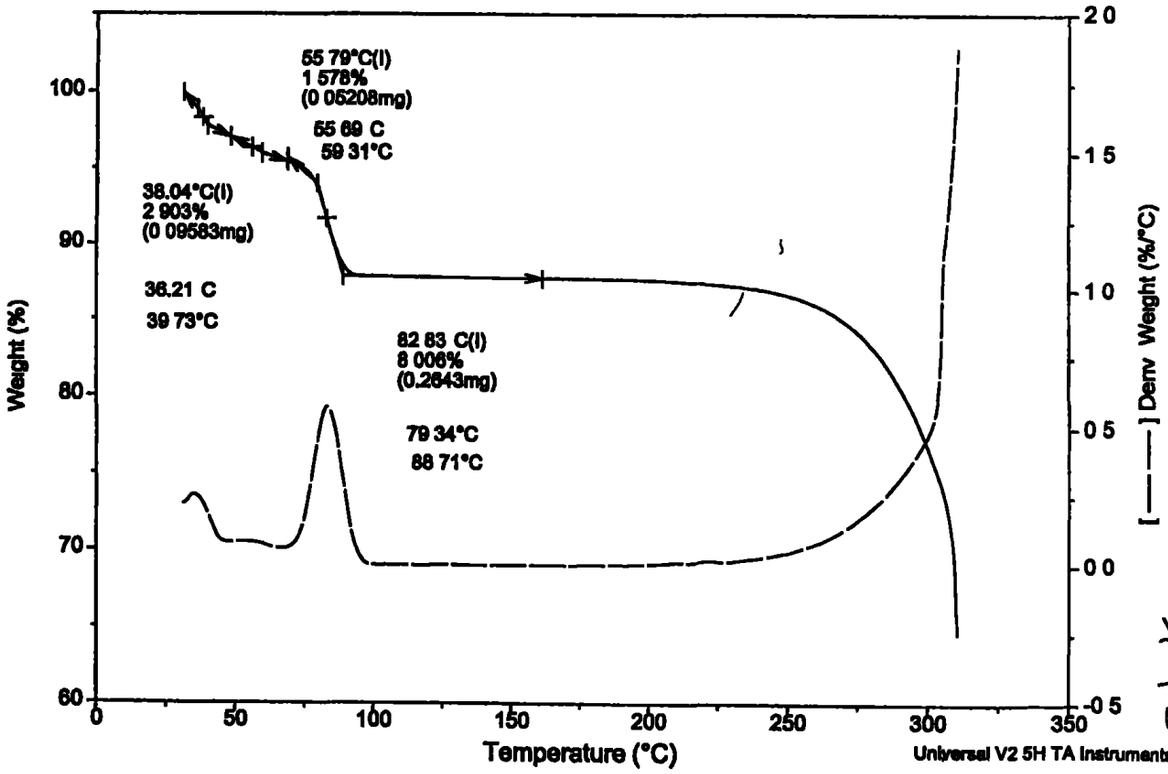


Figure 6 B

Size 13.4190 mg
Method 10 deg ramp to 300 deg

n-propyl alcohol solvate

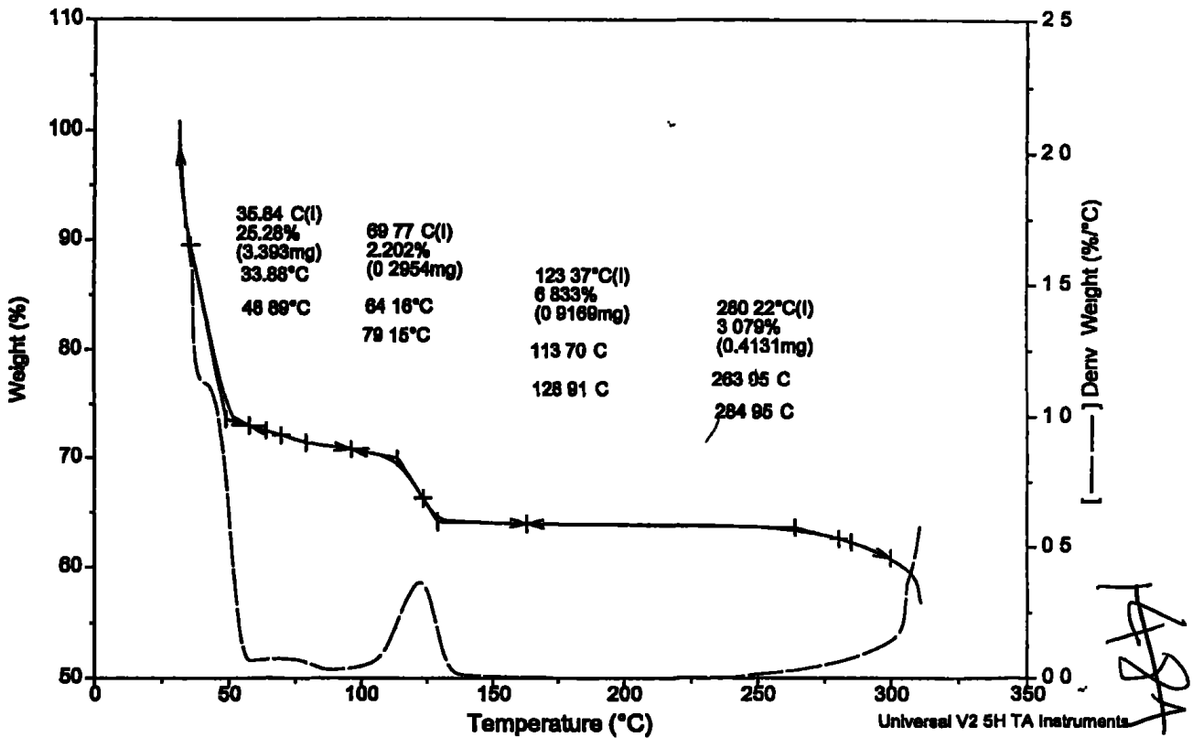
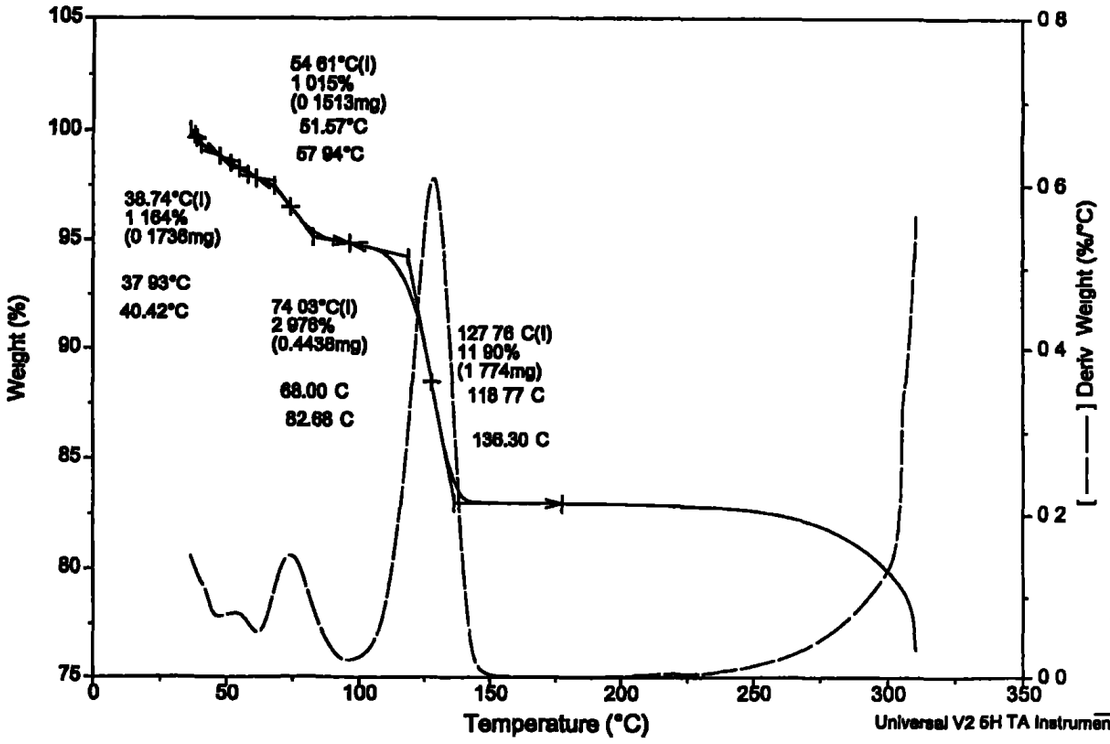


Figure 6-C

1471

Size 14 9110 mg
Method 10 deg ramp to 300 deg

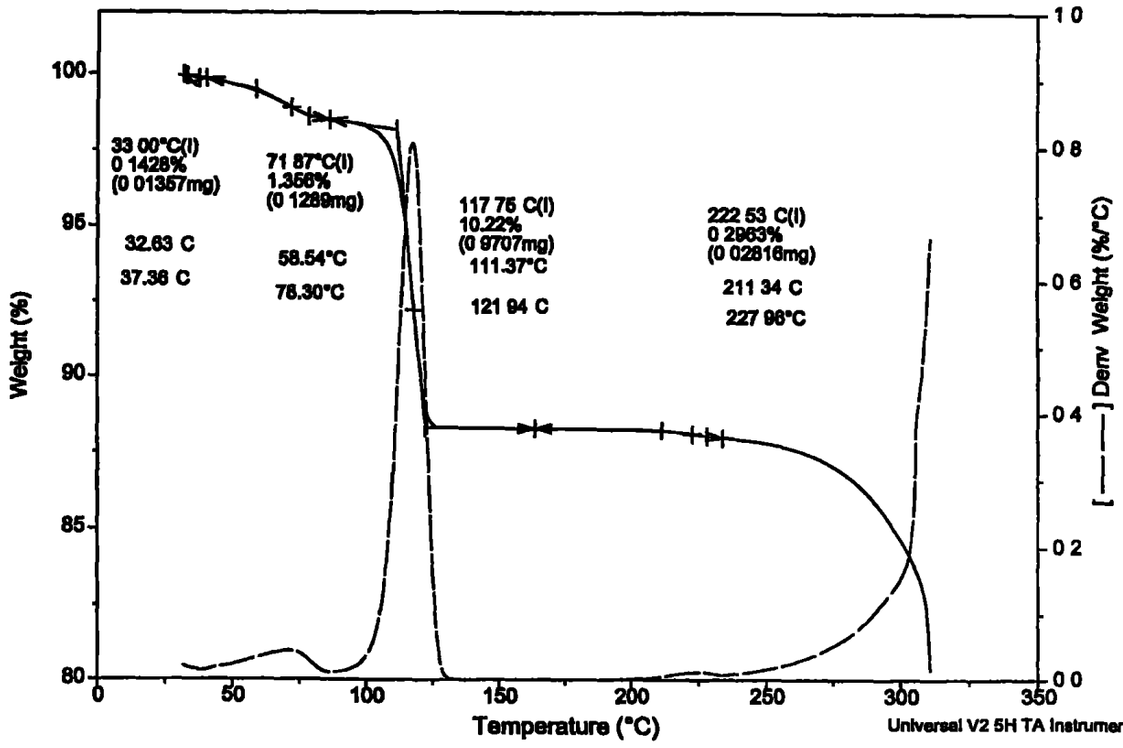
tetrahydrofuran solvate



1492

Size 9 5030 mg
Method 10 deg ramp to 300 deg

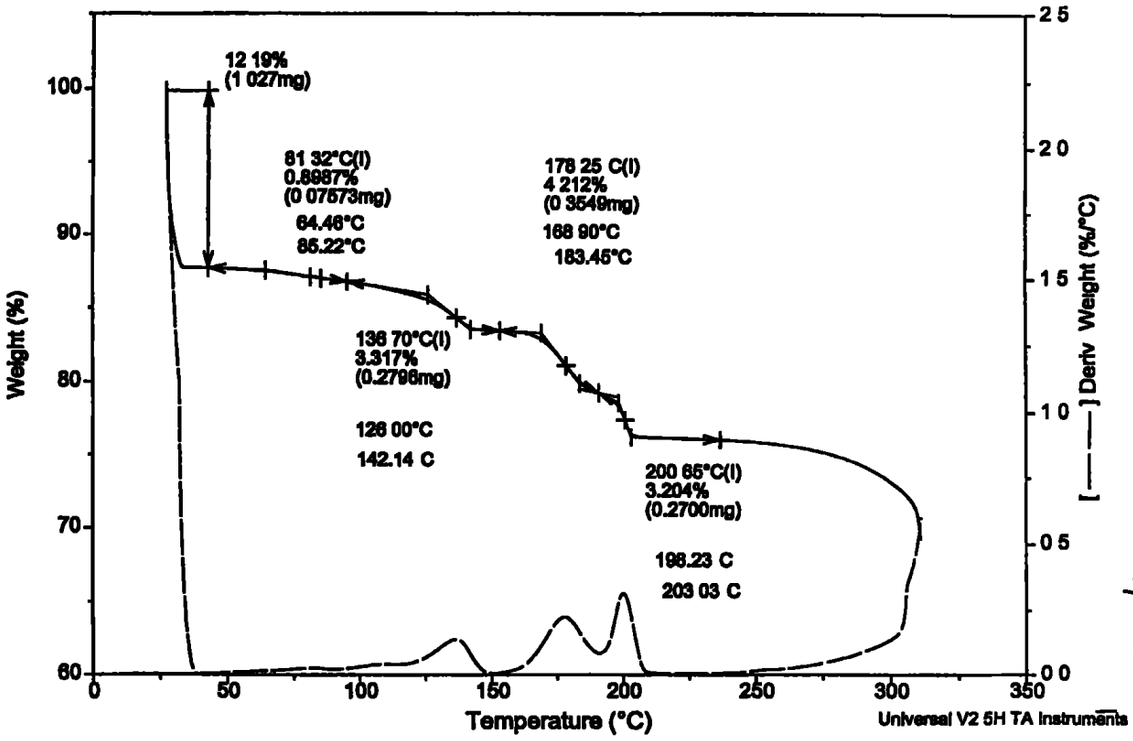
ethyl propionate solvate



1484

Size 8.4270 mg
Method 10 deg ramp to 300 deg

acetic acid solvate

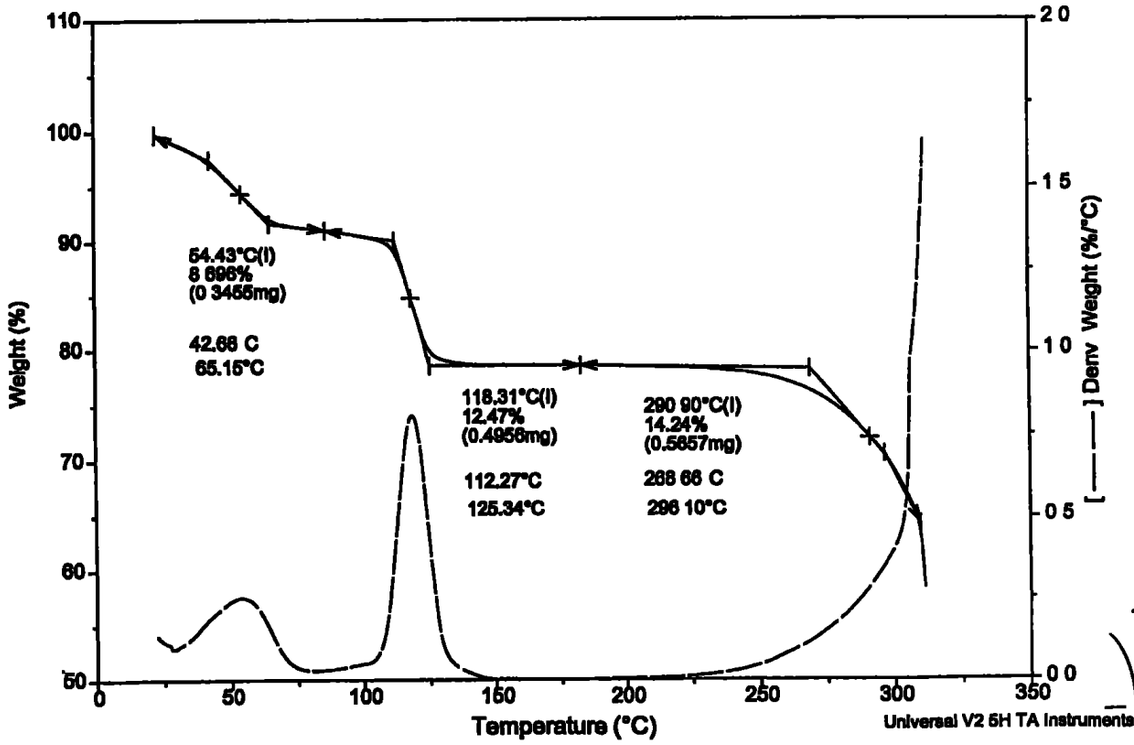


1474

Figure 6-F

Size 3.9730 mg
Method 10 deg ramp to 300 deg

chloroform solvate



Size 2 2040 mg
Method 10 deg ramp to 300 deg

acetone solvate

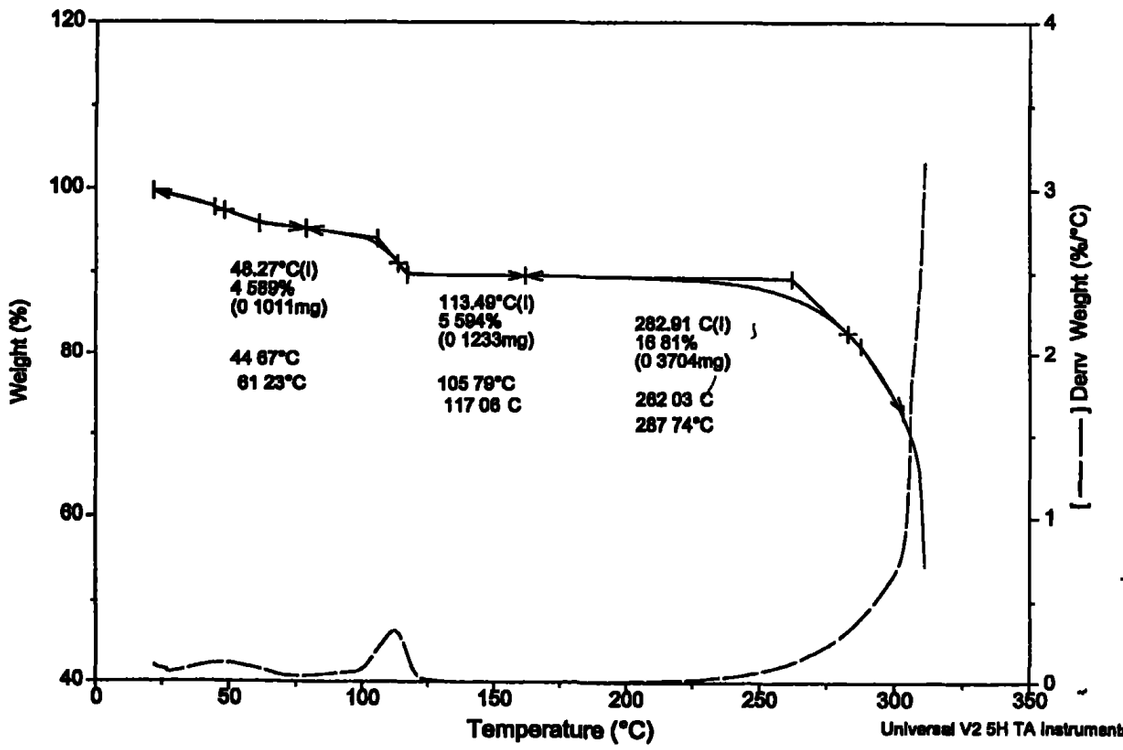


Figure [redacted]

66 027 [redacted] 69T09

Size 1 7480 mg
Method 10 deg ramp to 300 deg

toluene solvate

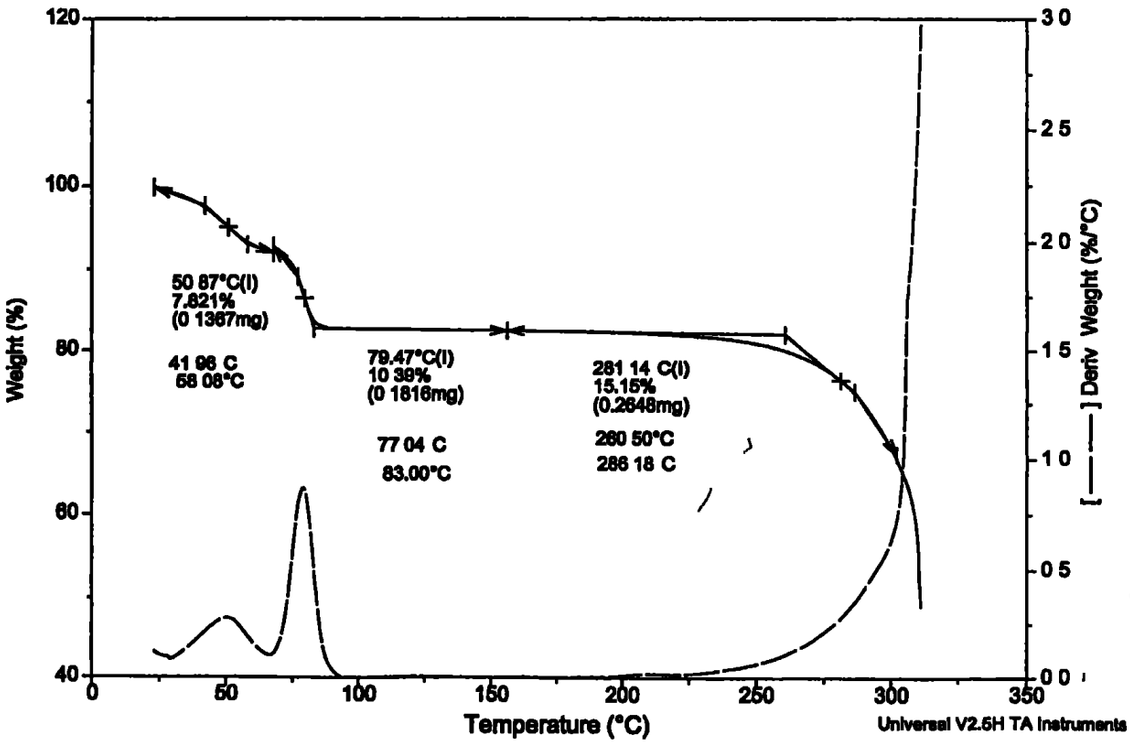
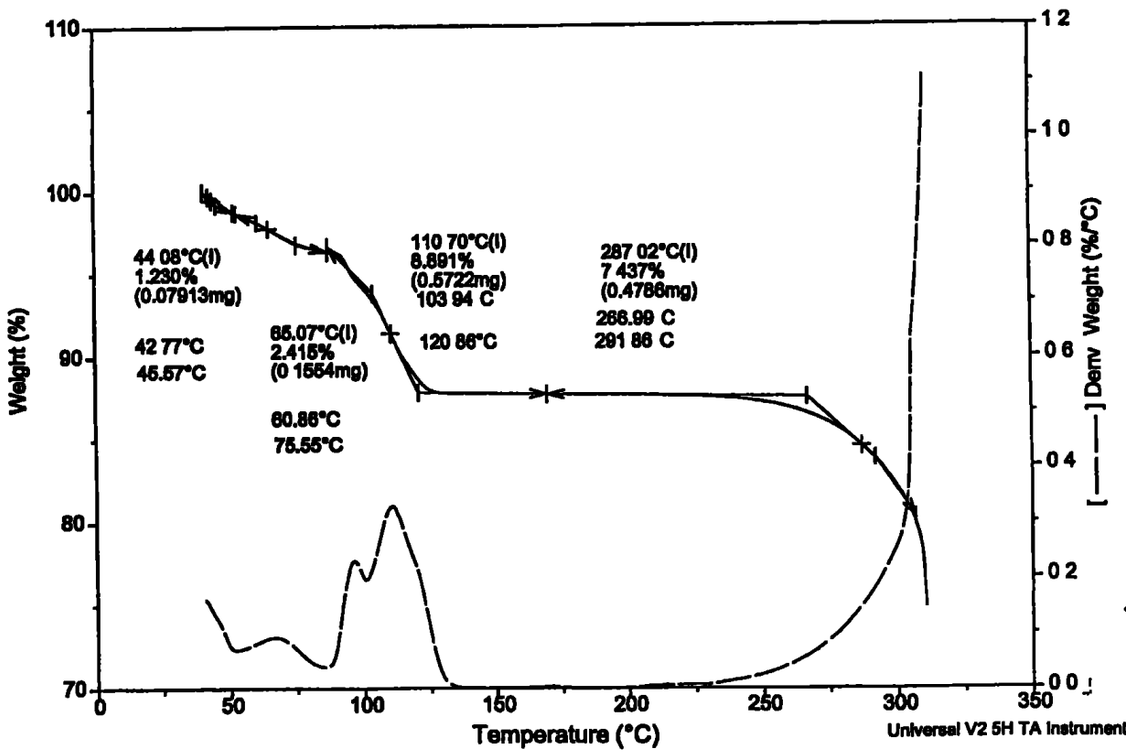


Figure 6
668021 20 6 TO

Size 6 4350 mg
Method 10 deg ramp to 300 deg

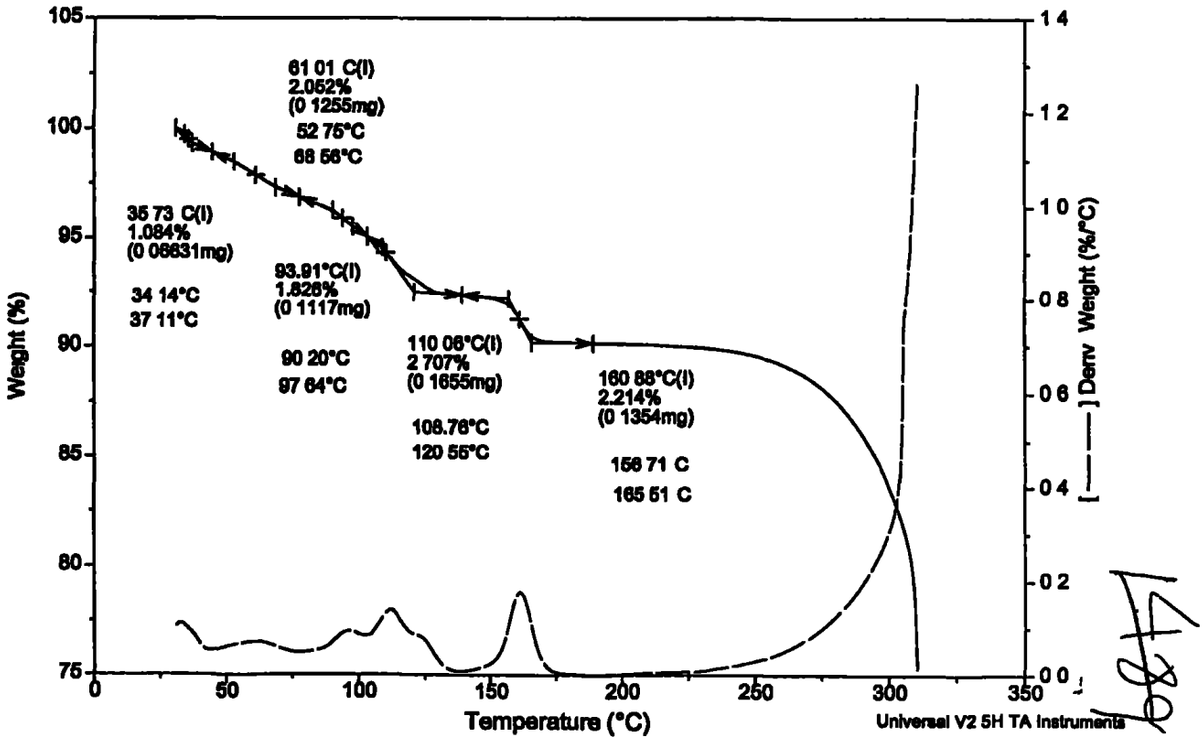
isopropanol solvate



1478

Size 6 1160 mg
Method 10 deg ramp to 300 deg

ethanol solvate



1478

Figure 6K

668027 0869709

1479

Size 4 9710 mg
Method 10 deg ramp to 300 deg

isobutyl acetate solvate

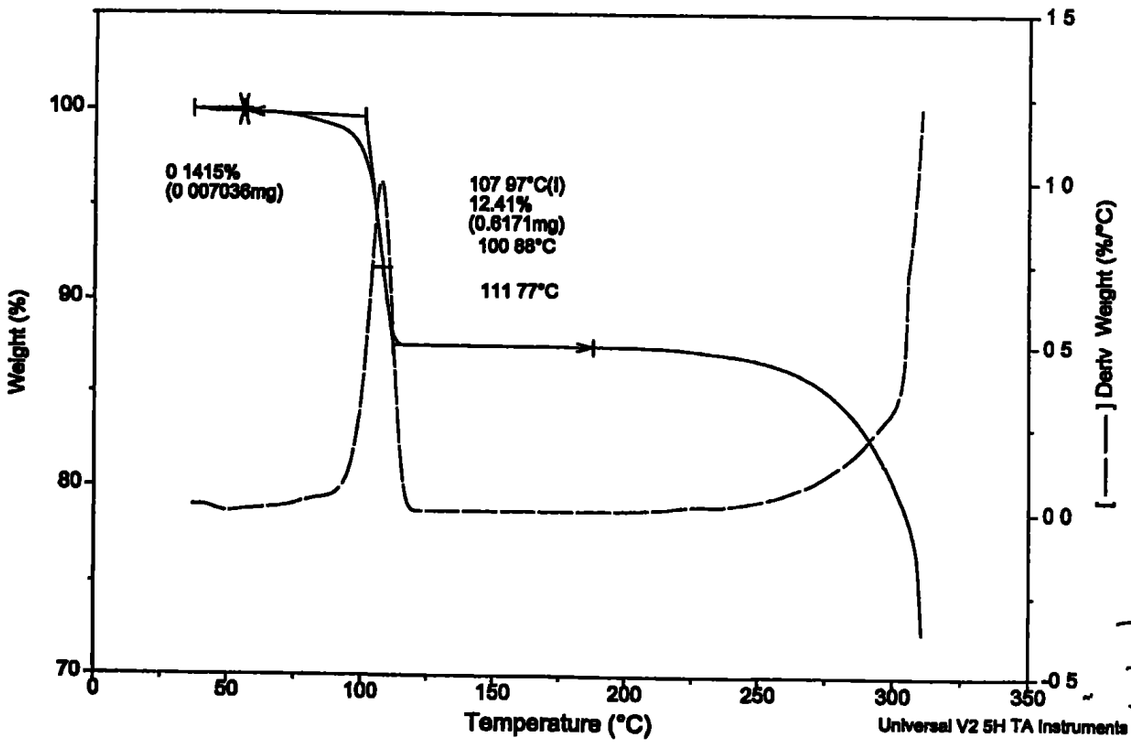


Figure 6-L

668021 20869109

1480

Size 2 3240 mg
Method 10 deg ramp to 300 deg

butyl acetate solvate

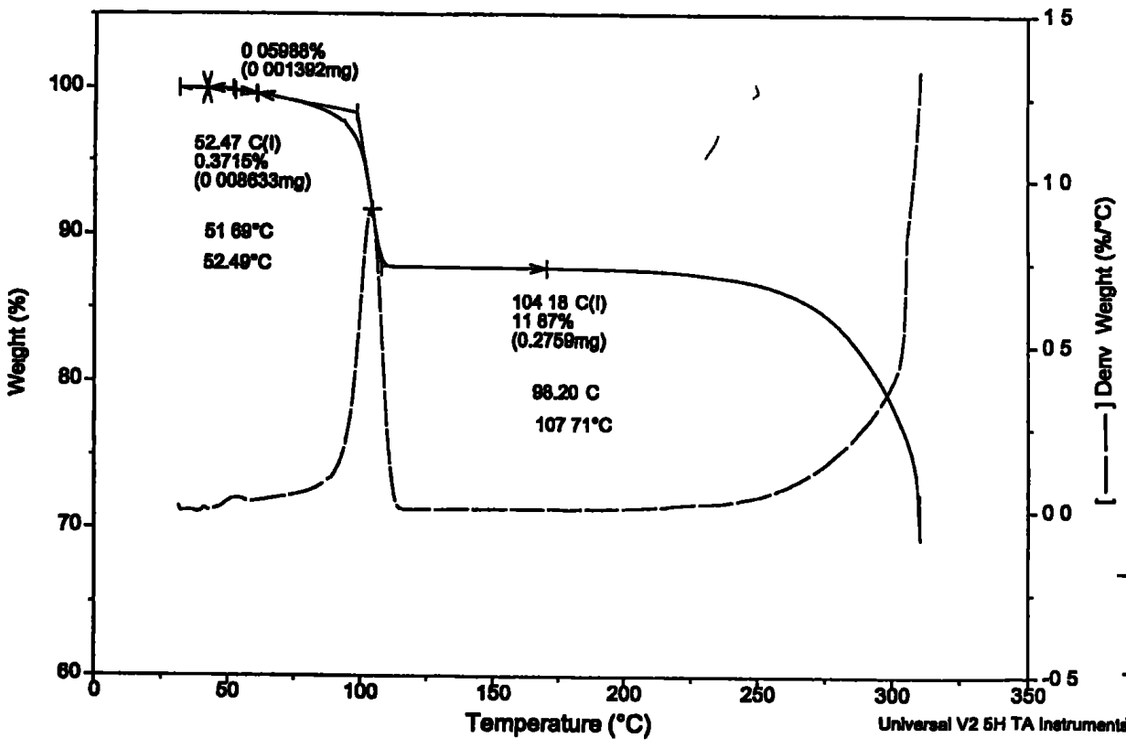


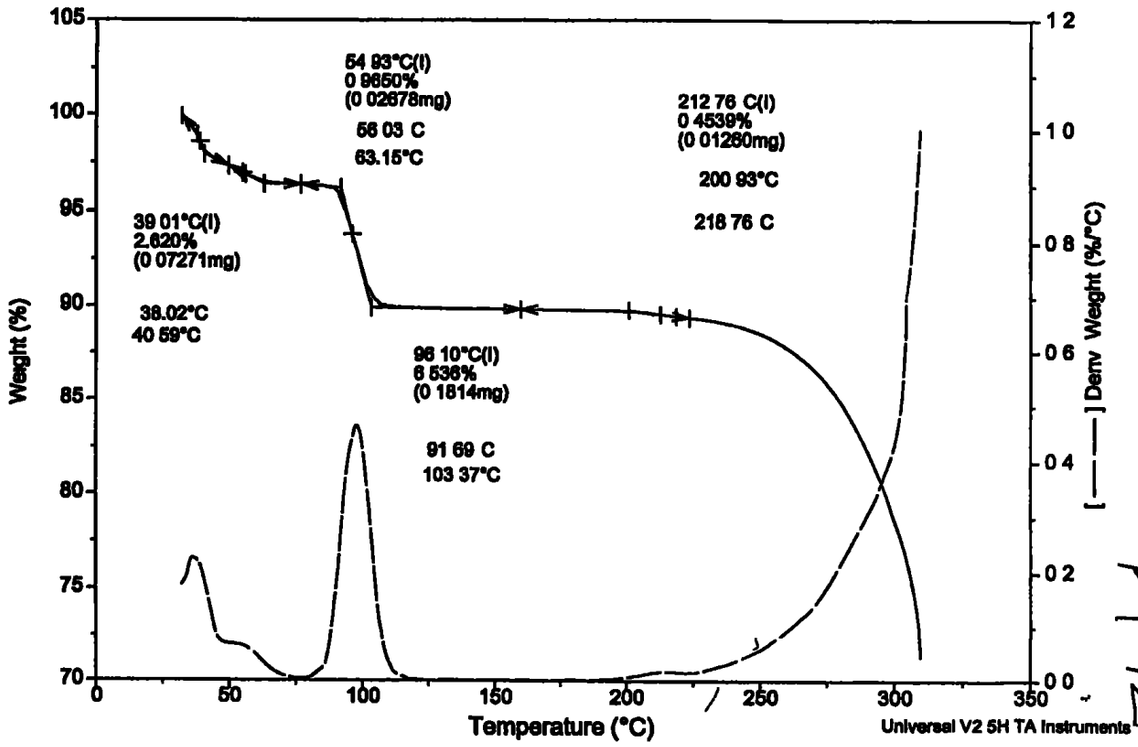
Figure 6-M

668021 0869709

1481

Size 2 7750 mg
Method 10 deg ramp to 300 deg

methyl acetate solvate



1492

Figure 6-N

Size 4 4780 mg
Method TG 10D/M TO 300

propyl acetate solvate

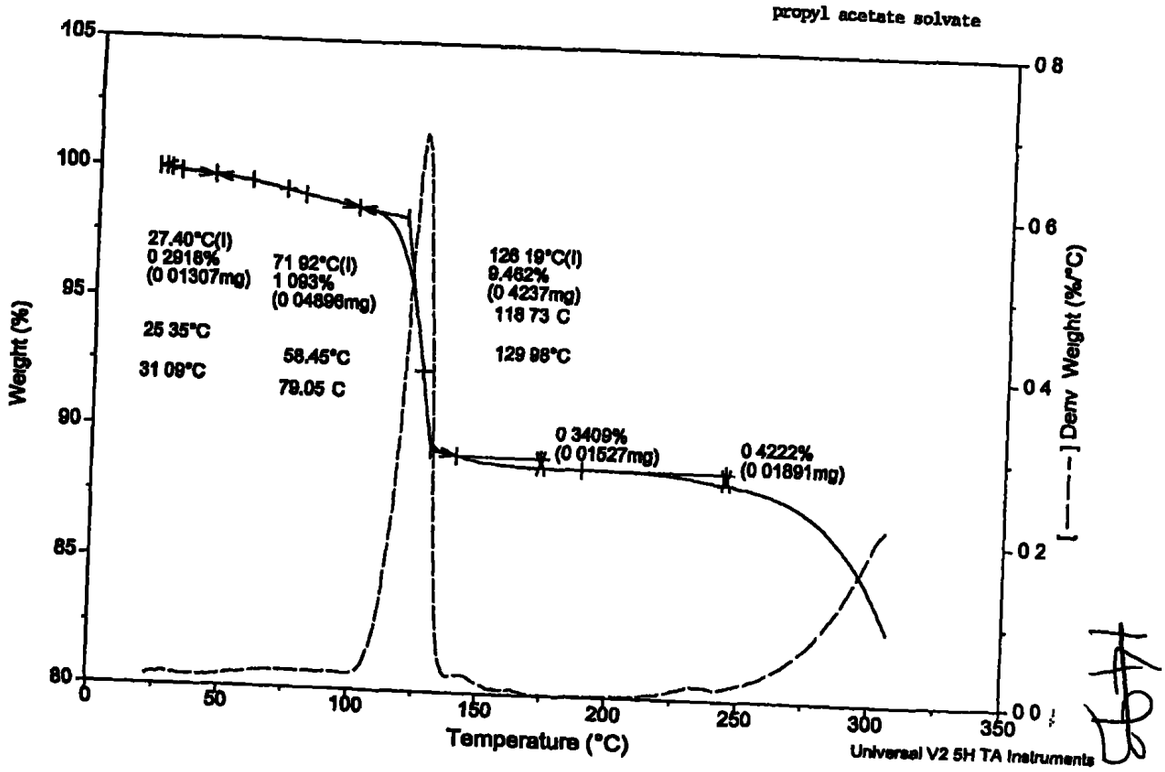


Figure 6-0

propylene glycol solvate

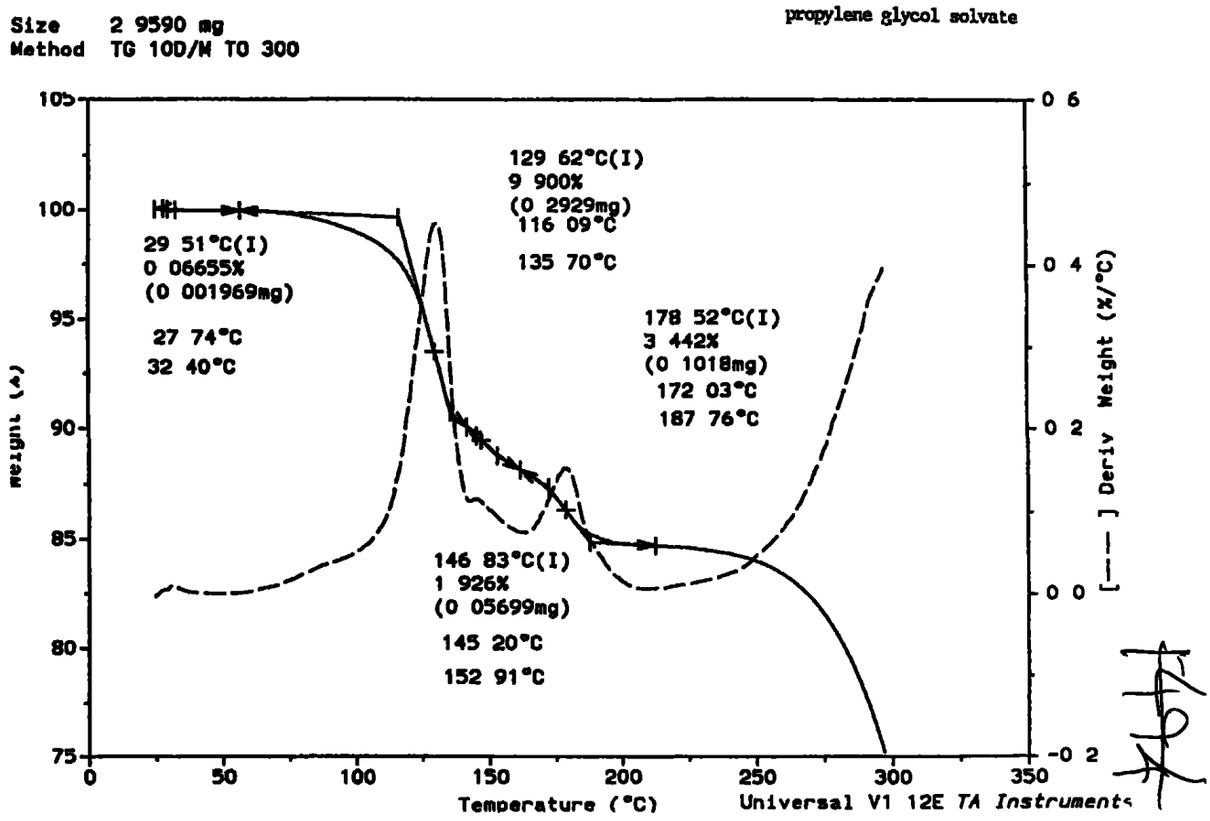


Figure 6-P

n-butanol solvate

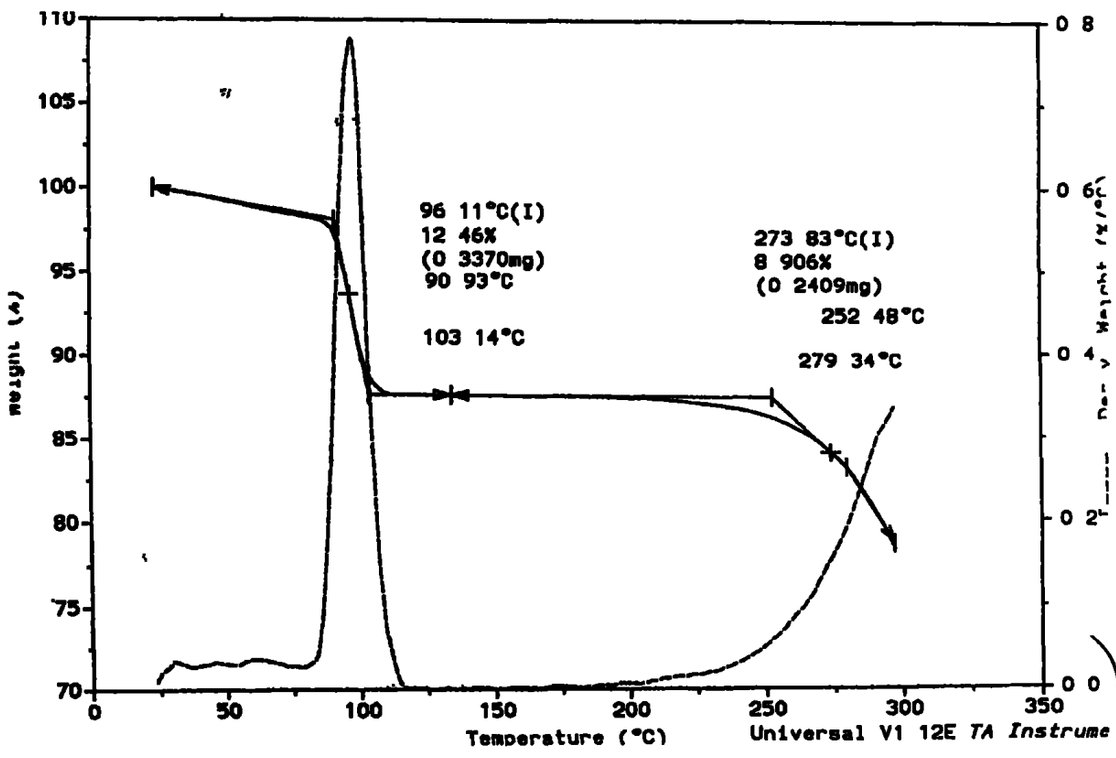
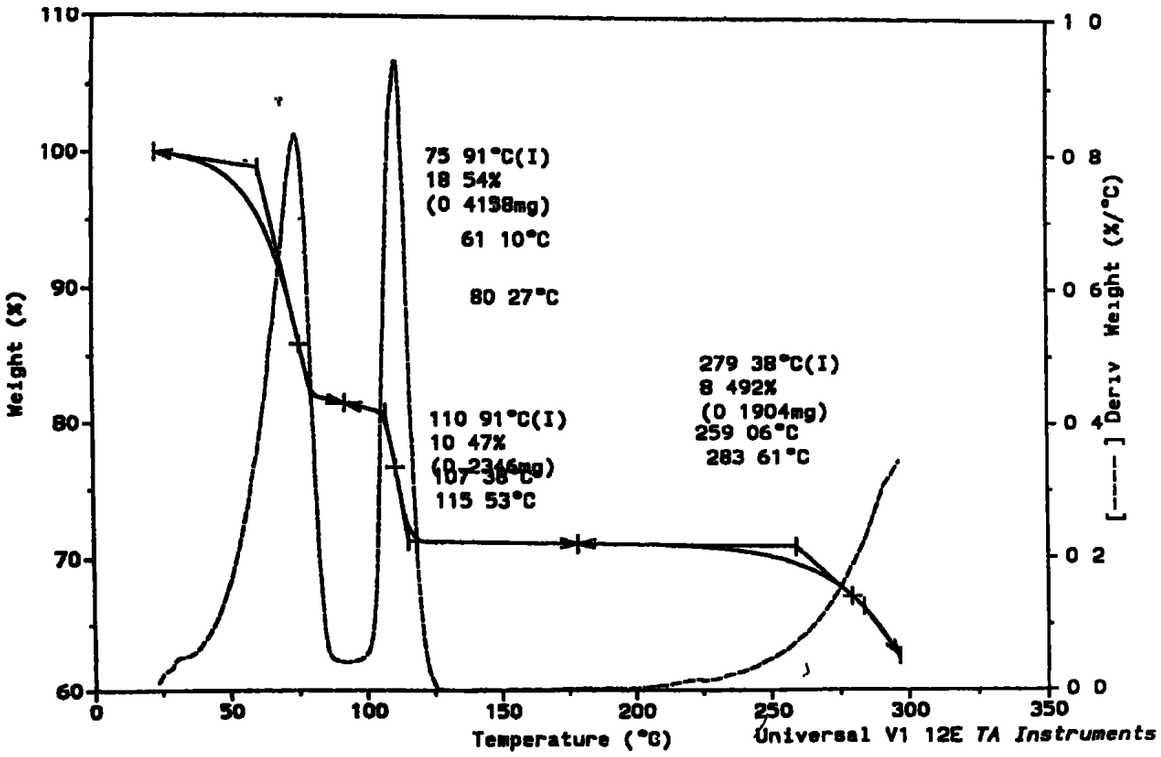


Figure
668021 2086910

1485

Size 2 2420 mg
Method TG 10D/M TO 300

n-octanol solvate



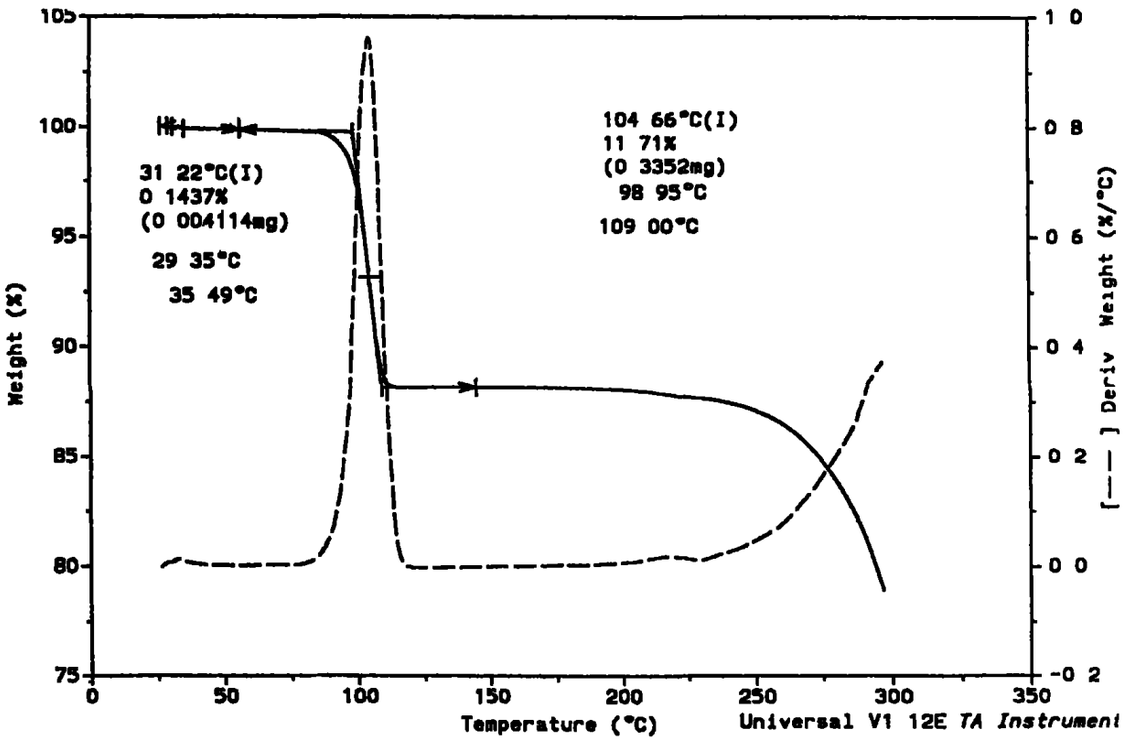
1494

Figure [redacted]

bb8021 20869109

Size 2 8630 mg
Method TG 10D/M TO 300

t-butyl acetate solvate



1487

6688021 - 1208899

Figure 7

Figure 8

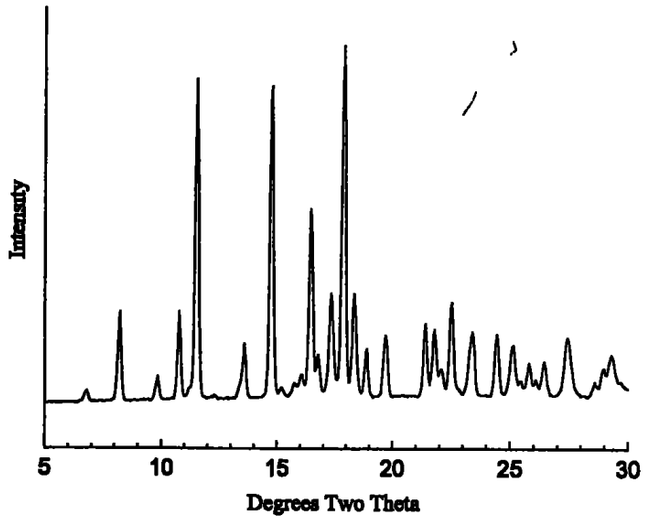


Figure 9

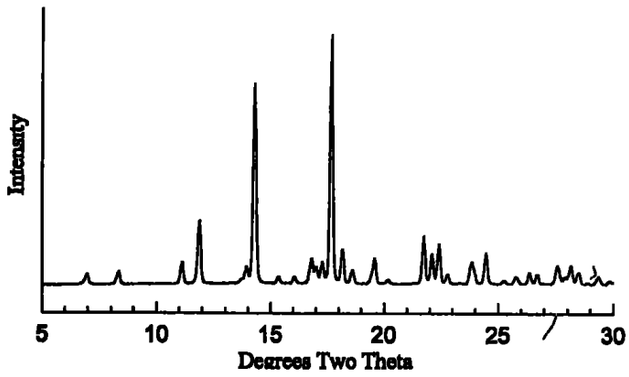


Figure 10

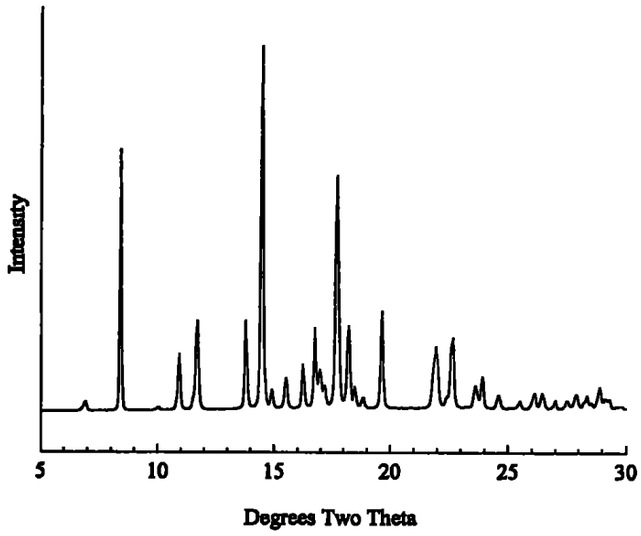


Figure 11

4502
1491

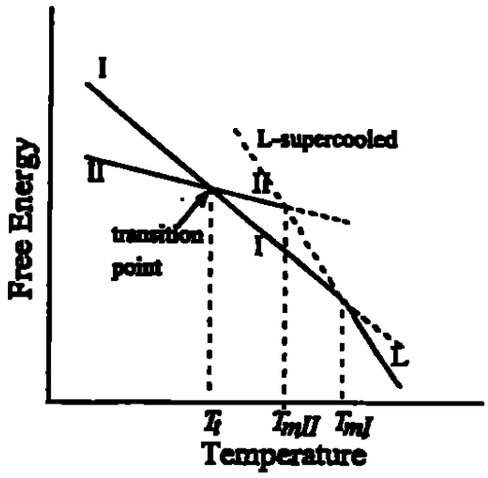


Figure 12

99 021-20869705

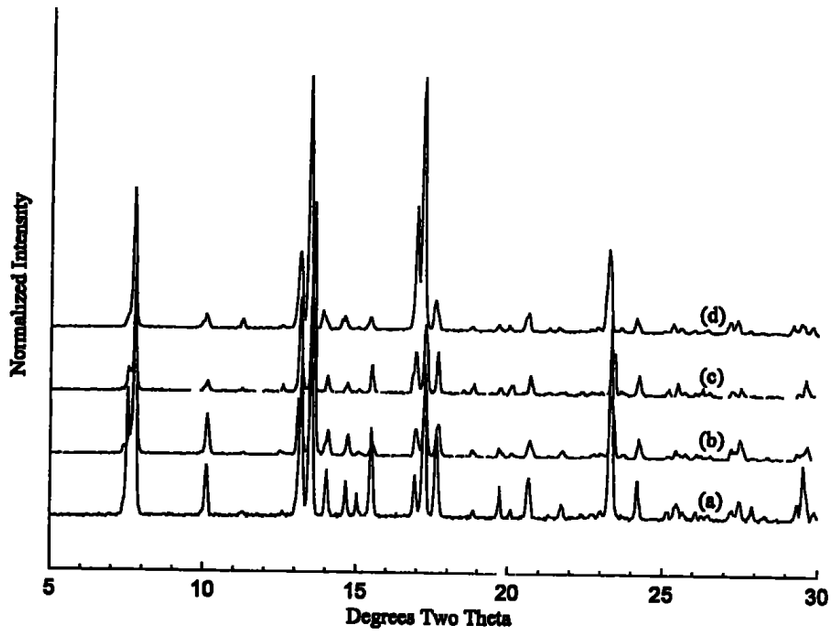


Figure 13

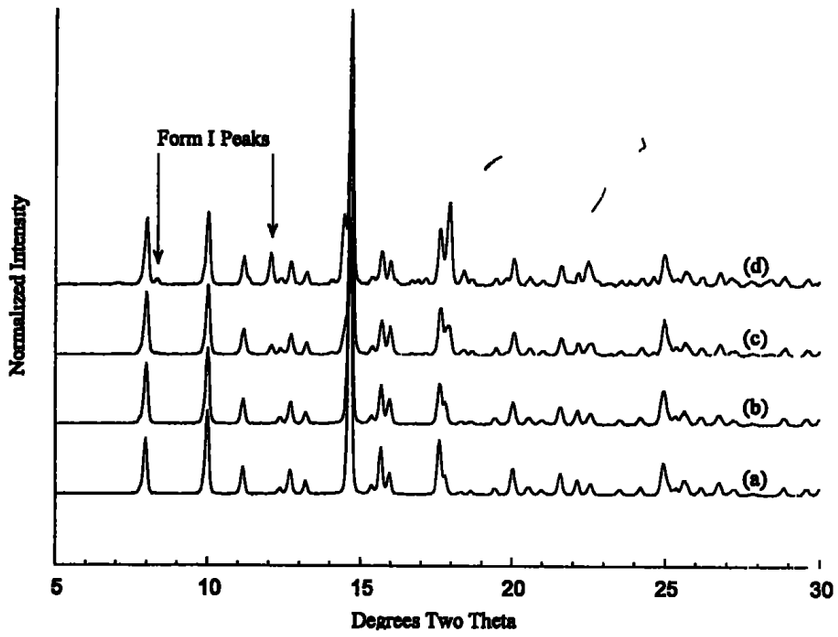

Form I Peaks
Normalized Intensity
(d)
(c)
(b)
(a)
5
10
15
20
25
30
Degrees Two Theta

Figure 14

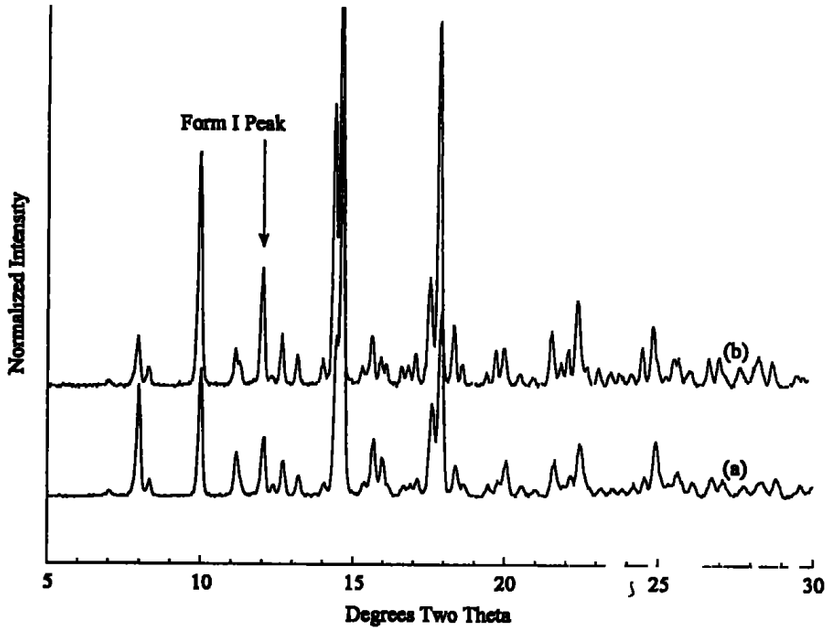

Form I Peak
Normalized Intensity
(b)
(a)
5
10
15
20
25
30
Degrees Two Theta

Figure 15

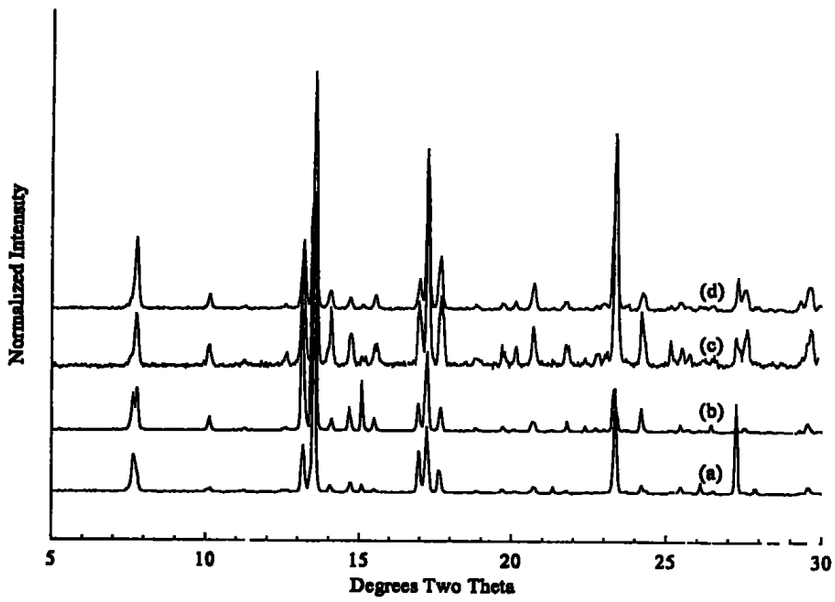


Figure 16

1495

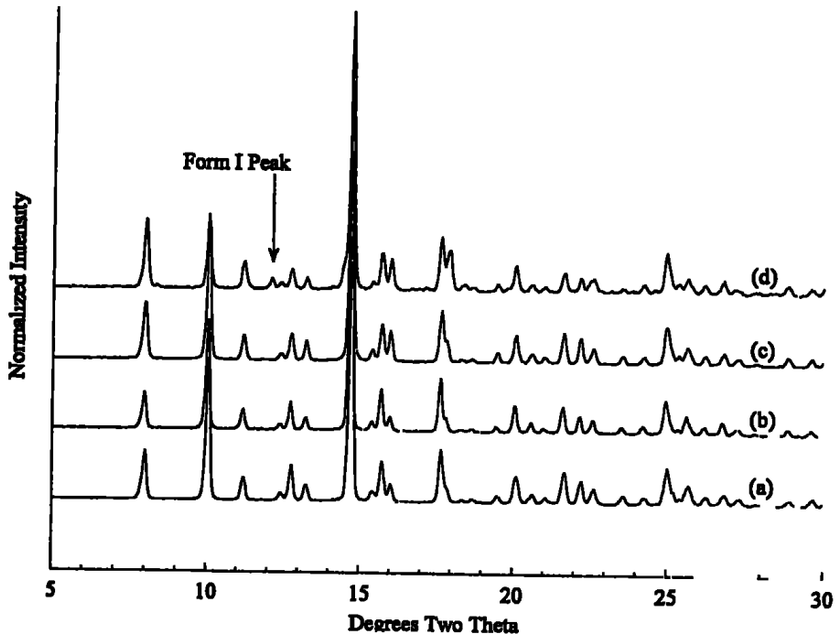

Form I Peak
Normalized Intensity
(d)
(c)
(b)
(a)
5
10
15
20
25
30
Degrees Two Theta

Figure 17

Figure 18

1508.
1497

6680210 .1208999 501

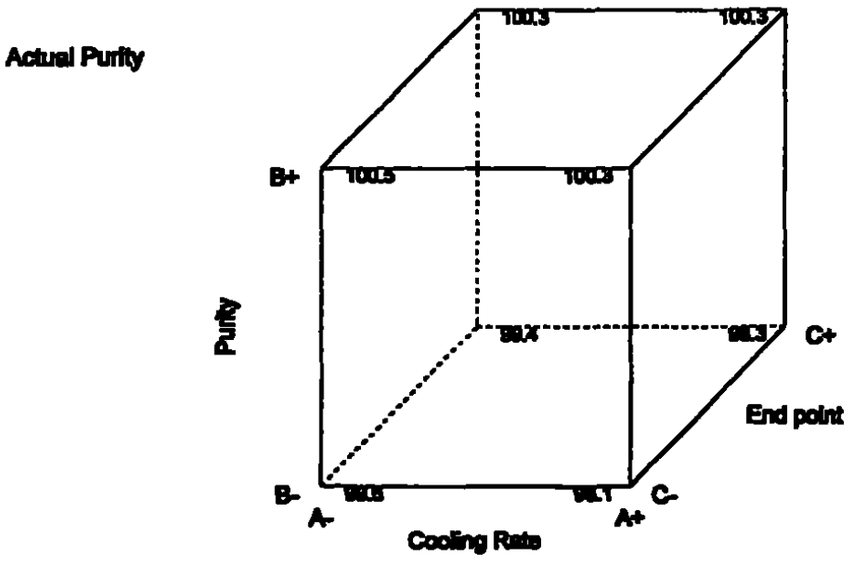


Figure 19

1509
1498

6668021 20869809

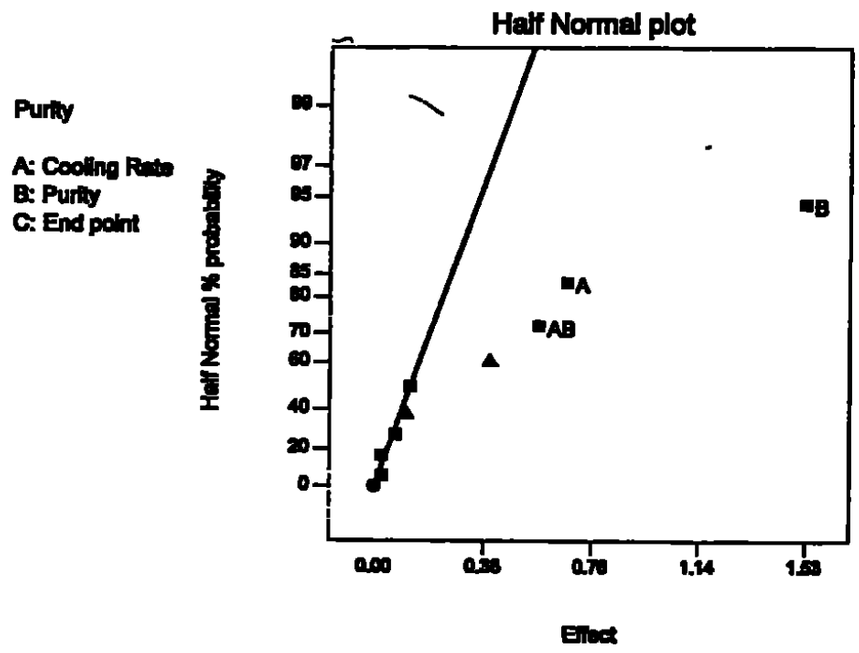


Figure 20

ISA 1499

6668027 .20899 5 16988

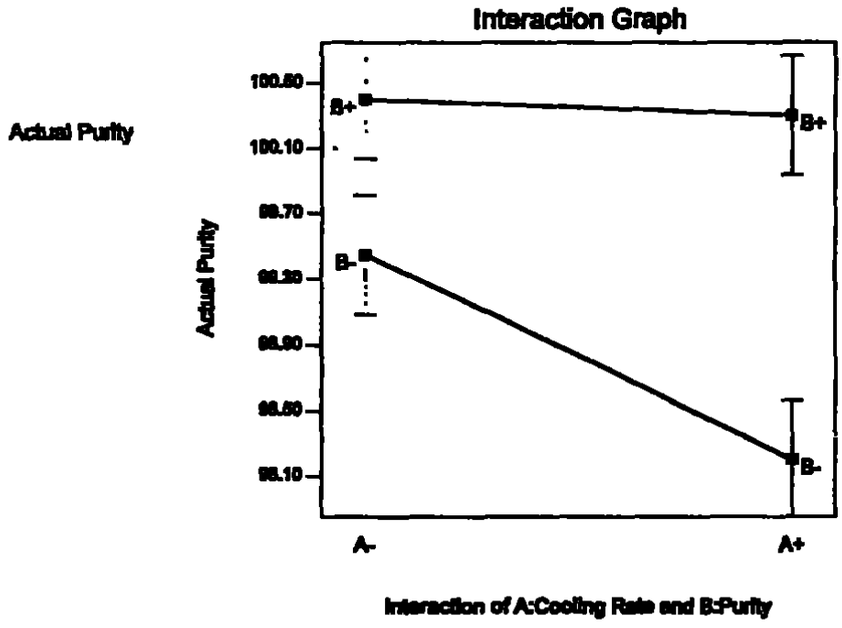
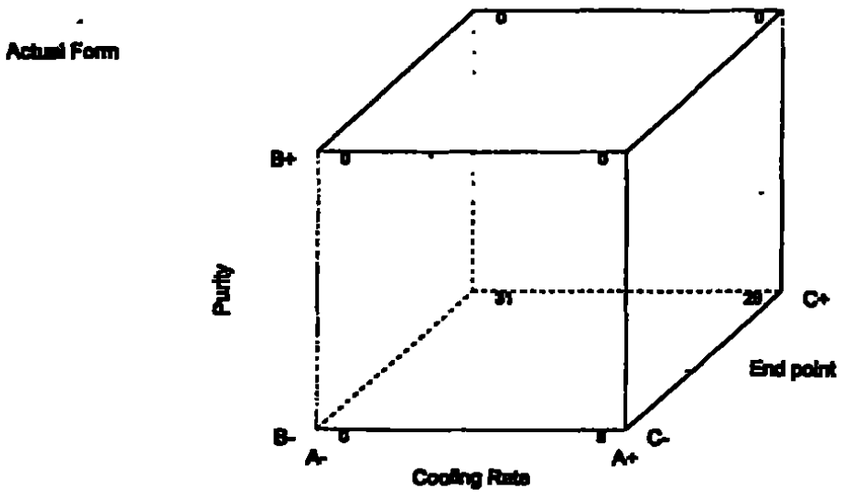


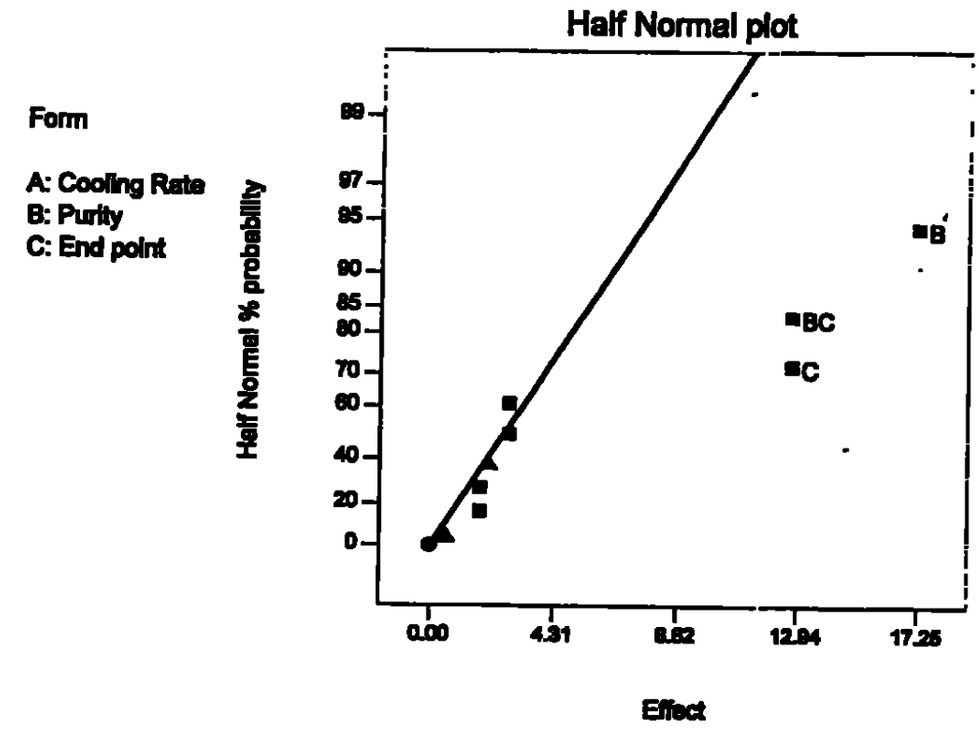
Figure 21



6680217 20869105

Figure 22

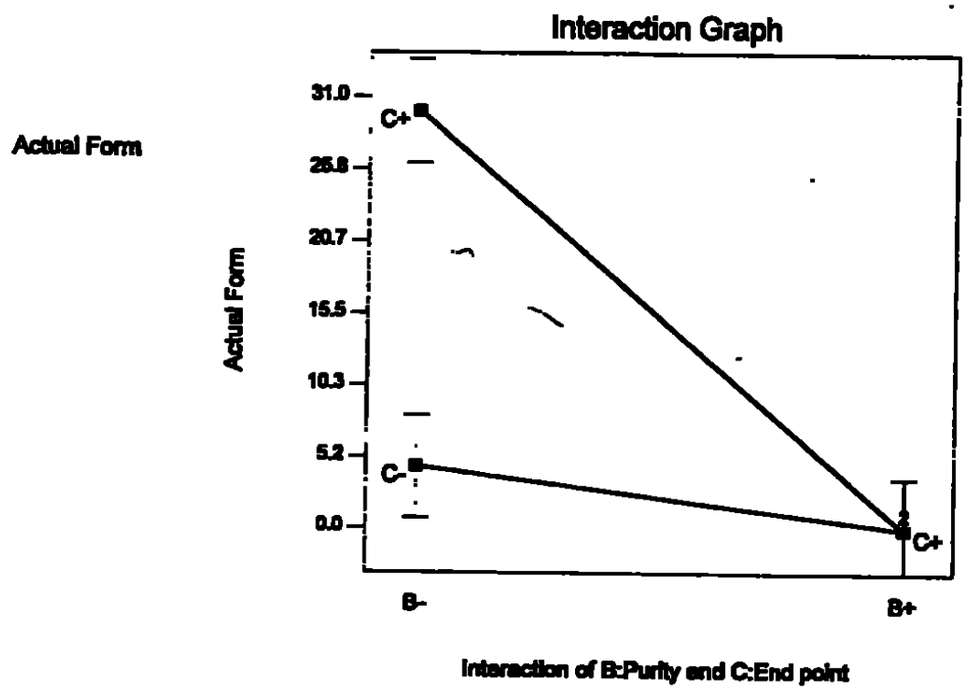
1512
1501



6680271-20869105

Figure 23

1SA3
1502



6680217.20869705

Figure 4

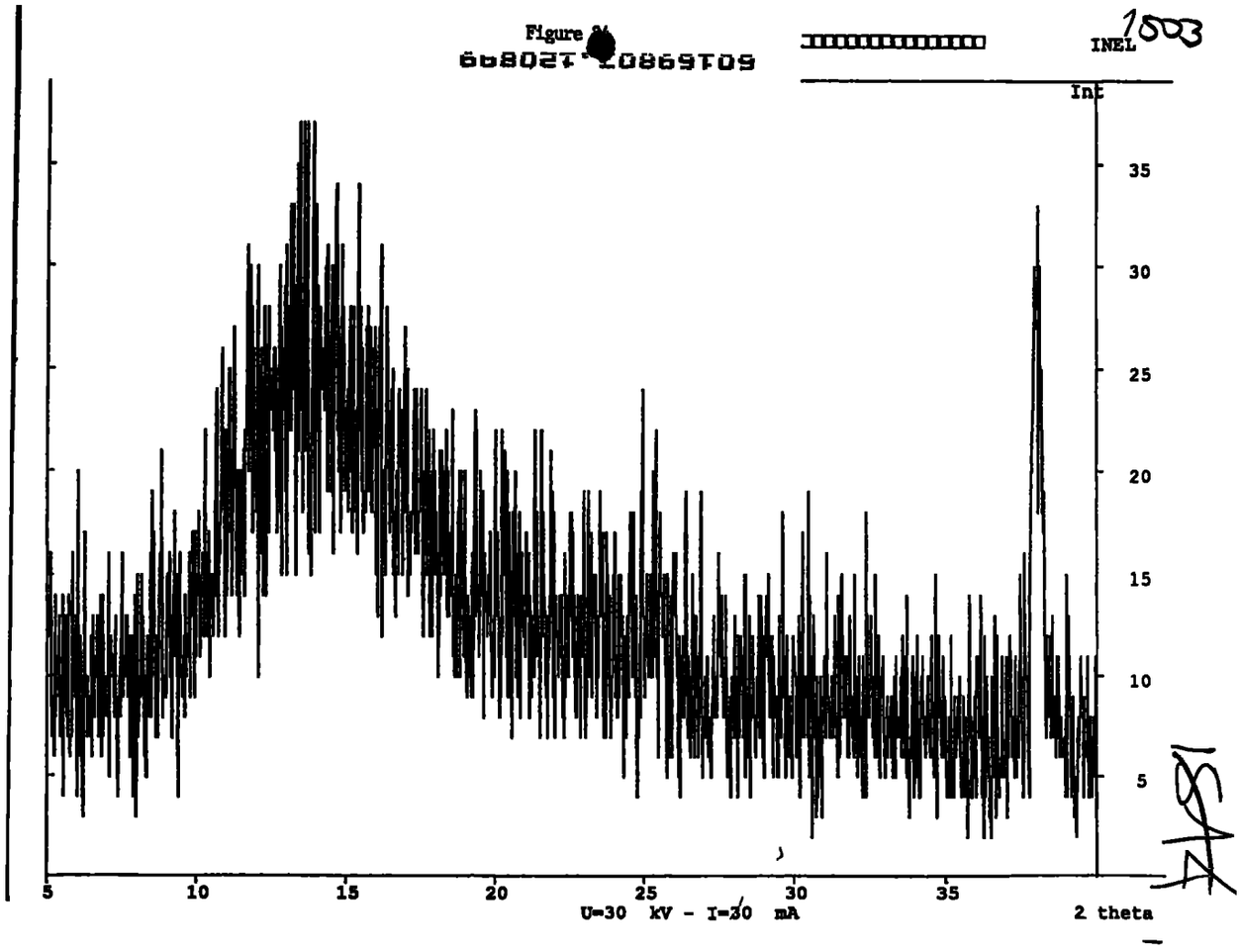
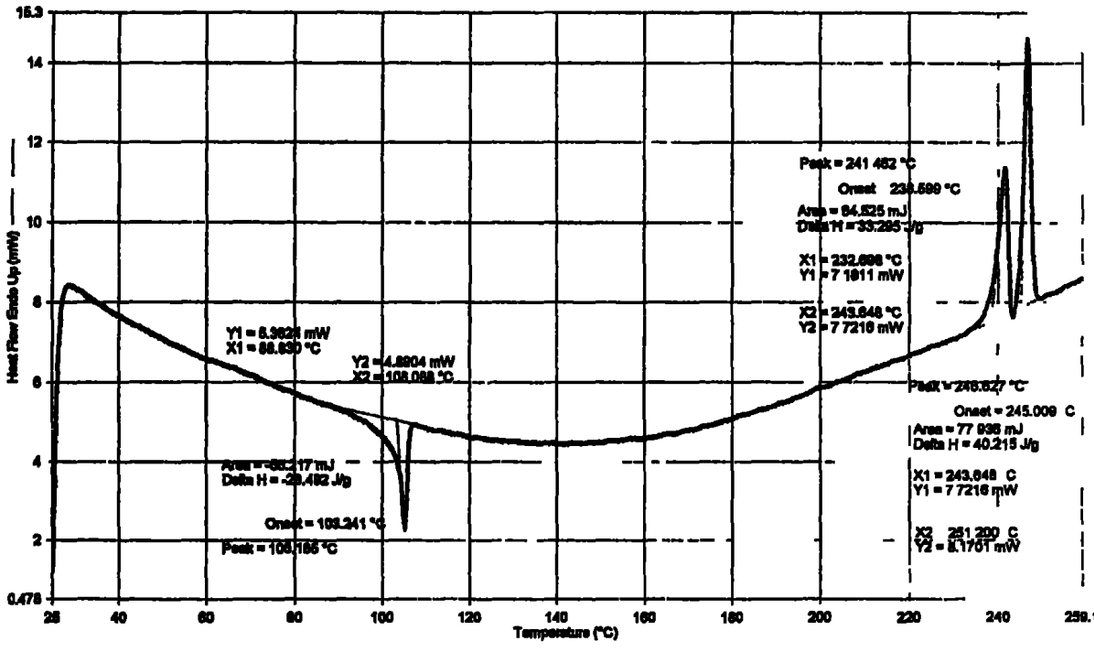


Figure 6

1504

Sample Weight: 1.936 mg
Comment: Eplerenone
Amorphous by freeze dry in water



1546
1505

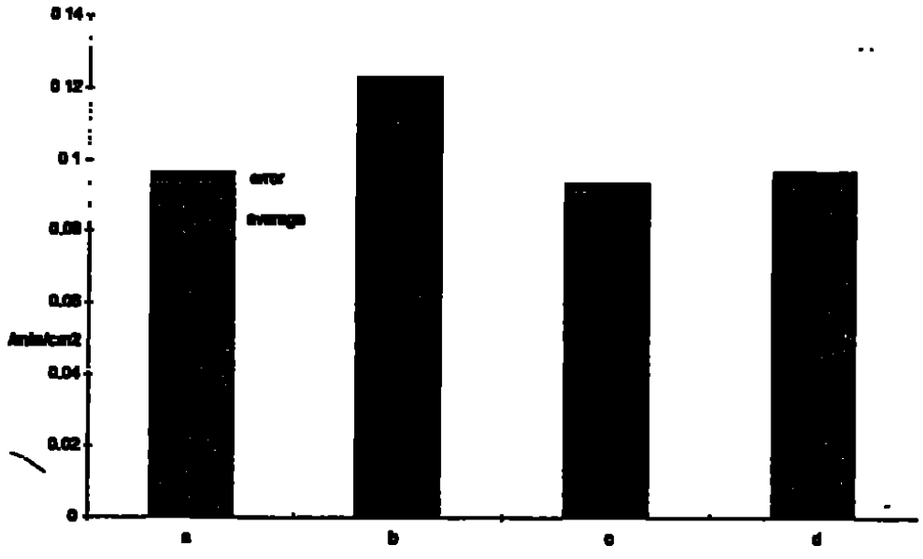


Figure 26:

6016999 07/12/0899

1517.
1506

PR 125661

LOS ESTADOS UNIDOS DE AMERICA

A TODOS AQUELLOS A QUIENES LA PRESENTE CONCIERNE:
DEPARTAMENTO DE COMERCIO DE LOS ESTADOS UNIDOS

Oficina de Patentes y Marcas de los Estados Unidos

5 de enero de 2001

LA PRESENTE ES PARA CERTIFICAR QUE LA ANEXA A ESTA ES UNA COPIA FIEL DE LOS REGISTROS DE LA OFICINA DE PATENTES Y MARCAS DE LOS ESTADOS UNIDOS DE LOS DOCUMENTOS DE LA SOLICITUD DE PATENTE IDENTIFICADA MAS ADELANTE QUE SATISFACEN LOS REQUISITOS PARA QUE SE OTORGUE UNA FECHA DE PRIORIDAD BAJO 35 USC 111..

SOLICITUD NUMERO: 60/169,807

FECHA DE PRESENTACION: ***8 de diciembre de 1999***

Por la Autoridad del
COMISIONADO DE PATENTES Y MARCAS

Firmado por
H. PHILLIPS

Funcionario Certificador

REIVINDICACIONES

1.Una forma cristalina de eplerenona que comprende un solvente, donde dicha forma no comprende metilen cloruro, etil éter o isopropanol.

2.La forma de la reivindicación 1 donde dicha forma no comprende metilen cloruro, etil éter, isopropanol, metil etil cetona, o etanol.

3.La forma de la reivindicación 1 donde dicha forma comprende un solvente seleccionada del grupo que consiste de metiletilcetona, 2-pentanona, ácido acético, acetona, butil acetato, cloroformo, etanol, isobutanol, isobutil acetato, metil acetato, etil propionato, n-butanol, n-octanol, n-propanol, propil acetato, propilen glicol, t-butanol, tetrahidrofurano, tolueno, y t-butil acetato.

4.La forma de la reivindicación 1 donde dicha forma comprende un solvente seleccionado del grupo que consiste de 2-pentanona, ácido acético, acetona, butil acetato, cloroformo, etanol, isobutanol, isovutil acetato, metil acetato, etil propionato, n-butanol, n-octanol, n-propanol, propil acetato, propilen glicol, t-butanol, tetrahidrofurano, tolueno, y t-butil acetato.

5.La forma de la reivindicación 1 donde dicha forma comprende etanol.

6.La forma de la reivindicación 1 donde dicha forma comprende metiletil cetona.

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7.La forma de la reivindicación 6 la cual tiene un patrón de difracción de polvos en rayos X con un pico de 13.6 ± 0.2 grados dos theta.

8.La forma de la reivindicación 6 la cual tiene un patrón de difracción de polvos en rayos X con picos de 7.6 ± 0.2 y 13.6 ± 0.2 grados dos theta.

9.La forma de la reivindicación 6 la cual tiene un patrón de difracción substancialmente al mostrado en la figura 1-C.

10. La forma de la reivindicación 6 que tiene un sistema cristalino ortorómbico.

11.La forma de la reivindicación 6 que tiene un espacio de grupo $P2_12_12_1$.

12.La forma de la reivindicación 6 que tiene valores de unidad de celda a, b y c de 23.53 Å, 8.16 Å y 13.08 Å, respectivamente.

13.La forma de la reivindicación 6 que tiene valores de unidad de celda α,β y γ cada uno de 90°.

14.Partículas de la forma de la reivindicación 1 donde al menos 90% de dichas partículas tienen un tamaño de partícula menor de 400 micras.

15.Partículas de la forma de la reivindicación 1 donde al menos 90% de dichas partículas tiene un tamaño de partícula menor de 200 micras.

16. Las partículas de la reivindicación 14 donde al menos 90% de dichas partículas tienen un tamaño de partículas desde 25 hasta 400 micras.

17. Las partículas de la reivindicación 14 donde al menos el 90% de dichas partículas tienen un tamaño de partícula desde 50 hasta 15 micras.

18.Las partículas de la reivindicación 14 donde al menos 90% de dichas partículas tienen un tamaño de partícula desde 40 hasta 100 micras.

19.Las partículas de la reivindicación 14 donde al menos 90% de dichas partículas tienen un tamaño de partícula desde 30 a 50 micras.

20.Eplerenona que comprende la forma cristalina de cualquiera de las reivindicaciones 1 a 6, donde dicha eplerenona tiene una fase de pureza de al menos el 50%.

21.La eplerenona de acuerdo a la reivindicación 20 donde dicha eplerenona tiene una fase de pureza de al menos 90%.

22.La eplerenona de acuerdo a la reivindicación 20 donde dicha eplerenona es sustancialmente una forma de base pura.

23.la composición farmacéutica que comprende una cantidad terapéuticamente efectiva de la eplerenona de acuerdo a cualquiera de

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las reivindicaciones 1 a 22 en asociación con al menos un portador, adyuvante o diluyente farmacéuticamente aceptable. 1510

24.La composición de la reivindicación 23 donde la única forma cristalina de eplerenona presente en la composición es la forma de acuerdo a cualquiera de las reivindicaciones 1 a 6.

25.El método para tratar o prevenir una condición o desorden mediada por aldosterona en un sujeto, dicho método comprende administrar a un sujeto que tenga o sea susceptible a dicha condición o desorden una cantidad terapéuticamente efectiva de eplerenona de acuerdo a cualquiera de las reivindicaciones 1 a 22.

26.El método de la reivindicación 25 a ser usada en el tratamiento de hiperaldosteronismo.

27.El método de la reivindicación 25 para ser usado en el tratamiento de hipertensión.

28.El método de acuerdo a la reivindicación 25 para ser usada en el tratamiento de fallas de corazón.

29.El método de acuerdo a la reivindicación 25 para ser usada en el tratamiento de cirrosis del hígado.

30.El método de acuerdo a la reivindicación 25 para ser usada en el tratamiento de una condición seleccionada del grupo consistente de

exceso de colágeno / fibrosis, hipertrofia benigna de próstata, y depresión.

31.Un método para preparar eplerenona que comprende la forma cristalina de acuerdo a cualquiera de las reivindicaciones 1 a 6, el método comprende la cristalización de la eplerenona de un solvente farmacéuticamente aceptable o mezcla de solventes para obtener dicha forma.

32.El método de la reivindicación 31 donde el solvente o mezcla de solventes comprende un solvente seleccionado de un grupo consistente de metiletil cetona, 2-pentanona, ácido acético, acetona, butil acetato, cloroformo, etanol, isobutanol, isobutil acetato, metil acetato, etil propionato, n-butanol, n-octanol, n-propanol, propil acetato, propilen glicol, t-butanol, tetrahidrofurano, tolueno, y t-butil acetato.

33.El método de acuerdo a la reivindicación 31 donde el solvente o la mezcla de solventes comprenden un solvente seleccionado de un grupo consistente de 2-pentanona, ácido acético, acetona, butil acetato, cloroformo, isobutanol, isobutil acetato, , metil acetato, etil propionato, n-butanol, n-octanol, n-propanol, propil acetato, propilen glicol, t-butanol, tetrahidrofurano, tolueno y t-butil acetato.

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34.El método de la reivindicación 31 donde el solvente o la mezcla de solventes comprenden un solvente seleccionado de un grupo que consiste de metiletil cetona y etanol.

35.El método de la reivindicación 31 donde la eplerenona usada en la cristalización tiene un pureza química de al menos 99%.

36.El método de la reivindicación 31 donde la cristalización es llevada a cabo en la presencia de un promotor de crecimiento de Forma II o un inhibidor de crecimiento de Forma I.

37.El método de la reivindicación 31 donde la cristalización es llevada a cabo en la presencia de un promotor de crecimiento de Forma I o un inhibidor de crecimiento de Forma II.

38.El método de la reivindicación 31 donde la cristalización es llevada a cabo en presencia del promotor de crecimiento de Forma I que tiene una única estructura cristalina sustancialmente idéntica a la única estructura cristalina de la forma de la reivindicación 1.

39.El método de la reivindicación 31 donde la cristalización es llevada a cabo en la presencia del promotor de crecimiento teniendo un patrón de difracción en rayos X, sustancialmente idéntico al patrón de difracción de la forma de la reivindicación 1.

40.El método de la reivindicación 31 donde la cristalización es llevada a cabo en la presencia de un compuesto seleccionado del grupo que

consiste de 7-metil hidrógeno 4α, 5α:9α, 11α-diepoxi-17 hidroxi-3-oxo-17α-pregnano-7α, 21-dicarboxilato, γ-lactona; 7-metil hidrógeno 11α,12α-epoxi-17-hidroxi-3-oxo-17-α-pregn-4-ene-7α, 21-dicarboxilato, γ lactona; y 7-metil hidrógeno 17-hidroxy-3-oxo-17α-pregna-4,9(11)-dien-7α,21-dicarboxilato,γ lactona.

41.El método de la reivindicación 31 donde la cristalización es llevada a cabo en la presencia de 7-metil hidrógeno 4α, 5α:9α, 11α-diepoxi-17 hidroxi-3-oxo-17α-pregnano-7α, 21-dicarboxilato, γ-lactona.

42.El método de la reivindicación 31 donde el solvente o mezcla de solventes es sembrado con una forma cristalina no solvatada de eplerenona antes de cristalizar la eplerenona.

43.El método de la reivindicación 31 donde al menos 50 por ciento en peso de la eplerenona cristalizada es dicha forma.

44.El método de la reivindicación 31 donde al menos 90 por ciento en peso de la eplerenona cristalizada es dicha forma.

45.El método de la reivindicación 31 donde al menos 99 por ciento de la eplerenona cristalizada es dicha forma.

46.El método de preparar eplererona que comprende una forma de cualquiera de las reivindicaciones 1 a 6, el método comprende la digestión de eplerenona en un solvente farmacéuticamente aceptable o mezcla de solventes para la obtención de dicha forma.

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47.El método de la reivindicación 46 donde el solvente comprende etanol, o metiletil cetona.

48.El método de la reivindicación 46 donde al menos 50 por ciento en peso de la eplerenona preparada es dicha forma.

49.El método de la reivindicación 46 donde al menos 90 por ciento de la eplerenona preparada es dicha forma.

50.El método de la reivindicación 46 donde al menos 99 por ciento de la eplerenona preparada es dicha forma.

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