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Señor

JEFE DE DIVISIÓN DE NUEVAS CREACION

Superintendencia de Industria y Comercio

E.

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 11-081403- -00008-0000

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ASUNTO: N° DE RADICACIÓN : 11-081403
TRAMITE : PATENTE DE INVENCION
ACTUACIÓN : RECURSO DE REPOSICIÓN
TOTAL DE FOLIOS : 8

REFERENCIA : SISTENSIS Y FORMAS DE SALES NOVEDOSAS DE..."

CARLOS MAYA RODRÍGUEZ, domiciliado en esta ciudad, identificado como aparece al pie de mi firma, Abogado en ejercicio y portador de la Tarjeta Profesional N° 17.402 del Consejo Superior de la Judicatura, obrando en calidad de apoderado de la sociedad **TARGACEPT, INC** según Poder que obra en el expediente de la referencia.

Encontrándome dentro del término legal comparezco a instaurar Recurso de Reposición en contra la resolución N° 43797, notificada mediante listado 31 Desfijado en Septiembre 06 de 2013, mediante la cual se niega el privilegio de Patente de Invención a la creación denominada "SINTESIS Y FORMAS DE SALES NOVEDOSAS DE (R)-5-((E)-2(PIRROLIDIN-3-ILVINIL)PIRIMIDINA". tramitada bajo el expediente citado en la referencia.

FUNDAMENTOS DE HECHO:

1°) La citada Resolución 43797 cita en sus considerandos primero a quinto los antecedentes del trámite y lo dispuesto en parte del artículo 14 de la Decisión 486 de la Comisión de la Comunidad Andina.

2°) En el considerando sexto concluye que "las reivindicaciones 1 a 13 no cumplen con el requisito de novedad y nivel inventivo" y continua con consideraciones al respecto.



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3°) Dichas consideraciones incluyen:

1. Objeto de la invención, que incluye un resumen de la solicitud.
2. Determinación del estado de la Técnica, donde se citan los documentos D1, D2 y D3, afirmando que estos se relacionan con la materia de la solicitud en estudio.
- 3.- Novedad, donde se cita el artículo 16 de la Decisión 486y menciona que el documento D1 indicando que de acuerdo con las características esenciales de la sal de la reivindicación 1 habían sido divulgadas previamente en D1.
4. Nivel inventivo donde se cita el Art. 18 de la Decisión 486 y se describen los documentos D1, D2 y D3 citados. Se concluye en forma negativa respecto del nivel inventivo de la solicitud de la solicitud.
4. Frente a las respuestas y argumentos aportado por el solicitante, su Despacho hace mención a que una invención de este tipo no puede encontrar nivel inventivo en simples modificaciones y condiciones que se deben tener en cuenta para la obtención de una sal de adición de un principio activo ya conocido y que para cualquier persona versada en la materia es conocido que una forma de obtener este tipo de productos consiste en el empleo de técnicas tales como por ejemplo reacciones ácido-base, reacciones de óxido-reducción o reacciones catalíticas, entre otras y concluye que en esta solicitud dicho proceso no presenta nivel inventivo alguno si no que por el contrario son comúnmente utilizados en el campo químico-farmacéutico que prácticamente constituyen los métodos de obtención mas deseados por su economía, conveniencia y por los rendimientos que genera.

4°) Se concluye con la parte Resolutiva de la resolución recurrida. Denegar la patente de invención. A la creación denominada "SINTESIS Y FORMAS DE SALES NOVEDOSAS DE (R)-5-((E)-2(PIRROLIDIN-3-ILVINIL)PIRIMIDINA"

5°) ARGUMENTOS:

En primer lugar se hace notar que ya se han presentado en tiempo y forma, argumentaciones como las que siguen, a fin de demostrar el nivel inventivo, así



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como las condiciones de patentabilidad de la presente solicitud. Sin embargo, se procede a reiterar esos argumentos ya elevados por el solicitante, sin agregar materia o información nueva ni modificaciones a ésta solicitud, a efectos de fundamentar ésta declaración del solicitante, destinados a solicitar respetuosamente, se revoque la Resolución recurrida que niega la patente de invención de la presente solicitud, y además manifiesta:

Ante la imposibilidad de presentar nuevas reivindicaciones, el Solicitante manifiesta respetuosamente lo siguiente:

Acompañamos 3 informes técnicos en medio magnético relacionados con la presente invención, con el fin de demostrar su patentabilidad, sustentándola en los ensayos que allí se divulgan.

El documento "AVE3183" es un informe respecto a un estudio polimorfo sobre la sal hemigalactarato (que es la sal identificada en los documentos D1 - D3).

Nos gustaría dirigir la atención del Sr. Examinador hacia la Figura 16, que es un curva DSC (Calorimetría de Barrido Diferencial) de la sal hemigalactarato, específicamente la Forma polimórfica A. La Figura 16 es un ejemplo que muestra las pobres características de la sal hemigalactarato como una composición farmacéutica. No hay nada que predecir sobre la capacidad de las características de formas salinas. Más bien, aunque el hemigalactarato inicialmente pareció proporcionar material cristalino adecuado, tras el último análisis, el hemigalactarato mostró inestabilidad.

Se observan tres (3) endotermas - con un inicio medido, respectivamente, en 10.3, 110.2 y 177.5 °C. La primer endoterma corresponde a la deshidratación de la Forma A (dihidrato) que se transforma en forma anhidra B. Esta transformación sólido-sólido podría ser directa, o indirecta a través de un estado transitorio (Forma C).

Si es indirecta, la entalpía de transición de la forma C a la forma B podría estar oculta en la gran e intensa endoterma de deshidratación. El fenómeno empieza



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cerca de 0 °C, confirmando que la pérdida de moléculas de agua se produce muy poco después del comienzo de la medición.

La segunda endoterma, más bien pequeña, corresponde a la transición sólido-sólido de la forma B a la forma C. Las formas B y C son ambas anhidras. La entalpía de la transición es de 8,2 Joules/gramo con una Tmax a 112,6 °C; este valor ΔH está en consonancia con una transición sólido-sólido. La reversibilidad de esta transición sólido/sólido se confirma por el registro DSC de un ciclo de calentamiento/enfriamiento con un límite superior de temperatura de 130 °C. El último pico endotérmico se atribuye a la fusión de la forma C.

Por lo tanto, bajo condiciones de DSC, la forma A se transforma en una forma C anhidra, que se transforma luego en la forma anhidra B. La figura 39 proporciona una buena ilustración del resumen de la inestabilidad del hemigalactarato. El hemigalactarato no es apropiado para el desarrollo de una composición farmacéutica. La caracterización física del hemigalactarato muestra que la forma sólida es muy sensible a las condiciones ambientales de temperatura y humedad relativa.

En contraste, las sales de la presente solicitud proporcionan un material cristalino estable que es adecuado para su uso en una composición farmacéutica. Como se señaló en la solicitud tal como fue presentada, la base libre de (R)-5-((E)-2-pirrolidin-3-ilvinil)pirimidina (en la forma de base libre) es un aceite viscoso con limitada solubilidad en agua y estabilidad. La invención se basa en nuestra investigación para formas salinas que muestran propiedades mejoradas de: pureza, estabilidad, solubilidad y biodisponibilidad. Estas características aumentan la facilidad o la eficiencia de la fabricación del ingrediente activo y su formulación para un producto comercial. Por último, hemos trabajado sobre formas polimórficas estables que permiten un aumento de la facilidad o la eficiencia en la fabricación del ingrediente activo y su formulación para un producto comercialmente viable.

A manera de ejemplo, el documento "R1083_001BF02JUL08TC-6499-221" es un informe sobre un estudio de polimorfo de la sal citrato. Puede observarse en las Figuras 14 y 17 de dicho documento, que son curvas XRPD (difracción de



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polvo de rayos X) y DSC, respectivamente, de la sal citrato, específicamente la forma polimórfica 2. Como se muestra en estas figuras, la forma 2 de la sal citrato no es sensible a la temperatura y la humedad (en contraste con el hemigalactarato de D1-D3). La forma 2 es una forma polimórfica anhidra de la sal mono-citrato que se funde y se degrada a temperatura por encima de 164 °C. La forma 2 no es sensible a un alto nivel de humedad (97% HR a 25 °C y 75% HR a 40 °C) y no es higroscópica. El mono-citrato, por lo tanto, presenta una mejora sobre la técnica anterior, y las características mejoradas de esta sal citrato no se podrían haber predicho.

Por último, en relación con el alcance total de la reivindicación 1 actual, el archivo adjunto en medio magnético "R880_002VDP07JAN08" proporciona los resultados de un gran proceso de selección de sal utilizado para identificar sales con mejores características que el hemigalactarato del arte previo. Las tablas 9 y 10 de este anexo proporcionan resúmenes tabulares para la mono y hemi sales. Como se señaló en la solicitud tal como fue presentada, las formas salinas preferidas son el citrato, el orotato, y el maleato. La reivindicación original 2, por lo tanto, proporciona la lista preferencial de sales con las características adecuadas.

Aclaramos a su Despacho que en medio magnético se están aportando los documentos citados en el presente escrito, al igual que las traducciones de las partes de estos documentos a que se hace referencia.

Por otra parte, el solicitante además respetuosamente manifiesta su predisposición a presentar una solicitud divisional enfocada sobre la sales citrato, orotato y maleato, o incluso sobre el citrato en particular, si su Despacho lo considera pertinente.

PETICIÓN:

De acuerdo a lo antes expuesto, comedidamente solicito a ese Despacho se estudie nuevamente el presente caso, se revoque en su totalidad la resolución 43797,



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notificada notificada mediante listado 31 Desfijado en Septiembre 06 de 2013, que
niega la solicitud radicada bajo el expediente del asunto.

Como consecuencia de esta actuación se **CONCEDA** el privilegio de patente, a la creación denominada "SINTESIS Y FORMAS DE SALES NOVEDOSAS DE (R)-5-((E)-2(PIRROLIDIN-3-ILVINIL)PIRIMIDINA", teniendo en cuenta las razones expuestas en el presente escrito.

FUNDAMENTOS DE DERECHO :

Fundo mi petición en los artículos 50 y siguientes del C.C.A.; Decisión 486 de la Comisión de la Comunidad Andina y demás normas concordantes sobre la materia.

NOTIFICACIONES :

Mi Poderdante y yo recibiremos notificaciones en la Secretaría de su Despacho o en la Oficina ubicada en la Carrera 13 A N° 28-38 Ofc. 230 de la ciudad de Bogotá D.C.

Señor Superintendente de Industria y Comercio

CARLOS MAYA RODRIGUEZ

C.C. N° 17.117.818. de Bogotá

T.P. N° 17.402.del C. S. de la J.



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EXPLICACION DE LOS ADJUNTOS TRADUCIDOS

Adjuntamos al presente las traducciones de las figuras a las que se hace referencia en el escrito del Presente Recurso, así:

Las figuras corresponden a:

AVE3183 **Figura 16**

AVE3183 **Figura 39**

R1083_001BF02JUL08TC-6499-221 - **Figura 14**

R1083_001BF02JUL08TC-6499-221 - **Figura 17**

R880_002VDP07JAN08 - **Tabla 9**

R880_002VDP07JAN08 - **Tabla 10**

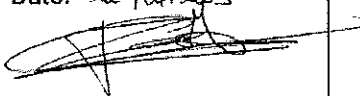
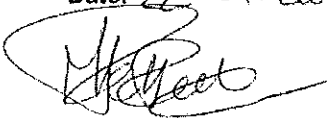
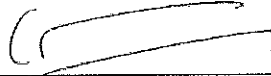
Vitry-Alfortville Research Center
Global Chemistry, Manufacturing & Control
Analytical Sciences Department

POLYMORPHISM STUDY

AVE3183A, hemigalactarate

Physical Characterization of Anhydrous and Hydrated Forms

Analytical services / physical characterization 1

Signature Lionel Zaske Researcher	Date: 22/04/05 
Signature Marc-Antoine Perrin Research Investigator	Date: 22-04-2005 
Signature Carole Neves Head of Analytical Services	Date: 22/04/05 

Document Number: CMC-PA-2004-04435 EN 2.0

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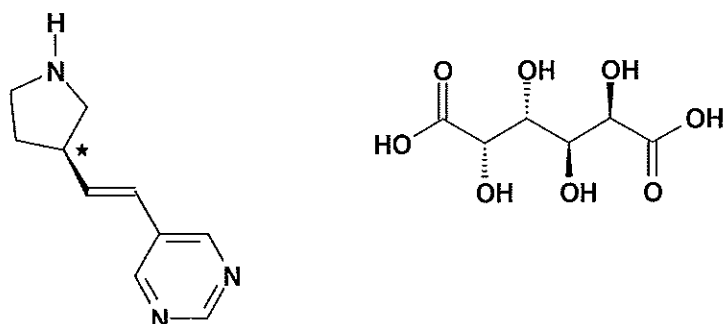
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Molecular formula: $C_{10}H_{13}N_3 \cdot \frac{1}{2}C_6H_{10}O_8$

Molecular weight : 280.30

Chemical name (IUPAC): 5-(2-Pyrrolidin-3-yl-vinyl)-pyrimidine, hemi-galactarate

Common names: AVE3183A, RPR263183B, A002940847F, LC30418

1 INTRODUCTION

AVE3183A belongs to the therapeutic class of nicotinic receptor agonist¹ developed to be effective in the area of CNS (Central Nervous System) neurodegeneration disease. The action mechanism lies in the selective activation of nicotinic acetylcholine receptors (nAChR), which are particularly implicated in Alzheimer's disease.

AVE3183A is the "follow-up" compound that has emerged from a collaborative research agreement with the Targacept biopharmaceutical company². This partnership has already given rise to previous drug substance molecules (XRP8557B³ and its "backup" AVE6608⁴), which are also active in treating CNS pathologies.

¹ Agonist: A drug that binds to a receptor of a cell and triggers a response by the cell. An agonist often mimics the action of a naturally occurring substance. An agonist produces an action. It is the opposite of an antagonist which acts against and blocks an action.

² <http://www.targacept.com/wt/page/index>

³ Gresda report ref.: A30203 FR, September 14th 2000, M-A Perrin, L. Zaske, "XRPD characterization of RPR238557B (hemi-D-p-di-toluytartrate)".

⁴ Gresda report ref.: A43130 FR, August 22th 2001, M.-A. Perrin, A. Rameau, "XRPD characterization of AVE6608 (hemi-galactarate)".

Previous work was done in the Pharmaceutical Sciences Department⁵ at Vitry in 2003 aimed at checking out the behavior of this new chemical entity from a physical viewpoint. So far, at room temperature, AVE3183 as a free base has only been obtained as an oil. Salt formation suggested itself as a way to isolate this compound in crystalline form(s). After the salt selection process (Med. Chem. Vitry), galactarate salt was chosen. During the pre-EDC (Early Development Candidate) study, partially amorphous compound mixed with free crystallized galactaric acid was observed, suggesting the need for improvement in crystallization in the post-EDC steps.

Nevertheless, some preliminary results have been obtained. They lead predominantly to the identification of a hydrated form of the hemi-galactarate salt. This form appeared very often during the initial and reduced screening in ambient conditions of pressure, temperature and relative humidity. At that time the behavior of this form under variable relative humidity (RH) rate was not fully understood and the corresponding pressure-temperature-RH stability domain was not determined.

Due to the potential of this "follow-up" substance, the Project Team Management then recommended studying the polymorphism of this hemi-galactarate salt. The study was carried out in collaboration between Vitry API-O/Process Development team for the experimental screening and ASD Vitry -Analytical Services (ASD/AS/PC-1) for the physical characterization.

The results of the detailed physical characterization of the solid forms of the AVE3183A hemi-galactarate obtained so far (both hydrated and anhydrous) are presented in this report.

This kind of study not only makes it possible to ensure that the forms observed actually exist and are correctly identified, but also to look into how they evolve under different conditions of ambient temperature and relative humidity, and mechanical constraints (grinding). With this information their respective ranges of thermodynamic stability can be assessed, and the form most appropriate for development (as to physical stability) can be recommended.

2 INSTRUMENTATION & METHODS

2.1 X-RAY POWDER DIFFRACTION (XRPD)

XRPD analyses are carried out on a Siemens-Bruker D5000 Matic powder diffractometer using the Bragg-Brentano (vertical θ - 2θ configuration) parafocusing geometry. A sample feeder makes it possible to automate the work. If enough of the product is available, the powder is top-loaded on a concave stainless steel sample holder. Otherwise, a thin layer of the product is deposited on a single-crystalline silicon wafer, cut out according to Si(510) crystallographic orientation that, by systematic extinction, impedes any Bragg reflection. A sealed cobalt anode X-ray tube running at 40 kV and 30 mA levels is used. Two lines are typically emitted: $\text{CoK}\alpha_1$ ($\lambda = 1.7890 \text{ \AA}$) and $\text{CoK}\alpha_2$ ($\lambda = 1.7929 \text{ \AA}$). An Iron β -filter, placed between the detector and specimen, does not

⁵ Project LC30418 / RPR263183B – GPD Assessment Ref: NEC/JoG03006, February 12th 2003.

altogether eliminate $\text{CoK}\beta$ ($\lambda = 1.6208 \text{ \AA}$) radiation, which still contributes about 1% of the diffracted beam at the detector (manufacturer's data). The primary beam passes through a parallel plate collimator (0.2 mm Soller slits), then through a divergence slit (0.2 mm). A Braun 50 M multicanal linear detector completes the setup. It has a 10° -wide detection window in angle 2θ . Diagrams should be recorded in the following conditions: a 1.5 to 50.0° scan in angle 2θ , 10 and 100 seconds counting time per degree in 2θ , according to the amount of powder to be analyzed, and ambient conditions of pressure, temperature and relative humidity (RH).

2.2 VARIABLE TEMPERATURE AND HUMIDITY X-RAY POWDER DIFFRACTION (T-XRPD)

Tests are carried out on a Siemens-Bruker D5000 diffractometer equipped with the Bragg-Brentano para-focusing (θ - θ) geometry and an Anton-Paar TTK temperature chamber. For certain tests a dry nitrogen or RH streams is used. The powder is deposited in a concave stainless steel sample holder. A sealed cobalt anode X-ray tube running at 40 kV and 30 mA levels is used. Two lines are typically emitted: $\text{CoK}\alpha_1$ ($\lambda = 1.7890 \text{ \AA}$) and $\text{CoK}\alpha_2$ ($\lambda = 1.7929 \text{ \AA}$). An Iron β -filter, placed between the detector and specimen, does not altogether eliminate $\text{CoK}\beta$ ($\lambda = 1.6208 \text{ \AA}$) radiation, which still contributes about 1% of the diffracted beam at the detector (manufacturer's data). The beam is sighted using Soller slits, to improve its parallelism. Variable divergence slits keep the illumination area of the sample constant. A 1 mm collimator limits diffusion between the tube and the sample. A Braun 50-M multicanal linear detector completes the setup. It has a 10° -wide detection window in angle 2θ . Temperature is allowed to rise at a rate of 0.05° C/sec . Diagrams are recorded in the following conditions: a 1.5 to 50.0° degree scan in angle 2θ , 10 to 30 seconds counting time per degree in 2θ . Data are acquired in isotherm mode when the requested temperature is reached. A similar procedure is used to take measurements under controlled relative humidity (RH). As the humidity generator runs manually, it is difficult to select a precise degree of RH and then maintain it over a prolonged period of time.

2.3 HIGH-RESOLUTION X-RAY POWDER DIFFRACTION (HR-XRPD)

2.3.1 BRAGG-BRENTANO GEOMETRY

High-resolution diagrams are recorded at ambient conditions on a Philips Analytical X'Pert Pro MPD powder diffractometer using the Bragg-Brentano (vertical θ - 2θ configuration) para-focusing geometry coupled with a X'Celerator detector. A sealed copper anode X-ray tube is used, running at 45 kV and 40 mA levels. An incident beam monochromator (Johansson type: a symmetrically cut curved germanium (111) crystal) produces pure $\text{Cu K}\alpha_1$ radiation ($\lambda = 1.54060 \text{ \AA}$). A thin layer of the product is deposited on a single-crystal silicon wafer, cut out according to Si (510) crystallographic orientation that, by systematic extinction, impedes any Bragg reflection. In order to bring more crystallites into the diffraction position and thus reduce the influence of particle statistics on the measurements, a sample spinner is used. The spinner rotation speed is set at 1 revolution per second. The angular range extends from 2° to 50° in 2θ , with a 0.02° step size in 2θ . According to the powder quality a variable counting time from 250 to 3500 seconds per step was used.

2.3.2 DEBYE-SCHERRER GEOMETRY

High resolution diagrams are recorded at ambient conditions on a Philips Analytical X'Pert Pro MPD powder diffractometer using the Debye-Scherrer transmission geometry (vertical θ - 2θ configuration) designed with a capillary spinner stage and coupled with a X'Celerator detector. A sealed copper anode X-ray tube is used, running at 45 kV and 40 mA levels. An incident beam hybrid monochromator produces a monochromatic parallel beam ($\text{Cu } K\alpha_1$ radiation $\lambda = 1.5406 \text{ \AA}$). The sample is slightly ground in an agate mortar before filling the capillary (Lindeman type, internal diameter comprises between 0.5 and 1 mm). The angular range extends from 1.5 to 50° in 2θ , with a 0.02° step size in 2θ . A counting time of 2500 seconds per step is used.

2.4 X-RAY SINGLE CRYSTAL DIFFRACTION (XRSCD)

XRSCD data are recorded at ambient conditions on a Bruker Smart Apex single crystal diffractometer. A sealed molybdenum anode X-ray tube is used, running at 50 kV and 40 mA levels. An Mo $K\alpha$ line is typically emitted ($\lambda = 0.710731 \text{ \AA}$). To reduce the extraneous $K\beta$ radiation, a graphite crystal monochromator is inserted in the incident beam path just after the safety shutter. A 0.5 mm collimator increases the output flux that hits the sample. A Charge-Coupled Device (CCD chip: 4K, 62 mm) area detector positioned at 6.0 cm completes the setup. The crystal is mounted on a quartz capillary rod. A full Ewald sphere of reflections has been collected (3 x 600 frames with a frame width of 0.3°). Due to the large size of the single crystal, accumulation time has been set at 10 seconds, during each frame to be acquired. The orientation matrix and unit cell were established using the SMART v5.63 program. 3D reflection profile and the integration of all reflections were done with the SAINT+ v6.45 program. The SADABS program was then used to correct for Lorentz and polarization effects and for absorption due to the sample. The provisional space group was determined with the XPREP program. The SHELXTL v6.14 program suite was then applied to solve the structure by direct methods and refine the solution with least squares method. As part of the data is weak, refinement based on F2 has been preferred. This gives more chemically reasonable bond lengths, bond angles, and displacement parameters than F-based refinements do.

2.5 DIFFERENTIAL SCANNING CALORIMETRY (DSC)

Analyses are carried out under a nitrogen stream with a T.A. Instruments DSC2010 and Q1000 analyzers. The calorimeters are temperature-calibrated with indium and lead (onset temperatures of 156.6 and 327.5°C respectively). Energy calibration is done with a certified indium calibrator (melting enthalpy of 28.45 J/g). A mechanical compressor is used to obtain and equilibrate the temperature program: from RT to 230°C at a rate of 5°C/min under a constant nitrogen stream of 55 mL/min . The quantity of product analyzed lies between 1.5 and 5 mg , and is placed in an open aluminum sample pan.

2.6 THERMAL GRAVIMETRIC ANALYSIS (TGA)

Analyses are carried out on a T.A. instruments TGAQ500 analyzer. Mass calibration is performed with 10 and 100 mg certified masses and the instrument is temperature-calibrated with alumel and nickel standards (Curie points of respectively 154 and 354°C). Samples are exposed to a constant nitrogen stream of 60 mL/min and temperature ranges from 25 to 210°C at a 5°C/min rate. The quantity of product lies between 2 and 5 mg. The powder is deposited in an open aluminum sample pan, which is itself placed in a platinum pan.

2.7 SIMULTANEOUS TGA COUPLED MASS SPECTROMETER (TGA -MS)

Analysis are carried out using a SETSYS thermal analysis system produced by the Setaram corporation. This system allows simultaneous differential scanning calorimetric and thermogravimetric analyses to be performed. The gases emitted as the sample is heated are carried off by a transfer capillary tube heated to 150 C 200°C to a Pfeiffer mass spectrometer. This instrument can analyze molecular fragments at « m/e » ratios between 1 and 200 uma. A sample with a mass between 5 and 10 mg is deposited in an open concave aluminum crucible (75 µL capacity). The analysis is effected under a nitrogen stream. The sample is heated from 25 to 165°C at a rate of 5°C/min.

2.8 WATER-ACTIVITY ISOTHERM

All experiments were performed on a DVS-1 automated gravimetric vapor sorption analyser (Surface Measurement Systems Ltd., London, UK). The DVS-1 measures the uptake and loss of vapor gravimetrically using a Cahn D200 recording ultra-microbalance with a mass resolution of $\pm 0.1 \mu\text{g}$. The relative humidity around the sample was controlled by mixing saturated and dry carrier gas streams using mass flow controllers. The temperature was maintained constant, $\pm 0.1^\circ\text{C}$, by enclosing the entire system in a temperature-controlled incubator. A sample size between 4 and 10 mg was used. Prior to being exposed to any water vapor the samples were dried at 0% relative humidity (RH) to remove any surface water present and establish a dry, baseline mass. Next, the samples were exposed to the following relative humidity profile: 0%, 10%, 20%, ... 90%, 80%, ... 0% RH. At each stage, the sample mass was allowed to reach equilibrium before the relative humidity was increased or decreased (considering that equilibrium was established when dm/dt ratio ($m = \text{mass}$; $t = \text{time}$) did not exceed the value of $3.3 \cdot 10^{-4} \text{ mg/s}$ during 30 minutes). If equilibrium state was not reached, the change in relative humidity took place automatically after 600 minutes. Two consecutive cycles were recorded. From the complete moisture sorption and desorption profile an isotherm was calculated using the DVS Advanced Analysis Suite v3.6. All experiments were performed at 25° C.

2.9 OPTICAL MICROSCOPY

The observations are made with a Leica DMRB microscope under polarized light with a magnification of $G = 50X$, $G = 100X$, $G = 200X$ et $G = 400X$. The microscope is calibrated for size with a reticule ($2\text{mm} \Leftrightarrow 200$ graduations). The powder is deposited on a slide and immersed in silicon oil « 47V20 » in order to disperse the particle agglomerates, prior to covering with another micro-cover glass.

3 RESULTS & DISCUSSION

3.1 POLYMORPHISM SCREENING

The batch used for the polymorphism study is referenced P-31959-094-3. It corresponds to a hydrated crystalline form denoted **form A**.

During the polymorphism screening of the AVE3183A hemi-galactarate performed by the Vitry API-O/Process Development team, various crystallization assays were made. Only the samples presenting a distinct XRPD diagram were sent to our group (Vitry ASD/AS/PC-1) for physical characterization.

These assays, along with the crystallization conditions used, are listed in Table 1. The corresponding crystalline form or mixture of forms, as identified afterwards by Vitry ASD/AS/PC-1, are also indicated.

Table 1 – Summary of batches examined during polymorphism screening

Crystallization conditions	Solvents	Samples	Form(s)	Assays
recrystallization in solvent / non solvent mixtures containing a small amount of water (reflux crystallization)	water 25% / acetone 75%	TLL487-A	A + C	ZXS235A
	water 20% / MeOH 80%	TLL487-B	A + B	ZXS236A
	water 12% / MeOH 88%	TLL489-B	C + A	ZXS247A
	water 20% / EtOH 80%	TLL487-C	A	ZXS237A
	water 9% / EtOH 91%	TLL489-C	A	ZXS244A
	water 15% / THF 85%	TLL487-D	A	ZXS238A
	water 7% / THF 93%	TLL489-D	A	ZXS245A
	water 17 % / acetone 83 %	SGU01-094A	A	LZK0955A
	water 14 % / acetone 86 %	SGU01-094B	A	LZK0956A
	water 8 % / methanol 92 %	SGU01-098	A + B + C	LZK0957A
redissolution in azeotropic mixtures and then recrystallization when water is eliminated by distillation (solvent transfert)	water 5% / THF 95%	TLL488-A	A	ZXS241A
	water 15% / ACN 85%	TLL488-B	D + A	ZXS242A
		TLL492	B + A	ZXS248A
	water 11% / MEK 89%	TLL488-D	A	ZXS243A
	water 17% / propanol-1 83%	TLL488-C	D	ZXS246A
		TLL495	A + D	ZXS255A
		TLL497	D + B + A	ZXS260A
lyophilization	water	-	B + A + D	ZXS253A
precipitation	propanol-1	SGU001	A	ZXS256A
	acetonitrile	SGU002	A + B	ZXS261A

Four different crystalline forms of AVE3183A hemi-galactarate, denoted A through D according to their order of appearance, were identified during polymorphism screening and physical characterization studies. The detailed physical characterization of each of these 4 forms (A, B, C and D) is given respectively in paragraphs 3.2 to 3.5.

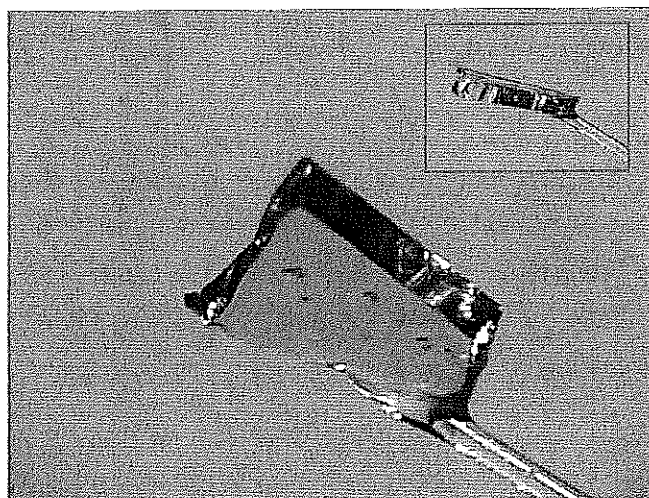
3.2 FORM A

Form A was obtained either pure or mixed with other crystalline forms in 19 of the 20 crystallization assays carried out (Cf. Table 1). The batch P-31448-030-1 was used to carry out the physical characterization of form A.

3.2.1 Crystal structure determination from X-ray single crystal diffraction (XRSCD) data

After recrystallization of batch P-31448-030-1 by water vapor diffusion in a sealed container at room temperature, a single crystal (with a plate-like morphology) was isolated, then cut and glued with Araldite™ on a quartz rod (Cf. Figure 1). The structure was determined at room temperature (293 K) by direct methods and least squares refinement — full-matrix least squares based on F^2 — ($R(\text{int}) = 0.036$ and $R(\text{sigma}) = 0.028$, 422 parameters)⁶. Details of the structure determination can be found in Appendix 1.

Figure 1 - Photograph of the single crystal used for structure determination, ref: amp044b



Form A is a **dihydrate** and crystallizes in an orthorhombic system (space group $P2_12_12_1$). Cell parameters, at room temperature (293 K), are as follows:

$$\begin{array}{ll} a = 8.2447(7) \text{ \AA}, & V = 3130.6(5) \text{ \AA}^3 \\ b = 10.2493(9) \text{ \AA}, & \text{density} = 1.342 \\ c = 37.047(3) \text{ \AA} & Z = 4 \text{ formula units} \end{array}$$

⁶ After refinement of the model structure, the final “residual” values were: $R_1 = 7.4\%$ for 4473 $F_o > 4\text{sig}(F_o)$ and 7.6% for all 4512 data, with $wR_2 = 20.2\%$ and $\text{GooF} = S = 1.27$ for all data.

The asymmetric unit (*Cf.* Figure 2) consists of two molecules of AVE3183, a molecule of galactaric acid and four molecules of water.

Atoms' positions and anisotropic atomic displacement parameters are given in Appendix 2. Crystal unit cell and crystal packing are shown in Figure 3 and Figure 4 respectively. The XRPD diagram simulated from the crystalline structure determined at ambient temperature is given in Figure 5.

Figure 2 - Form A of AVE3183A hemi-galactarate – asymmetric unit
(grey: carbon, red: oxygen, blue: nitrogen, white: hydrogen, orange: water molecule)

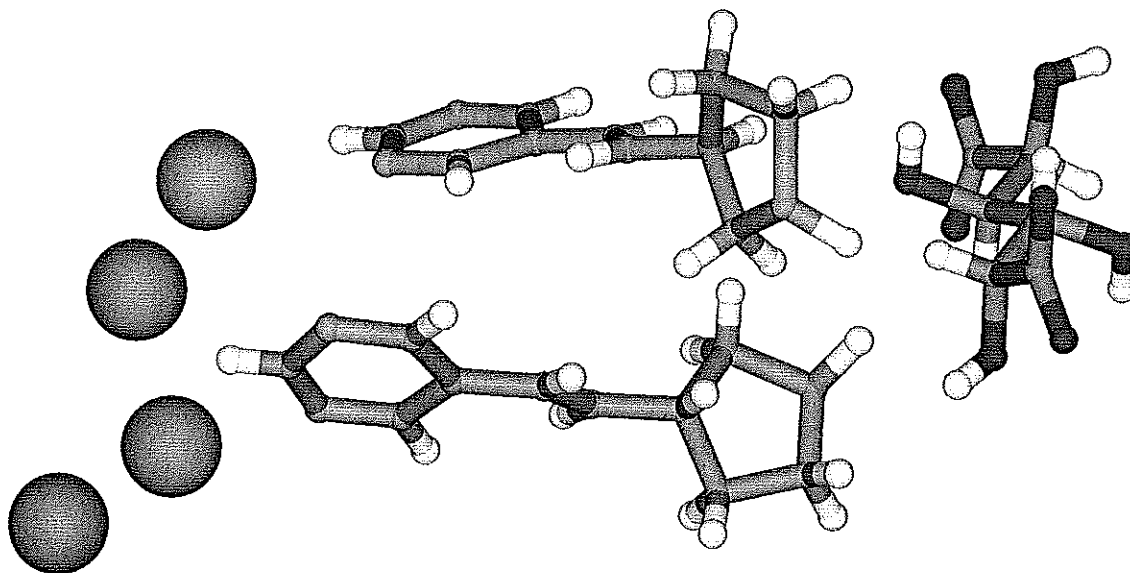


Figure 3 - Form A of AVE3183A hemi-galactarate – Crystal unit cell
(grey: carbon, red: oxygen, blue: nitrogen, white: hydrogen, orange: water molecule)

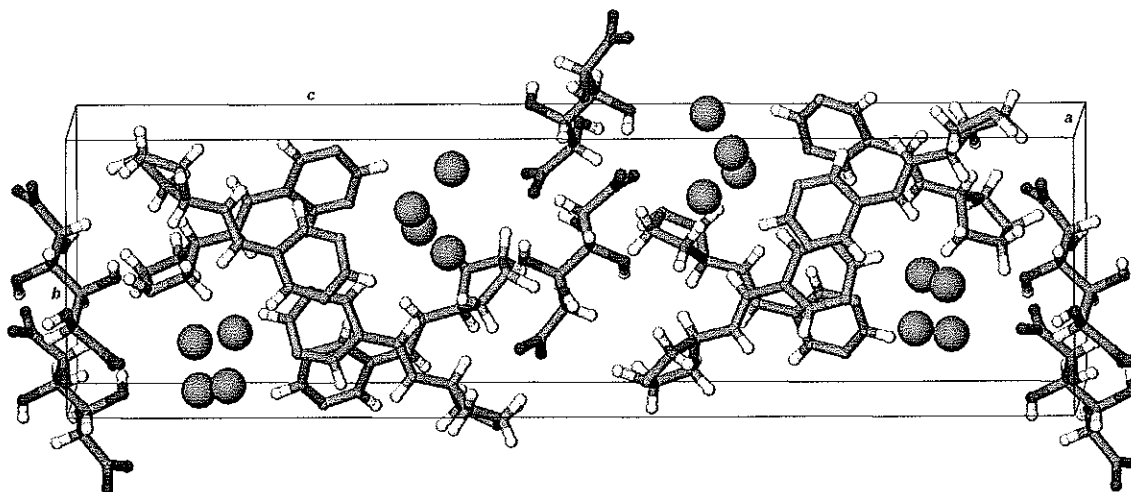
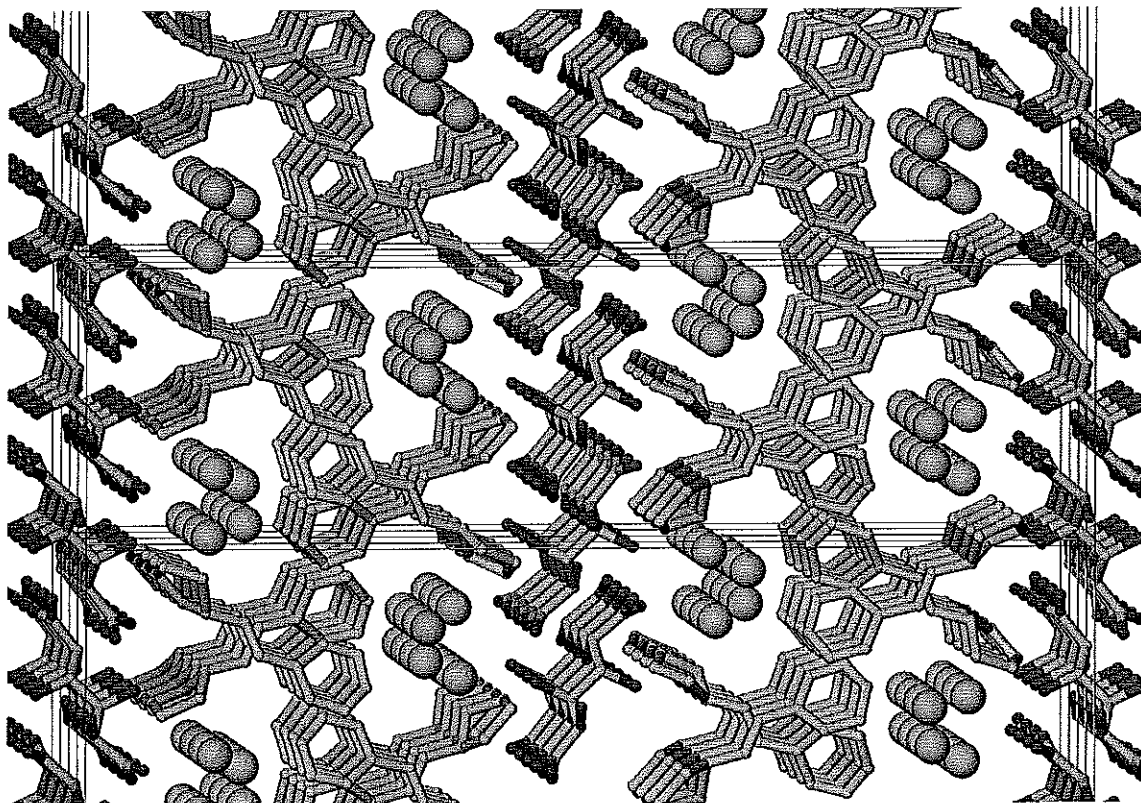


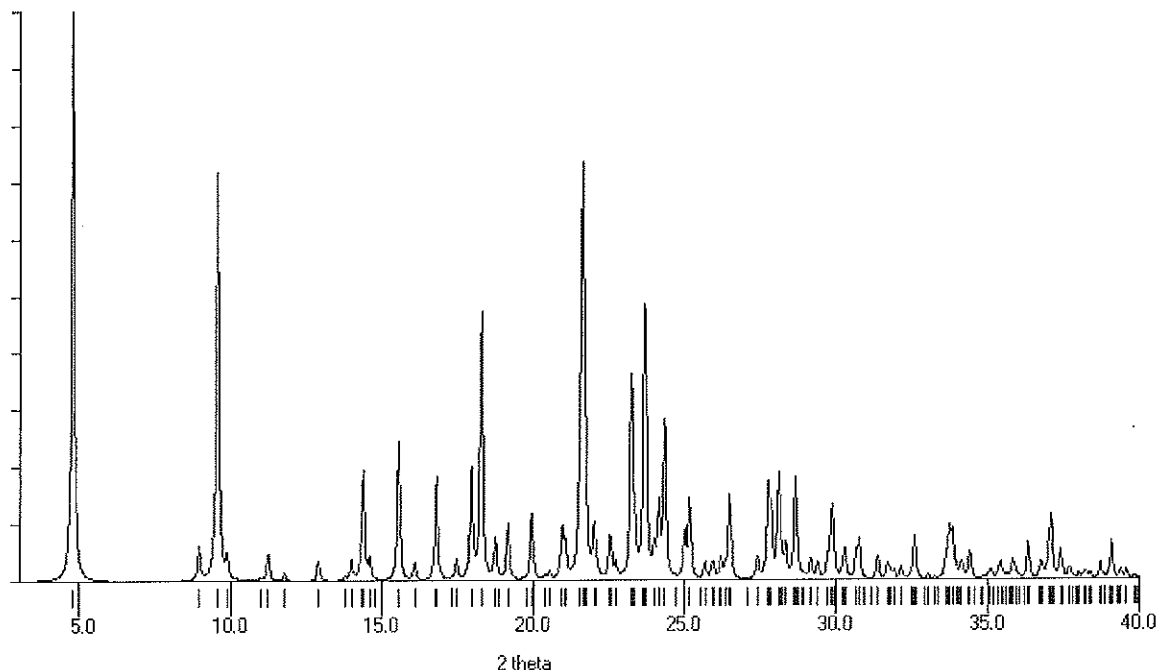
Figure 4 - Form A of AVE3183A hemi-galactarate – Crystal packing
(grey: carbon, red: oxygen, blue: nitrogen, orange: water molecule)
For easier readability hydrogen atoms are not represented



As clearly illustrated in Figure 4, the crystal structure of AVE3183A form A shows **layer stacking** with the layers developing perpendicular to the long cell axis. There are two distinct layers: On the one hand, galactarate molecules pack together in a strong 2-dimensional intermolecular H-bond network; while on the other hand AVE3183 molecules and water molecules interact closely in a wide, intricate bi-layer with AVE3183 molecules straddling in columnar edifices. These AVE3183 molecular edifices are stabilized by the strong intermolecular Van der Waals overlapping and " $\pi - \pi$ " stacking of both the vinylic insaturation and the pyrimidine cycle. The galactarate molecules provide the "bridge-layer" between the "AVE3183 + water" layers by sharing its two carboxylate groups, one on either side of the layer, with the pyrrolidinic group of the AVE3183 molecule through a strong H-bond.

The second noticeable structural characteristic of form A is its **network of straight parallel channels where water molecules are hosted**. The two water molecules of di-hydrated form A are ordered in these channels by forming a winding infinite H-bond chain between them. In addition, one of the two molecules develops a strong H-bond with a nitrogen atom of the AVE3183's pyrimidine cycle, while the other one interacts through a strong H-bond with a hydroxyl group of the galactarate molecule. This kind of water molecule arrangement strongly contributes to the global stability of the form A layer structure.

Figure 5 - XRPD Diagram (λ Cu K α 1 = 1,5406 Å) of Form A of AVE AVE3183A hemi-galactarate, simulated from the "single crystal" structure (red tick marks indicate the Bragg positions)



3.2.2 X-ray powder diffraction (XRPD) of form A

3.2.2.1 Indexing of form A

The indexing of an XRPD diagram is meant to identify the crystallographic form and to characterize its physical purity unequivocally. This procedure is particularly suited to the verification of batches for release or during stability studies.

To establish a batch's conformity one simply compares the **XRPD diagram of the sample to be analyzed** (control diagram) with the **reference XRPD diagram** furnished in this document⁷ (Cf. section 3.2.2.2). All the diffraction lines of the reference diagram should be found on the control diagram at the expected d-spacings (Cf. Appendix 3 for the complete indexing of the diffraction peaks of form A), within the limits of experimental uncertainty as to the angular positions. This uncertainty depends on how the diffractometer used is set⁸ and how the sample

⁷ The electronic reference XRPD diagram (Izk0999a) is available upon request from ASD/AS/PC1 Vitry.

⁸ Displacement of the goniometer's zero (Z): The effect on the 2θ positions is constant; the diagram is merely translated as a whole from Z° in angle 2θ .

being analyzed is positioned⁹. The simultaneous presence of angular displacements that are positive for certain lines and negative for others, with respect to the reference positions, is a sign of evolution in physical form. Similarly, if no lines coincide, there has obviously been a change in the batch's physical form. If extra, unindexed lines are detected, that is a certain indication of at least one new phase, either another physical form of the compound (a polymorph or a pseudo-polymorph) or an impurity. On the other hand, if only the intensity of certain lines varies, there is no reason to conclude that the physical form has been transformed¹⁰; it is more likely an effect of preferential orientation due to a difference in the morphology/texture of the particles (needles, platelets...), which can be reduced by light grinding¹¹. Finally, when the sample is analyzed "as such", the broadening of all the diffraction lines¹², along with a rise in the background noise, indicates the beginning of the crystalline form's amorphization.

Apposite indexing of a powder diagram requires the use of data that is irreproachable as to angular precision and instrumental resolution. The best way to accomplish this is to record a diagram in reflection mode (Bragg-Brentano geometry), performed with a properly aligned goniometer, using a monochromatic X-ray beam.

The high-resolution XRPD diagram shown in Figure 6 was recorded on batch P-31448-030-1. To avoid any risk of amorphization or polymorphic transition, the crystals were lightly ground by crushing under a glass slide directly on the silicon wafer used for the analysis. By analyzing the diagram with the "peak search" module of the Accelrys company's Materials Studio program, the 2θ angular position of each of the observable lines can be determined with precision. The best solution (the highest "figure of merit" FOM) given by the Accelrys company's "X-Cell" indexing program¹³ is subsequently optimized by Pawley refinement¹⁴. This treatment takes into account

⁹ Displacement of the sample plane (D): an effect in $D \cdot \cos(\theta)$ on the 2θ positions; of around 0.01° in 2θ at low angles ($\cos \theta$ close to 1), for a 15 μm displacement.

¹⁰ The rare instances where this comment does not apply (isomorphous non-stoichiometric hydrates, for example) will have been previously identified by the ASD/AS/PCI Vitry. The suggested identification method is adapted accordingly.

¹¹ If it is wished to reduce the impact on the XRPD diagram, the powder can be ground before analysis as long as the ASD/AS/PCI Vitry has previously determined that no physical transformation (amorphization, change in physical form) is induced by the resulting increase in energy. As the aim is rather to limit the anisotropy of the crystalline habit than to reduce the size of the crystallites (which entails an undesirable broadening of the diffraction lines by diminishing the size of the coherent diffraction domains), light grinding in an agate mortar for about a minute should be sufficient.

¹² However, broadening of all the lines of the diagram after grinding of the powder is a normal, expected phenomenon that reflects the smaller size of the coherent diffraction domains.

¹³ «X-Cell : a novel indexing algorithm for routine tasks and difficult cases» M.-A. Neumann, *J. Appl. Cryst.* (2003) 36, 356

¹⁴ «Unit-cell refinement from powder diffraction scans» G.S. Pawley, *J. Appl. Cryst.* (1981) 14, 357 : a "modern" alternative to the cell parameter refinement provided by the NBS*AIDS program, which deals only with the position of the lines.

the whole profile of the diagram and not simply the position of the lines. It seeks to reproduce the experimental diagram as closely as possible by treating the diffracted intensities as variables. The weaker the R_{wp} profile factors obtained, the higher is the quality of the solution.

For form A of compound AVE3183A hemi-galactarate, the indexing result, as also found by determination from X-ray single crystal diffraction data¹⁵ (Cf. section 3.2.1), at $T = 298\text{ K}$, is as follows:

the lattice is orthorhombic and conventional cell parameters are:

$$\begin{aligned}a &= 10.2864(3)\text{ \AA}, & V &= 3159.4(8)\text{ \AA}^3 \\b &= 37.1686(4)\text{ \AA}, & \text{density} &= 1.329 \\c &= 8.2651(3)\text{ \AA} & Z &= 4\text{ formula units}\end{aligned}$$

with a final R_{wp} of 3.4% after Pawley refinement (Cf. Figure 7).

Study of the systematic extinctions indicates that the space group is $P2_12_12_1$. This is a confirmation of the result obtained from single crystal data.

As all the lines present on the high-resolution XRPD diagram are indexed (Cf. Figure 7), **batch P-31448-030-1 (form A) is a pure crystalline phase**. Complete indexing of form A XRPD lines, given in d-spacings and in 2θ positions $\lambda_{Cu}K\alpha$ and $\lambda_{Co}K\alpha$, is to be found in Appendix 3.

¹⁵ The slight difference in the cell parameter values observed in the results of indexing carried out on powder and on single crystal derives from the different "statistical" nature of the samples studied. The powder is made up of a multitude of crystallites and is thus statistically more representative of the crystalline form than the single crystal, which is, by definition, singular.

Figure 6 - High-resolution XRPD diagram (λ Cu K α 1 = 1,5406 Å) of AVE3183A form A recorded at ambient temperature and RH (batch P-31448-030-1), ref: zxs254b

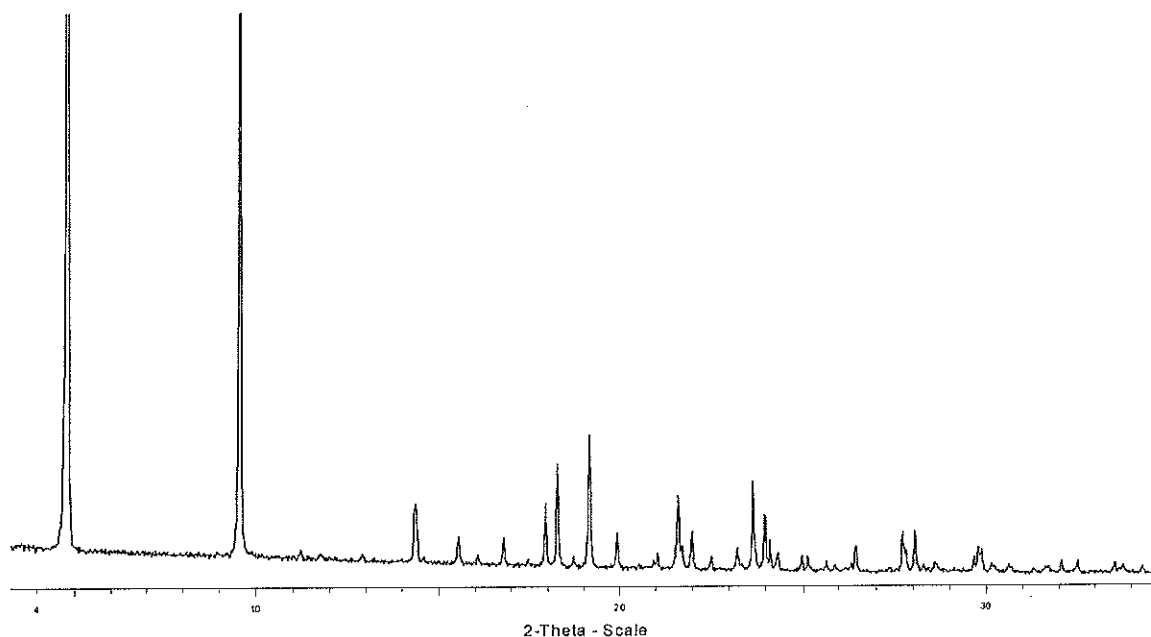
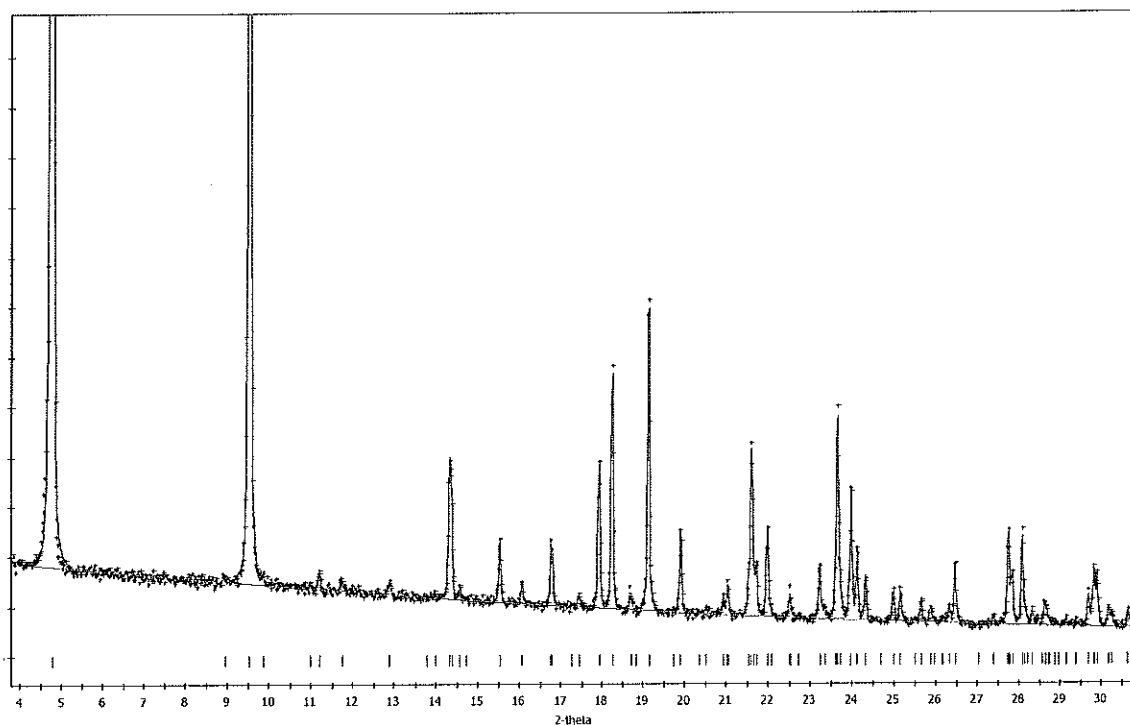


Figure 7 - Pawley refinement of the high-resolution XRPD diagram (λ Cu K α 1) of AVE3183A form A



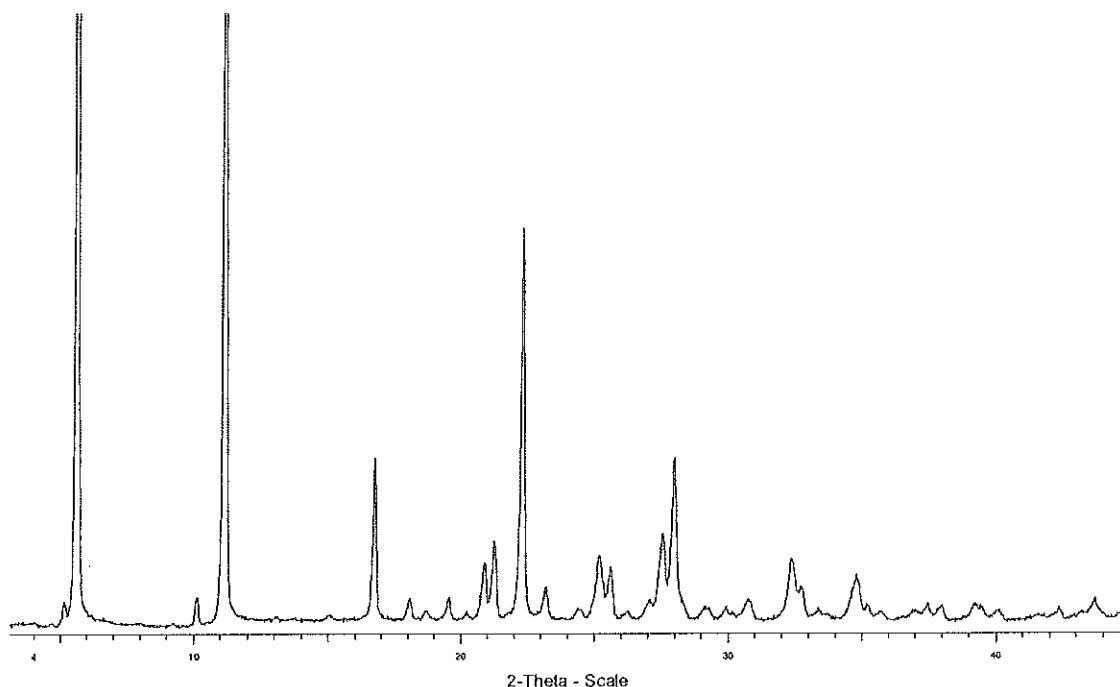
3.2.2.2 XRPD reference diagram of form A

The recording (*Cf.* experimental conditions section 2.1) of the XRPD reference diagram (ref: lzk0999a) of form A was carried out on a powder « as such » from a batch freshly made up at Neuville (batch 03N 0636B). The diagram is shown in Figure 8.

Four extra lines of weak intensity are due to incomplete filtration of the $K\beta$ radiation, as is indicated by the simulation results¹⁶ given in Figure 9 (*Cf.* black arrows). This undesirable instrumental effect is absent from the high-resolution diagrams, on which the radiation is rigorously monochromatic.

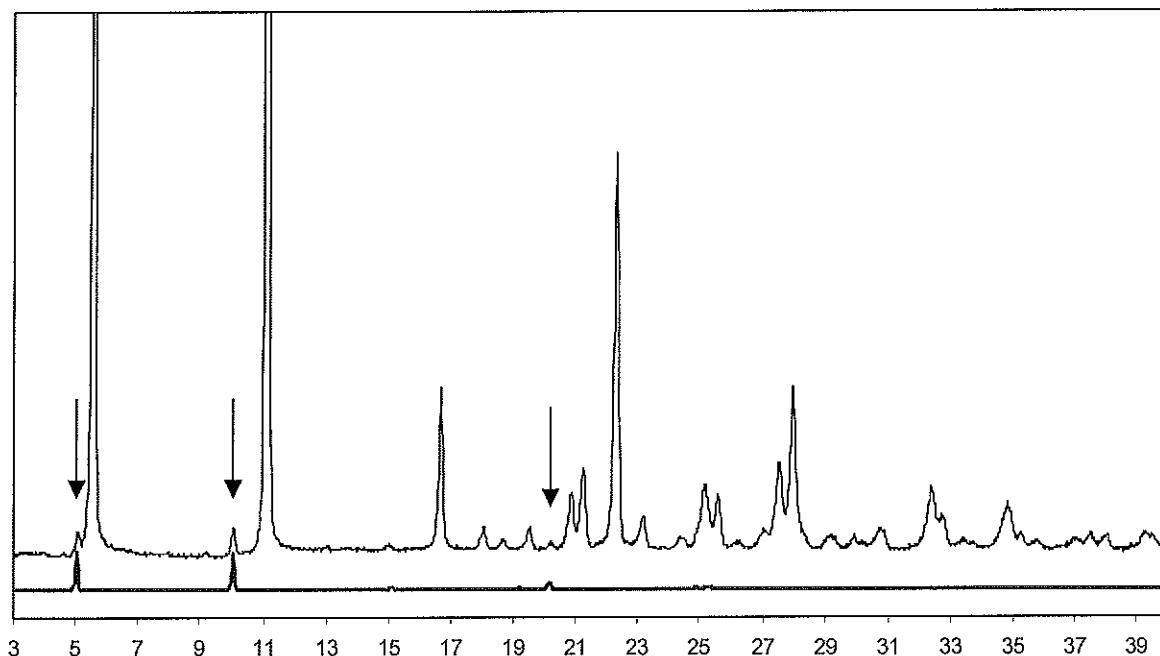
Analysis of this diagram indicates that the lines expected for form A are present and correctly positioned. **Batch 03N 0636B is made up of pure form A.**

Figure 8 - XRPD reference diagram ($\lambda_{Co} K\alpha1\alpha2$) of AVE3183A form A (batch 03N 0636B), ref: lzk0999a



¹⁶ As the cobalt $K\beta$ radiation is not totally filtered by the iron (*Cf.* section 2.1), it is important to know the position and intensity of the extra lines thus generated. This avoids wrongly attributing unindexed peaks to the presence of an impurity. An XRPD diagram was therefore calculated (*cf.* red curve in Figure 9) from the experimental diagram using the wavelength of cobalt $K\beta$ radiation ($\lambda = 1.6208 \text{ \AA}$). For each angular value in 2θ of the experimental diagram, a d-spacing was calculated from the formula of Bragg's Law, expressed in " $\lambda_{Co} K\alpha$ ", and then reintroduced, modified to " $\lambda_{Co} K\beta$ ", into the same formula so as to get the angular value in " $\lambda_{Co} K\beta$ ". The intensity retained is that of the corresponding " $\lambda_{Co} K\alpha$ " position, attenuated by a factor of 60. This value was determined by visual comparison. Under these conditions, the calculated $K\beta$ part of the experimental $K\beta$ lines is not detectable.

Figure 9 - XRPD diagrams of AVE3183A form A : experimental and calculated
 $\lambda_{Co} K\beta$ (1,6208 Å), in black and red respectively



3.2.2.3 Variable temperature T-XRPD analysis of form A

The structural behavior of form A (batch TLL488-D) under heating was studied in situ by variable T-XRPD in a low nitrogen flow (100 mL/min and $\approx$ 0.5 to 1 bar) (Cf. Figure 10). The temperature was raised by 5°C steps from room temperature up to 180°C.

Noticeable modifications of the XRPD diagram were observed at 35, 50 and 120°C (respectively the first blue, brown and green diagrams in Figure 10). Melting starts around 160°C. The diagrams recorded in the region from 50 to 110°C are quasi superimposable; the continuous heat-induced dilatation of the crystal lattice appears to be very weak. More surprisingly, the diagrams recorded between 35 to 45°C and over 120°C up to melting point are similar to each other.

Analysis of these XRPD data indicates that form A transforms into a new form at around 35°C. Then, close to 50°C this new form transforms in turn into another new form that appears to be stable up to 115°C. Surprisingly, the form briefly isolated at 35°C shows up again from over 115°C until the melting point of the substance.

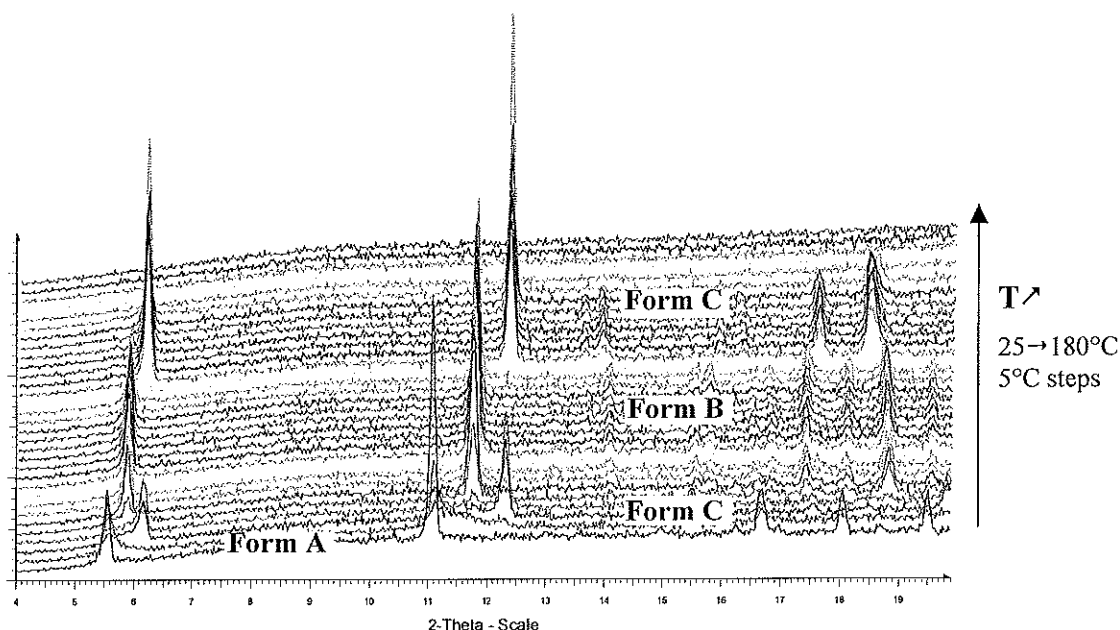
Since form A is a di-hydrate, these results tend to indicate that:

- 1 The first transformation, which takes place at the very beginning of the experiment (as the nitrogen stream starts to flow through the chamber), corresponds to the rapid dehydration of form A into a new, not well crystallized form, denoted form C.
- 2 The second transformation at 50°C is linked with the appearance of a well crystallized form, denoted form B.

- 3 The third transformation above 115°C is related to a solid-solid transition of form B into form C, which melts after 160°C. Melting terminates at around 185°C.

The physical characterization of forms B and C is treated respectively in sections 3.3 and 3.4 of this report. The XRPD diagrams of forms B and C were indexed (*Cf.* sections 3.3.1.1 and 3.4.1.1), proving their structural existence.

Figure 10 - T-XRPD diagrams (λCo Kα1α2) of AVE3183A form A (batch TLL488-D) recorded under a low nitrogen flow, from room temperature to 180°C, ref: lzk0970a

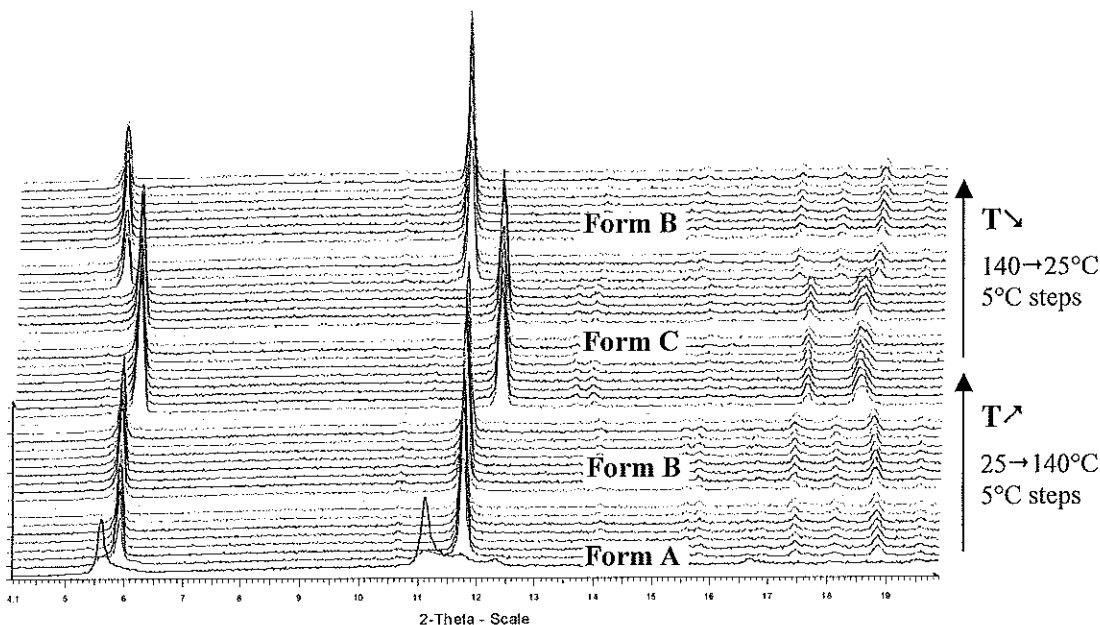


In order to check the reversibility of the form B → form C transformation, a second variable T-XRPD experiment was carried out heating a sample of form A only up to 140°C, followed by a return to room temperature. The diagrams recorded in 5° C steps are given in Figure 11. In this case, the nitrogen gas stream was set at usual flow and pressure (250 mL/min and ≈1 to 2 bars).

These data clearly indicate the reversibility of the form B → form C transformation. The inverse transformation starts at around 100°C.

As expected for a dehydrated sample analyzed under an anhydrous nitrogen flow, the Form A XRPD pattern is not observed on the way back down to room temperature. In addition, the first recorded XRPD diagram, at room temperature, already shows broadening and partial disappearance of form A peaks, weak traces of form C and the emergence of form B peaks. This clearly indicates that the dehydration of form A is faster than in the first experiment (*Cf.* Figure 10), since the nitrogen flow and thus the dehydration conditions are stronger in this case.

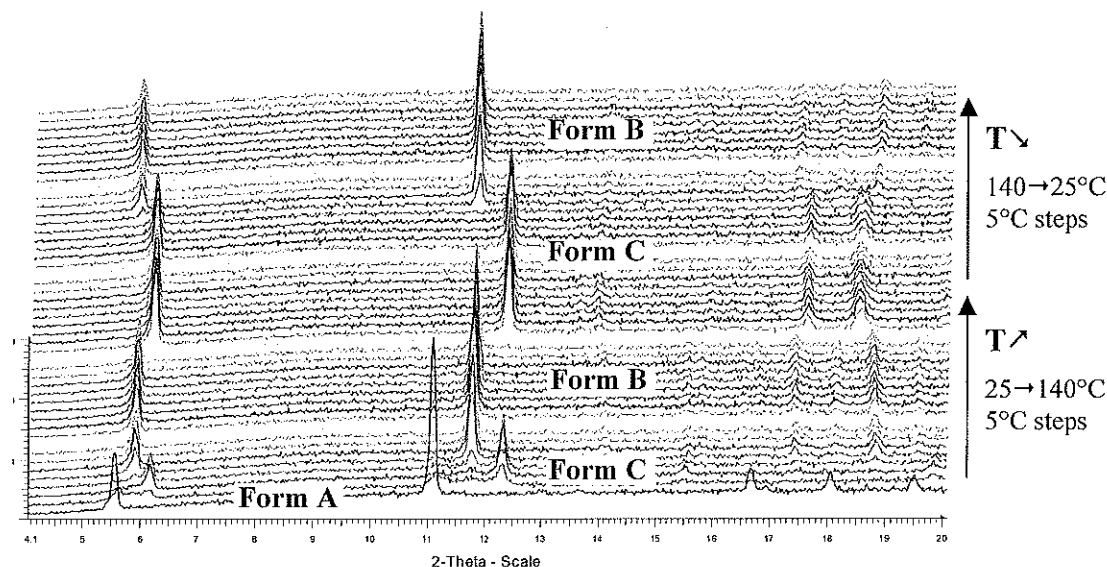
Figure 11 - T-XRPD diagrams (λ Co K α 1 α 2) of AVE3183A form A (batch TLL488-D) recorded under a normal nitrogen flow, from room temperature to 140°C and then back down to room temperature, ref: lzk0970e



In a third variable T-XRPD experiment similar to the second one just described no nitrogen flow was used. The diagrams recorded in 5° C steps are given in Figure 12. Here, the dehydration of form A was due solely to the increase in temperature. Compared to the previous variable T-XRPD experiment only the very first steps of heating are slightly different. Thus the change of form A into form C is more pronounced, as reflected by the XRPD diagram recorded at 35°C (in blue in Figure 12), which shows a form C of better crystallographic quality. Nevertheless, once again form C completely transforms into form B beyond 35°C. Finally, form A is again missing on the way back down to room temperature.

From these variable T-XRPD experiments, the dehydration process of form A upon heating and/or under nitrogen flow can be described as follows: At the very beginning of the experiments form A dehydrates (easily but more or less quickly depending on the strength of dehydration conditions) into form C, a kinetically favored transitory state in this low temperature range. Then beyond 35°C, metastable form C transforms rather promptly into form B, which appears to be a more thermodynamically stable product of form A dehydration in the low temperature range. Above 115°C, form B reversibly transforms into form C.

Figure 12 - T-XRPD diagrams (λ Co K α 1 α 2) of AVE3183A form A (batch TLL488-D) recorded without a nitrogen flow, from room temperature to 140°C then back down to room temperature, ref: lzk0970c



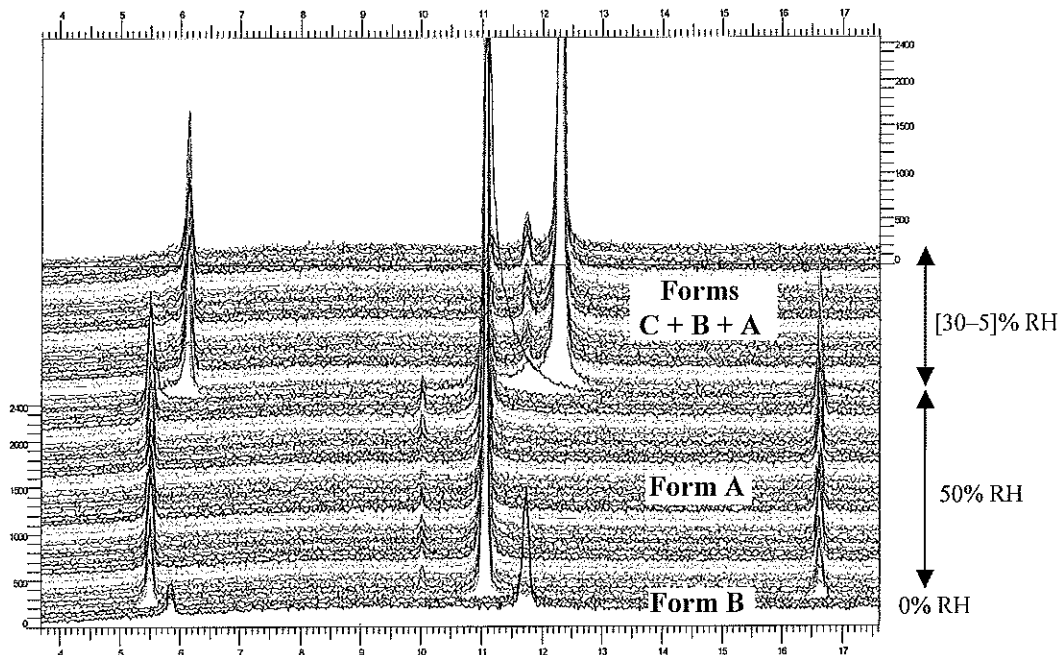
3.2.2.4 Variable relative humidity XRPD analysis of form A

A complementary XRPD experiment was done on form A (batch P-31448-030-1) in isothermal conditions (room temperature) and under a controlled variable relative humidity rate. The knowledge acquired from previous variable temperature XRPD experiments (*Cf.* section 3.2.2.3) suggested increasing the relative humidity rate from 0% to 50% RH and then lowering it back to close to 5% RH. The relative humidity rate was measured precisely in situ by inserting a thermo-hygrometer probe inside the X-Ray diffraction TTK-450 chamber. The diagrams recorded are given in Figure 13.

After RH had been held at 0% for 30 minutes, the XRPD diagram was characteristic of pure form B. As soon as the relative humidity rate was increased in the sample chamber up to 50% RH, the transformation of form B into form A was total and immediate. As long as the relative humidity was maintained at 50% RH, no further structural modifications were observed on the XRPD diagrams recorded. Then, slowly decreasing the relative humidity rate from 50% to around [30-5]% induces the transformation of form A into form C, with the presence of a low amount of form B and residual traces of form A.

Such mixtures of forms A, B and C, in various proportions depending on the ambient conditions of temperature, pressure and relative humidity, had already been observed by XRPD on many samples generated during polymorphism screening.

Figure 13 - XRPD diagrams (λ Co K α 1 α 2) of AVE3183A form A (batch P-31448-030-1) recorded at ambient temperature as a function of relative humidity and exposure time: 0% RH - 30 min, 50% RH - 400 min and finally at RH comprises from 30 to 5% - 300 min, ref: lzk0968a



3.2.3 Water activity isotherm (DVS) of form A

Sorption-desorption isotherms were recorded on batch TLL489-D. Figure 14 shows two consecutive sorption/desorption cycles recorded at 25° C; The experiment was started at 50% RH.

The two cycles are identical. A significant and stair-like hysteresis between the sorption and desorption phases is observed from 5 to 45% RH. Water sorption isotherms exhibit a water uptake of 13.5% w/w from 40% RH to a plateau around 50% RH. This result is in good agreement with the theoretical water uptake (11.4%) as anhydrous AVE3183A becomes a di-hydrated form.

The desorption curves show that the dehydration process takes place in two steps :

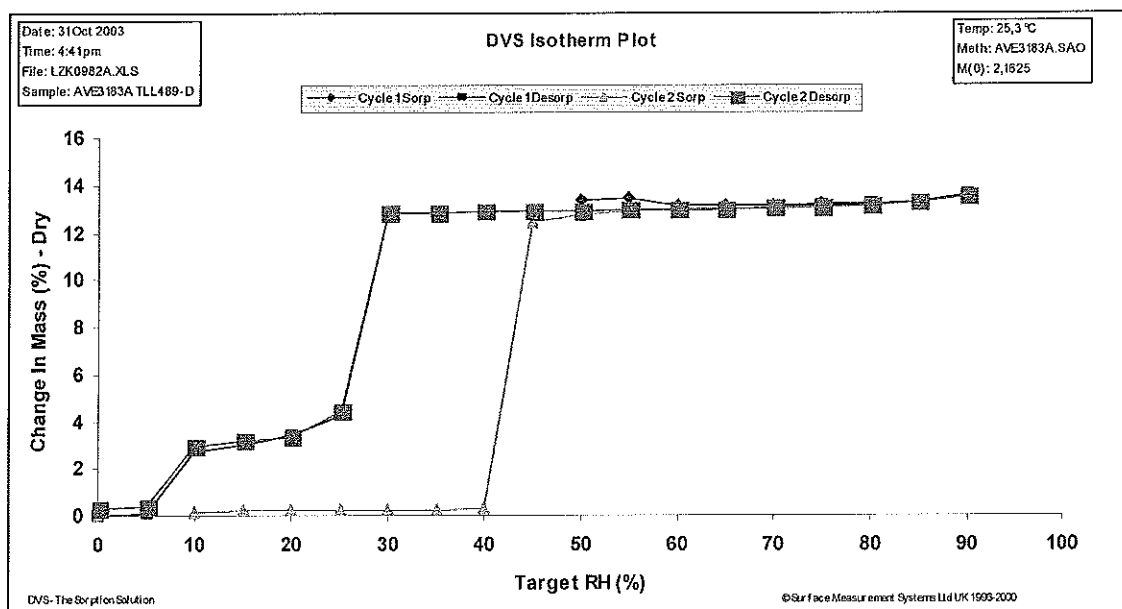
- a plateau of 2.8% w/w is recorded between 20 and 10% RH. As highlighted during variable relative humidity XRPD analysis of form A (*Cf.* section 3.2.2.4), this phenomenon is related to the incomplete transformation of form A into forms B and C during the desorption process that leads to a mixture of forms.
- below 10% RH, total dehydration of form A takes place.

XRPD analysis of the sample in ambient conditions, after the two DVS cycles, shows that there has been no structural modification, and that it is still dihydrated form A.

In addition, a form A sample stored in a desiccator maintained at 95% RH and 25°C) went into solution (deliquescence) after 3 days of residence. On the contrary, another form A sample placed for 4 days in a desiccator maintained at 85% RH and 25°C was physically unchanged as checked with XRPD.

Thus, at ambient temperature form A is a dihydrate that is physically stable from 50 to 85% RH.

Figure 14 – DVS sorption/desorption water isotherm of AVE3183A form A (batch TLL489-D) recorded at 25°C, ref: lzk0982a

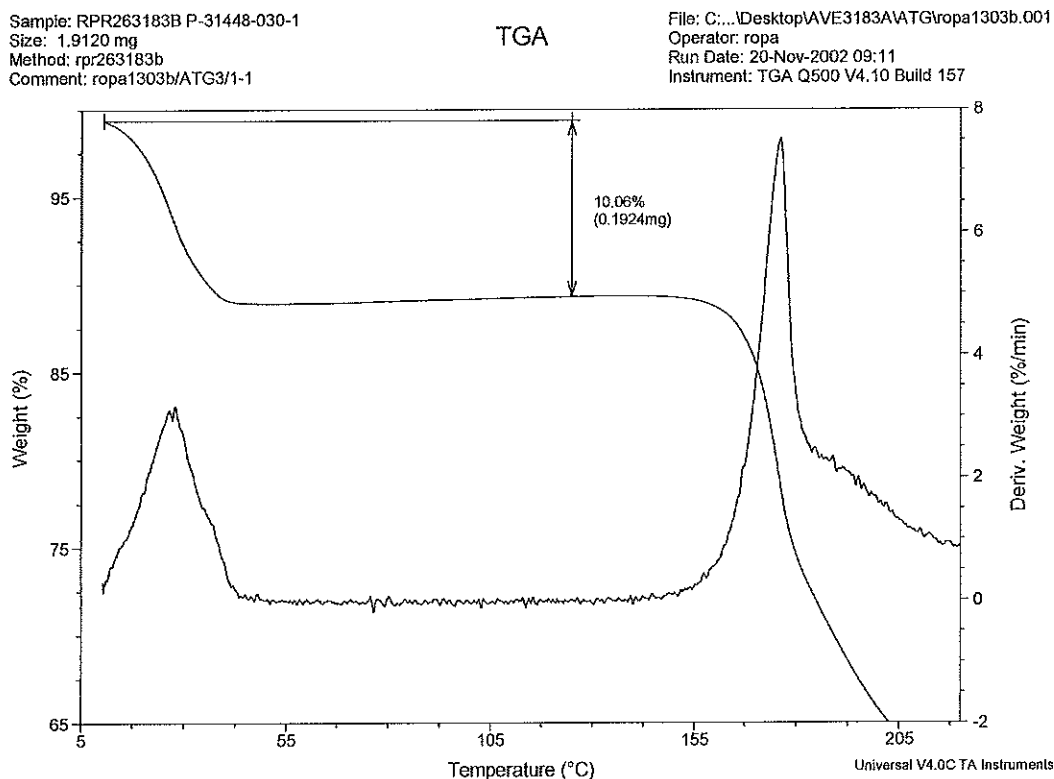


3.2.4 Thermal analysis of form A

3.2.4.1 TGA

A water loss of 10.1% w/w is observed in the temperature range comprised from 10 to 50°C (Cf. Figure 15). This loss is undervalued, since dehydration occurs immediately when the compound is simply exposed to a nitrogen flow (The theoretical water amount of a dihydrated form is 11.4%). Beyond 50°C and up to melting temperature, no other loss is recorded.

Figure 15 - TGA analysis of AVE3183A form A (batch P-31448-030-1), ref: ropa1303b



3.2.4.2 DSC

The DSC recording is given in Figure 16. Three endotherms are observed with an onset measured respectively at 10.3, 110.2 and 177.5°C. The first endotherm corresponds to the dehydration of form A (dihydrate) which transforms into anhydrous form B. This solid-solid transformation could be direct, or indirect through a transitory state (form C) as observed from T-XRPD experiments (*Cf.* sections 3.2.2.3 and 3.2.2.4). If indirect, the transition enthalpy of form C → form B would be hidden in the large and intense dehydration endotherm. The phenomenon begins close to 0°C, confirming that the departure of water molecules occurs very soon after the beginning of the measurement.

The second, rather small endotherm corresponds to the form B → form C solid-solid transition. Forms B and C are both anhydrous. The enthalpy of the transition is 8.2 J/g with a T_{max} at 112.6° C; this ΔH value is in keeping with a solid-solid transition. The reversibility of this solid-solid transition, as revealed by the variable T-XRPD experiment (*Cf.* Figure 11 and Figure 12), is confirmed by the DSC recording of a heating/cooling cycle with a high temperature limit of 130°C (*Cf.* Figure 17).

The last endothermic peak is attributed to the melting of form C.

Forms B and C are enantiotropically related. Form C is the high-temperature-stable anhydrous form.

Figure 16 - DSC curve of AVE3183A form A (batch P-31448-030-1), ref: ropal303b

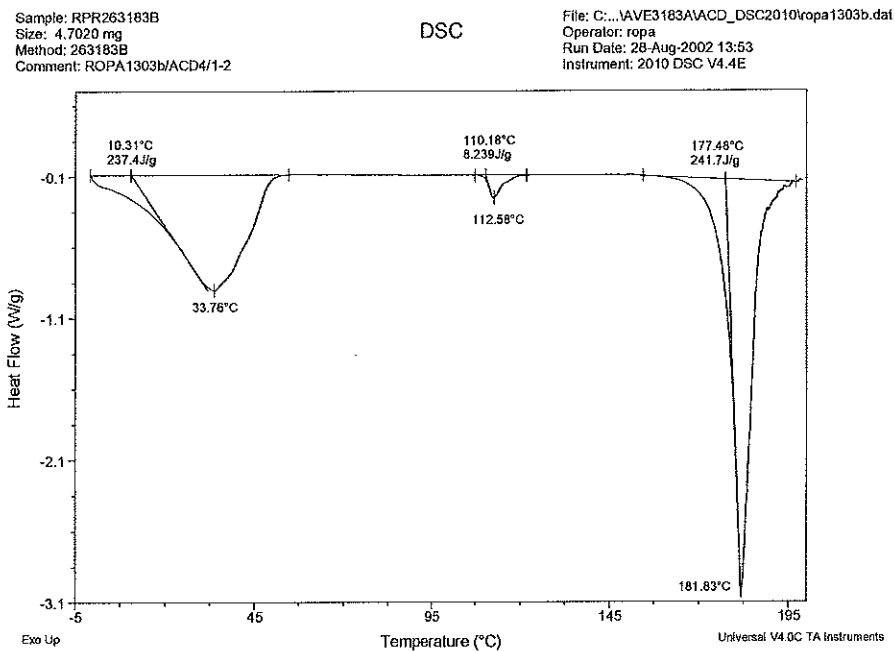
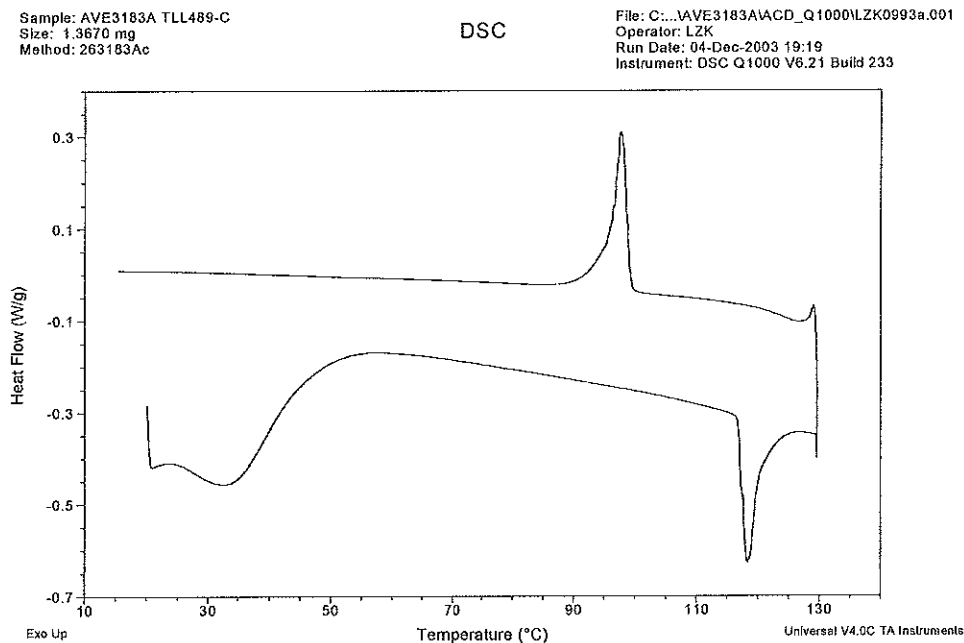


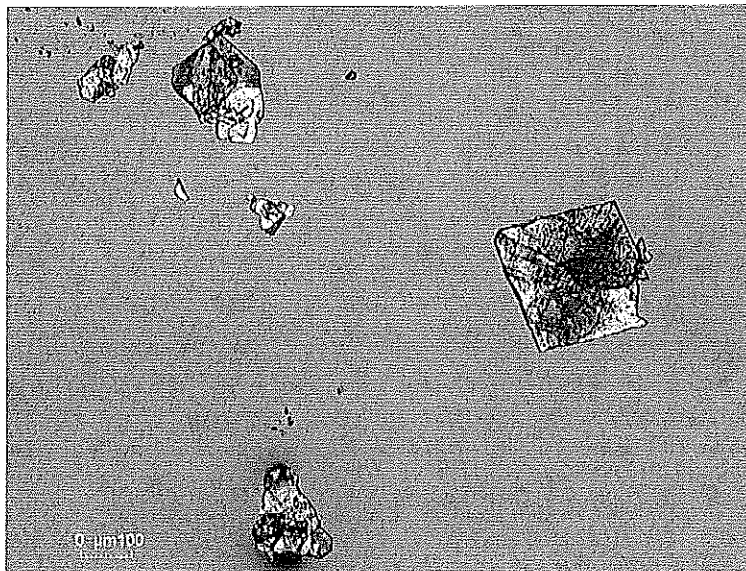
Figure 17 - DSC cycle curve of AVE3183A form A (batch TLL489-C), ref: lzk0993a



3.2.5 Optical microscopy of form A

The crystals of batch 03N-0636B (identified as physically pure form A), when observed under polarized light, are bi-refrident (*Cf.* Figure 18), which is a sign of crystallinity. Crystals have a plate-like morphology and very irregular shape; they agglomerate very easily with particle size ranging from 1 x 5 to 100 x 150 μm .

Figure 18 - Optical microscopy of AVE3183A form A (batch 03N-0636B) under polarized light, ref: lzk1000d



3.2.6 Complementary study: impact of grinding on form A

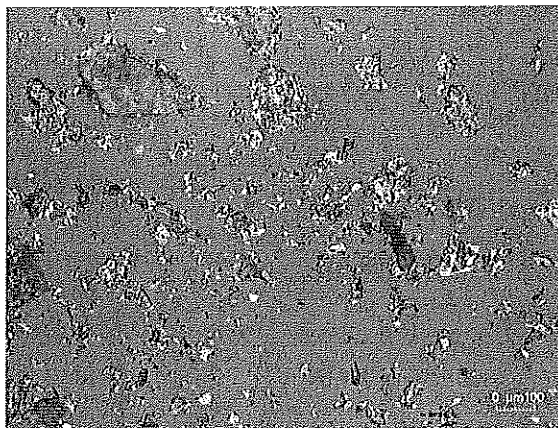
To evaluate the behavior of form A under mechanical stress and its propensity to amorphize, grinding assays were performed. The batches referenced TLL489-C and TLL489-D, available in much higher quantity and identified as physically pure form A, were used in these assays after 1 to 2 minutes of grinding in an agate mortar.

To evaluate the physical stability of this ground phase and its impact on the structural behavior of the powder in ambient conditions, a series of complementary analyses (microscopy, XRPD at ambient temperature and variable relative humidity rate, sorption/desorption DVS isotherms, DSC) was carried out.

3.2.6.1 Optical microscopy

Size reduction as a direct impact of grinding on crystal habit is clearly visible (*Cf.* Figure 19). The particles remain bi-refrident indicating that crystallinity has not been strongly altered. Numerous agglomerates are observed.

Figure 19 - Optical microscopy of AVE3183A form A (batch TLL489-C) after mortar-grinding, ref: lzk0990a

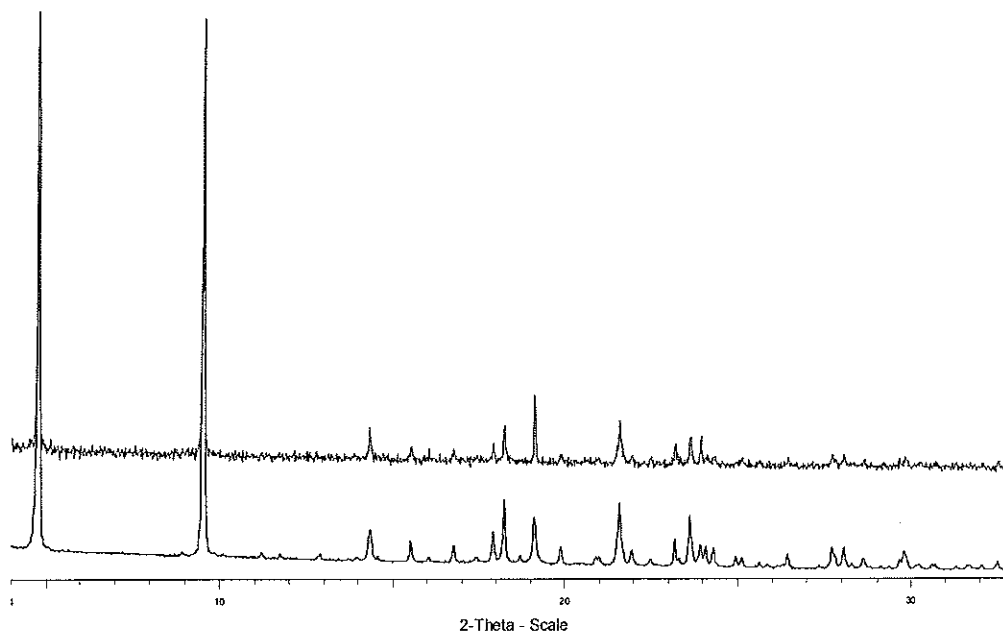


3.2.6.2 XRPD

The XRPD diagram of a ground form A sample is given in red in Figure 20. For purposes of comparison, the diagram of form A without any mechanical treatment is provided in black.

The XRPD peaks of both diagrams are very similar. Neither specific broadening nor any loss of resolution or increase in the background noise level is detected on the red diagram. The difference in signal/noise ratio is simply due to an XRPD recording time three times longer for the “as such” sample; This recording was used to perform the indexing of AVE3183A form A (Cf. section 3.2.2.1).

Figure 20 - XRPD (λ Co K α 1) diagrams of AVE3183A form A recorded at ambient T and RH: “as such” (batch P-31448-030-1) and mortar-ground (batch TLL489-C), refs.: zxs254b, lzk0985a



3.2.6.3 *Water activity isotherm (DVS)*

Two consecutive sorption/desorption cycles recorded at 25°C on ground batch TLL489-D were effected. The results are rigorously superimposable with the "as such" sample. XRPD analysis of the sample after the two cycles showed that there was no structural modification, *i.e.* that it remains di-hydrated form A.

3.2.6.4 *TGA*

Like the result obtained on the "as such" sample (*Cf.* section 3.2.4.1), a water loss of 11.27% w/w is observed in the temperature range comprised from 10 to 60°C. This weight loss is very close to the theoretical value of 11.38% for a di-hydrate form. To avoid the underevaluation of weight loss that occurred with the "as such" sample, no nitrogen flow was used in the TGA oven.

3.2.6.5 *DSC*

Here, too, the result was similar to those obtained on the "as such" sample. The milled and "as such" compounds present the same thermal behavior; this confirms the previous results indicating that no change is induced by grinding the substance.

3.2.6.6 *Conclusion for the grinding assay*

The behavior of form A under mechanical strain—in our study only 1-2 min. of mortar grinding—was examined. As shown by XRPD experiment, this mechanical treatment caused no observed polymorphic transition, and no appearance of amorphous phase was detected. Furthermore, no specific differences were observed between the physical behavior of the "as such" material and that of the compound after grinding.

3.2.7 *Physical characterization of form A - Conclusion*

Form A crystallizes in an orthorhombic system. Its XRPD diagram was indexed and its crystal structure was determined from SCD data. **It is a di-hydrate form that is physically unaltered from 50 to 85% RH. When placed in dehydrating conditions, form A transforms (easily but more or less quickly depending on the strength of dehydration conditions) into anhydrous form C, a kinetically favored transitory state in this low temperature range. Then metastable form C transforms rather promptly into anhydrous form B, which appears to be a more thermodynamically stable product of form A dehydration in the low temperature range. Above 115°C, form B reversibly transforms into form C. Forms B and C are enantiotropically related.**

When form A is subjected to a mechanical strain (e.g. mortar grinding), no structural modification takes place.

3.3 FORM B

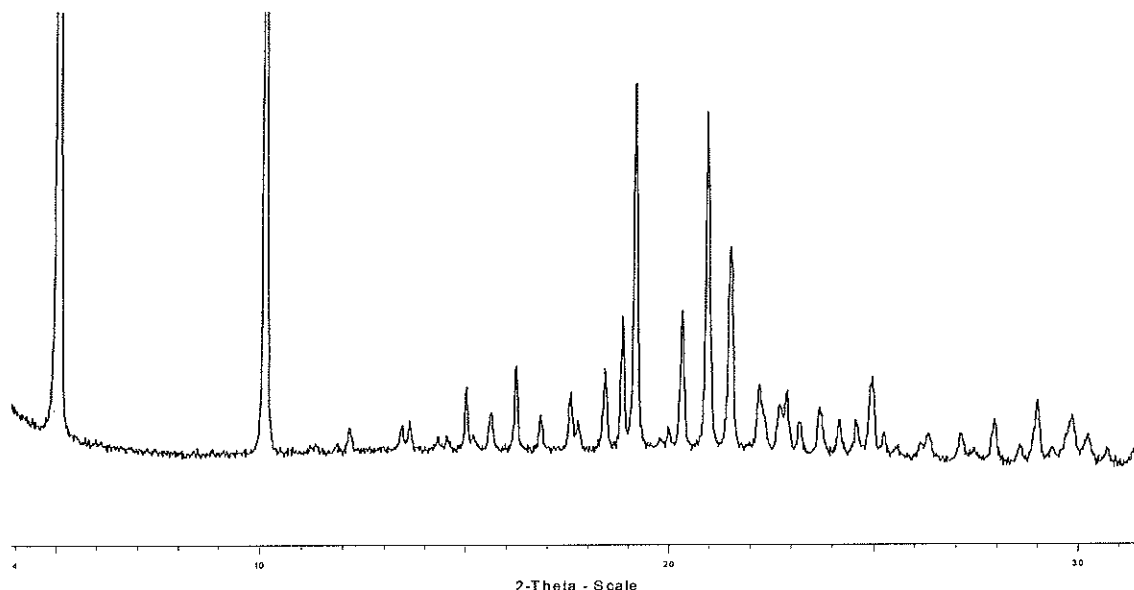
With the knowledge acquired on the physical behavior of AVE3183A form A under dehydration conditions, it was possible to set specific experimental conditions to prepare pure form B for structural investigations. Batch 03N-0636B corresponding to pure form A was used.

3.3.1 X-ray powder diffraction (XRPD)

3.3.1.1 Indexing of form B

A high-resolution XRPD Bragg-Brentano diagram of AVE3183A form B (*Cf.* Figure 21) was recorded from batch 03N-0636B (previously heated in situ to 100°C and cooled back down to room temperature) under 0% RH.

Figure 21 - High-resolution XRPD diagram (λ Cu K α 1) of AVE3183A form B recorded at ambient temperature under 0% RH (batch 03N-0636B), ref.: zxs258d



For form B of AVE3183 hemi-galactarate, the indexing result, at $T = 298$ K, is as follows:

the lattice is orthorhombic and conventional cell parameters are:

$$\begin{aligned}a &= 9.7542(9) \text{ \AA}, & V &= 2740.5(8) \text{ \AA}^3 \\b &= 35.0138(4) \text{ \AA}, & \text{density} &= 1.358 \\c &= 8.0243(7) \text{ \AA} & Z &= 4 \text{ formula units}\end{aligned}$$

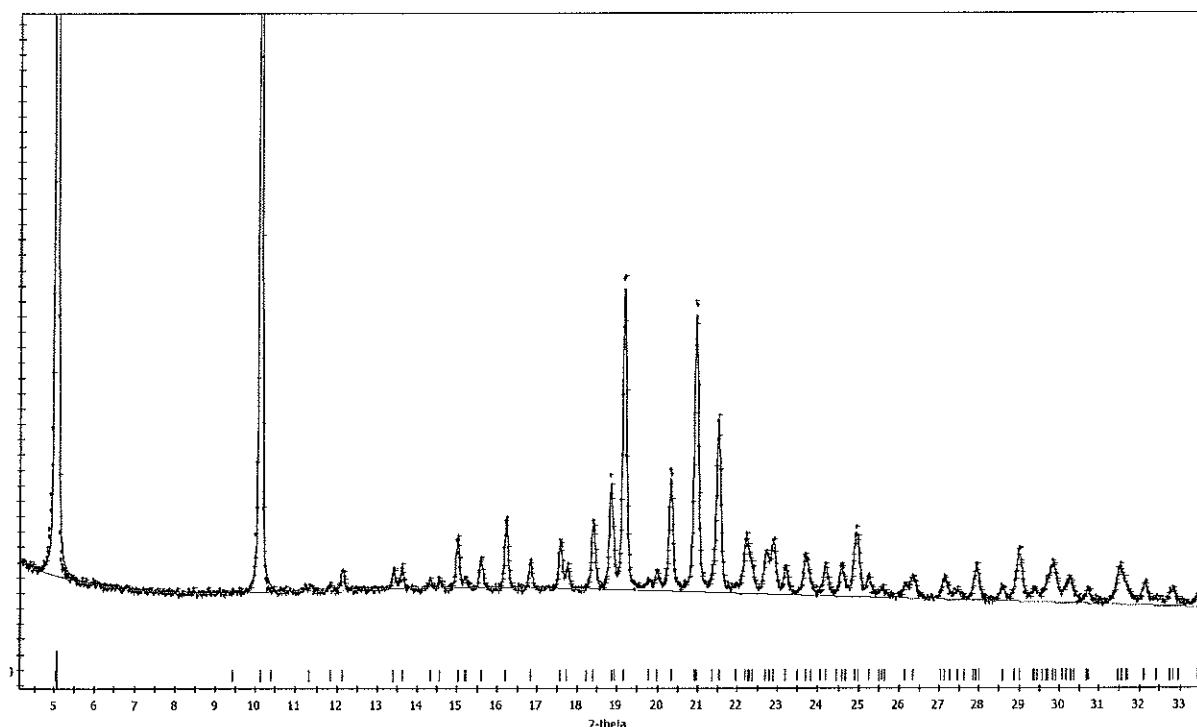
with a final R_{wp} of 4.9% after Pawley refinement (*Cf.* Figure 22).

Study of the systematic extinctions indicates that the space group is $P2_12_12_1$ ($Z = 4$ formula units).

As all the lines present on the high-resolution XRPD diagram, ref: zxs258d, are indexed (Cf. Figure 22), **form B** (obtained from batch 03N-0636B after dehydration process by heating at 100°C and then residence at 0% RH at room temperature) is a **pure crystalline phase**.

Complete indexing of AVE3183A form B XRPD lines, given in d-spacings and in 2θ positions $\lambda_{Cu}K\alpha$ and $\lambda_{Co}K\alpha$, is to be found in Appendix 4.

Figure 22 - Pawley refinement of the high-resolution XRPD diagram ($\lambda_{Cu} K\alpha 1$) of AVE3183A form B



3.3.1.2 XRPD crystal structure determination of form B

High-resolution X-ray powder diffraction (XRPD) is an alternative to single-crystal diffraction for determining the crystal structure of organic materials. In the case of drug compounds, for which it is difficult to obtain single crystals of suitable quality and size, and of metastable phases (polymorph or pseudo-polymorph), it is even the only solution. In the last 10 years, spectacular progress has been made in the development of direct space methods¹⁷. In this approach the difficult problem of extracting unambiguous integrated intensities is implicitly avoided by directly comparing diagrams calculated from postulated structural models with the envelope of the experimental powder diffraction pattern.

¹⁷ K.D.M. Harris and M. Tremayne, *Chem. Mater.* (1996) 8, 2554

In the present study the program *PowderSolve*¹⁸, as implemented under Accelrys's Materials Studio ReflexPlus module, version 3.1, was used. Due to the complexity of the AVE3183 hemigalactarate system (with 3 independent molecules in the asymmetric unit), the parallel tempering algorithm¹⁹ was chosen.

A realistic and relevant structural solution depends on the quality of two major XRPD diagram components: peak positions and intensities. Here, monochromatic Bragg-Brentano diffraction data guarantee the best resolution and precision in the determination of peak position, the key to successful indexing and thus to the obtainment of cell parameters (*Cf.* section 3.3.1.1). To collect the reliable intensities necessary to ensure the correct location of the atoms in the lattice, the preferred orientation effects of the crystallites have to be reduced to a minimum. To this end, the Debye-Scherrer transmission geometry was chosen, using a ground powder of form A introduced in a rotating, low-absorption Lindemann glass capillary of 0.5 mm diameter. The complete dehydration of form A (batch 03N-0636B) into form B was obtained by maintaining the filled glass capillary plunged into a beaker of hot water (365 K) under a dynamic primary vacuum for one night. The capillary was then flame-sealed to prevent unwanted rehydration of form B into form A.

The high-resolution XRPD Debye-Scherrer diagram of AVE3183A form B is shown in Figure 23. This is the sum of 6 consecutive equivalent recordings carried out to make sure that there was no structural modification over time.

As shown in Figure 23, scattering from the capillary glass induces strong nonlinear background noise in the pattern.

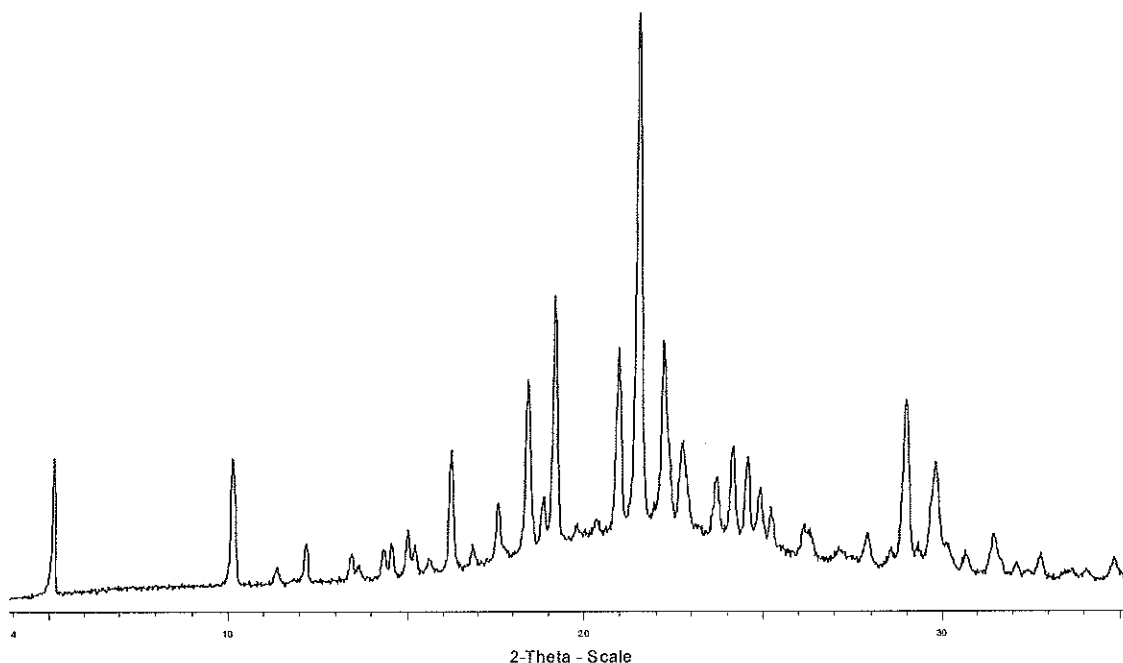
Based on the molecular geometries already obtained from form A single-crystal XRD data (*Cf.* section 3.2.1), trial crystal structures were then generated by stochastic movements of two AVE3183 molecules and one galactarate molecule within the previously indexed crystal cell (*Cf.* section 3.3.1.1). Intermolecular torsion angles were allowed to vary alternately with the orientation and position of the molecular fragment within the asymmetric unit. 27 structural degrees of freedom had to be taken into account: (3 molecular translations + 3 molecular rotations) for each of the 3 independent molecules + 9 intermolecular torsions (2 for each AVE3183 molecule and 5 for galactarate molecule). For each trial structure, a powder pattern was simulated and then compared to the experimental data. The acceptance criterion of each

¹⁸ G.E. Engel, S. Wilke, O. König, K.D.M. Harris, F.J.J. Leusen, *J. Appl. Cryst.* (1999) **32**, 1169

¹⁹ The parallel tempering procedure uses several reference structures in parallel, each evolving at a constant simulation temperature. The temperatures are chosen such that the system with the highest temperature is able to search the complete conformational space, whereas the system with the lowest temperature performs a local search for the best structure within the neighborhood of a deep minimum. Structures are exchanged between systems using so-called swap moves. The effect of swap moves is twofold. First, favorable structures found in systems with higher temperatures are passed to systems with lower temperatures and more local search horizons. Then, swap moves ensure that low temperature systems do not get trapped in a local minimum. For more details about the parallel tempering algorithm see: M. Falcioni and M.W. Deem, *J. Chem. Phys.* (1999), **110**, 1754.

successive structural model was defined by a lower R_{wp} profile factor. An acceptance probability is used for trial structures with an unfavorable R_{wp} profile factor.

Figure 23 - High-resolution XRPD "Debye-Scherrer" diagram (λ Cu K α 1) of AVE3183A form B, ref: lzk1002a



The Accelrys Materials Studio ReflexPlus module was then used to apply Rietveld²⁰ refinement in order to optimize the best solution, *i.e.* the one with the lowest R_{wp} profile factor.

The final structure model must fit satisfactorily both with the X-ray powder diffraction data and with the physical meaning of the molecular packing.

The final crystal structure of AVE3183A form B obtained had a Rietveld R_{wp} profile factor of 7.5%. Figure 24 shows the close agreement between the experimental XRPD diagram and the simulated pattern of the solution. As the Rietveld refinement was applied only to rigid bodies (defined by the torsions within the molecule), and not to individual atoms, even better agreement could most certainly have been obtained. Nevertheless, the absence of overlaps between molecules and the presence of appropriate hydrogen bonds in the physical model generated in this manner underpin the physical reality of the mathematical solution. The atoms' coordinates are given in Appendix 5. Crystal unit cell and crystal packing are shown in Figure 25 and Figure 26 respectively.

²⁰ « A profile refinement method for nuclear and magnetic structures » H.M. Rietveld, *J. Appl. Cryst.* (1969) 2, 65.

Figure 24 - Rietveld plot of AVE3183A hemi-galactarate form B (λ Cu $K\alpha$ 1)

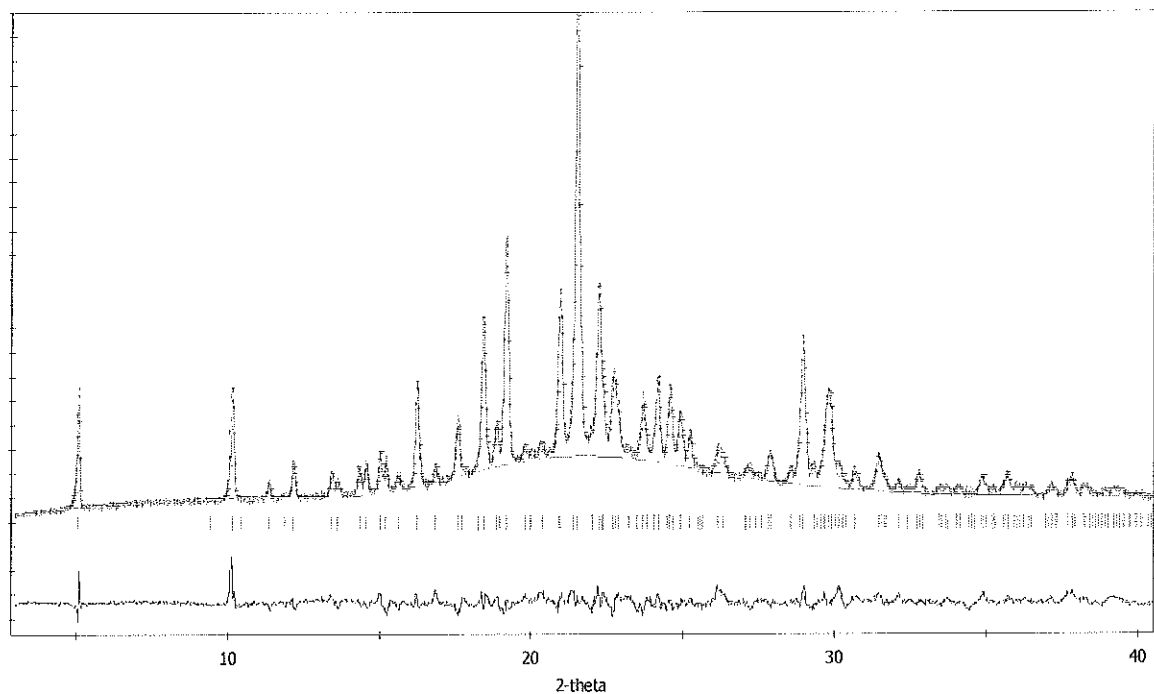


**Figure 25 - Form B of AVE3183A hemi-galactarate – Crystal unit cell
(grey: carbon, red: oxygen, blue: nitrogen, white: hydrogen)**

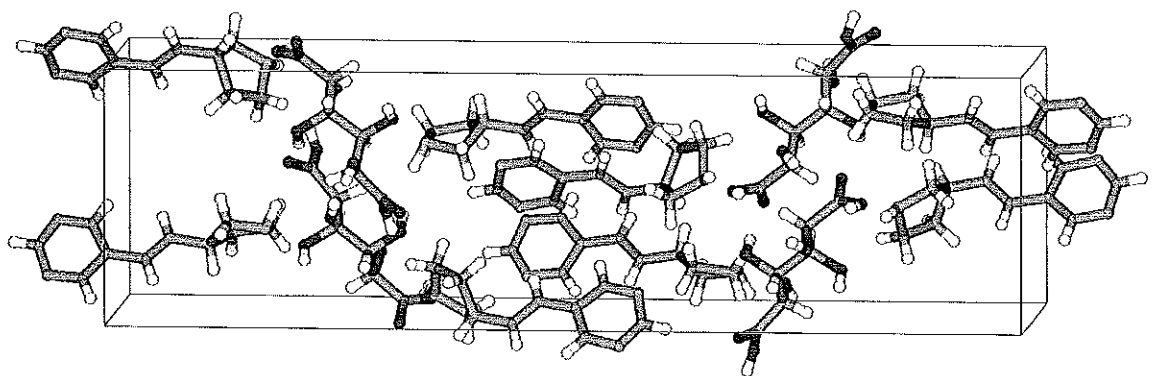
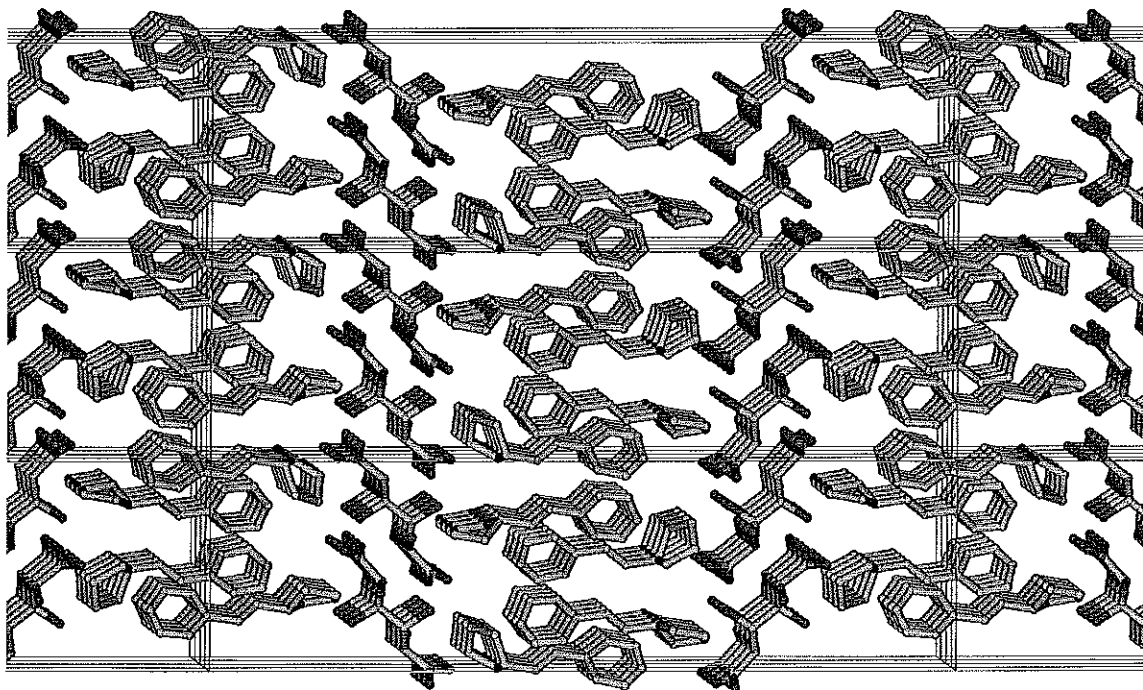


Figure 26 - Form B of AVE3183A hemi-galactarate – Crystal packing
(grey: carbon, red: oxygen, blue: nitrogen)
For easier readability hydrogen atoms are not represented



As for form A, the crystal structure of anhydrous form B shows layer stacking (*Cf.* Figure 26). The two distinct layers are again observed. Nevertheless, while the galactarate molecule layer is rigorously superimposable on those observed for form A, the AVE3183 molecular edifices have here undergone significant structural changes. Of course, the removal of the water molecules induces a reorganization of the AVE3183 molecule packing: the AVE3183 molecule layer loses thickness, and AVE3183 molecules no longer straddle each other. Instead, they now line up parallel and interact through strong intermolecular Van der Waals overlapping and “ $\pi - \pi$ ” stacking developed between the vinylic insaturation and the pyrimidine cycle rather than between identical functional groups as in form A.

In comparison with form A crystal structure this structural reorganization is expressed through a strong anisotropic contraction of the form B crystal cell along the long cell parameter (37.17 vs. 35.01 Å for the *c* cell parameter of form A and form B respectively).

3.4 FORM C

Dehydration of form A upon heating from room temperature gives anhydrous form B. Above 115°C, form B reversibly transforms into anhydrous form C. Forms B and C are enantiotropically related.

Below 130°C, it was impossible to obtain pure form C. We were able to produce form C by heating a form A sample (batch 03N-0636B) to 130°C in situ in an XRPD TTK-450 chamber. This, however, was with the Bragg-Brentano geometry, which is not suitable for XRPD crystal structure determination as was used to solve form B crystal structure (*Cf.* section 3.3.1.2). Thus, only XRPD diagram indexing was performed on form C.

3.4.1 X-ray powder diffraction (XRPD)

3.4.1.1 Indexing of form C

A high-resolution XRPD Bragg-Brentano diagram of AVE3183A form C (*Cf.* Figure 27) was recorded from batch 03N-0636B heated and maintained at 130 °C under 0% RH.

For form C of AVE3183 hemi-galactarate, the indexing result, at $T = 403$ K, is as follows:

the lattice is orthorhombic and conventional cell parameters are:

$$\begin{array}{ll} a = 10.2625(7) \text{ \AA}, & V = 2790.0(1) \text{ \AA}^3 \\ b = 33.1412(3) \text{ \AA}, & \text{density} = 1.334 \\ c = 8.0231(4) \text{ \AA} & Z = 4 \text{ formula units} \end{array}$$

with a final R_{wp} of 3.13% after Pawley refinement (*Cf.* Figure 28).

Study of the systematic extinctions indicates that the space group is $P2_12_12_1$ ($Z = 4$ formula units).

As all the lines present on the high-resolution XRPD diagram are indexed (*Cf.* Figure 28), **form C** (batch 03N-0636B after dehydration by residence at 130°C under 0% RH) **is a pure crystalline phase.**

**Figure 27 - High-resolution XRPD diagram (λ Cu K α 1) of AVE3183A form C
recorded at 130°C and 0% RH (batch 03N-0636B), ref.: lzk1159a**

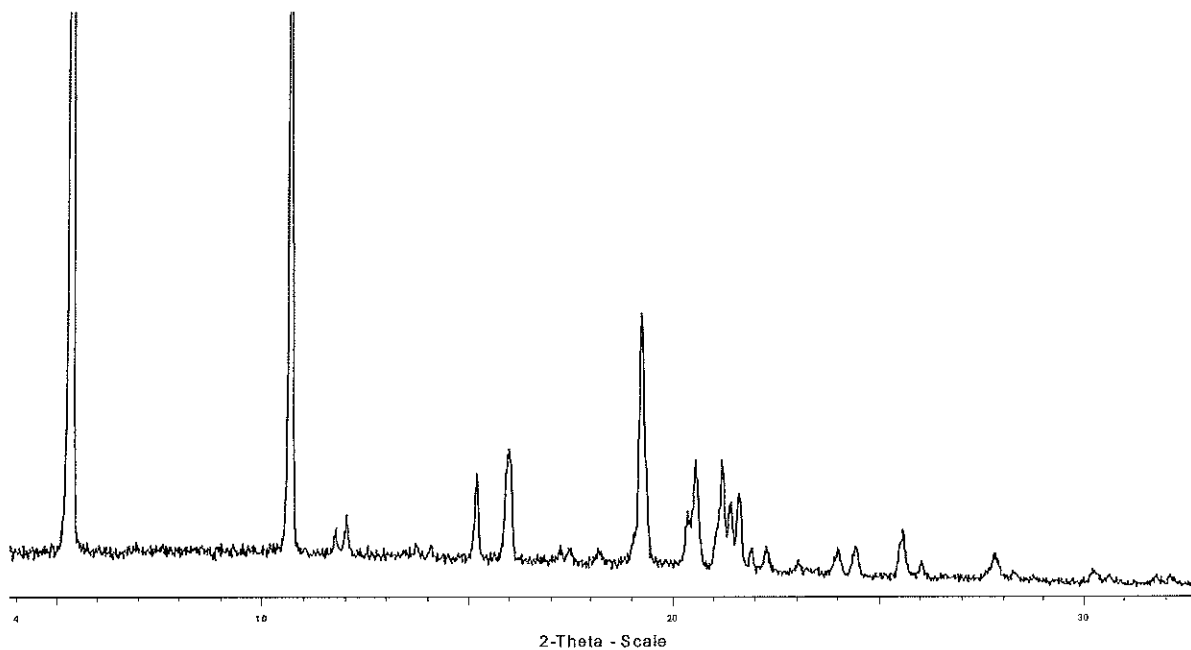
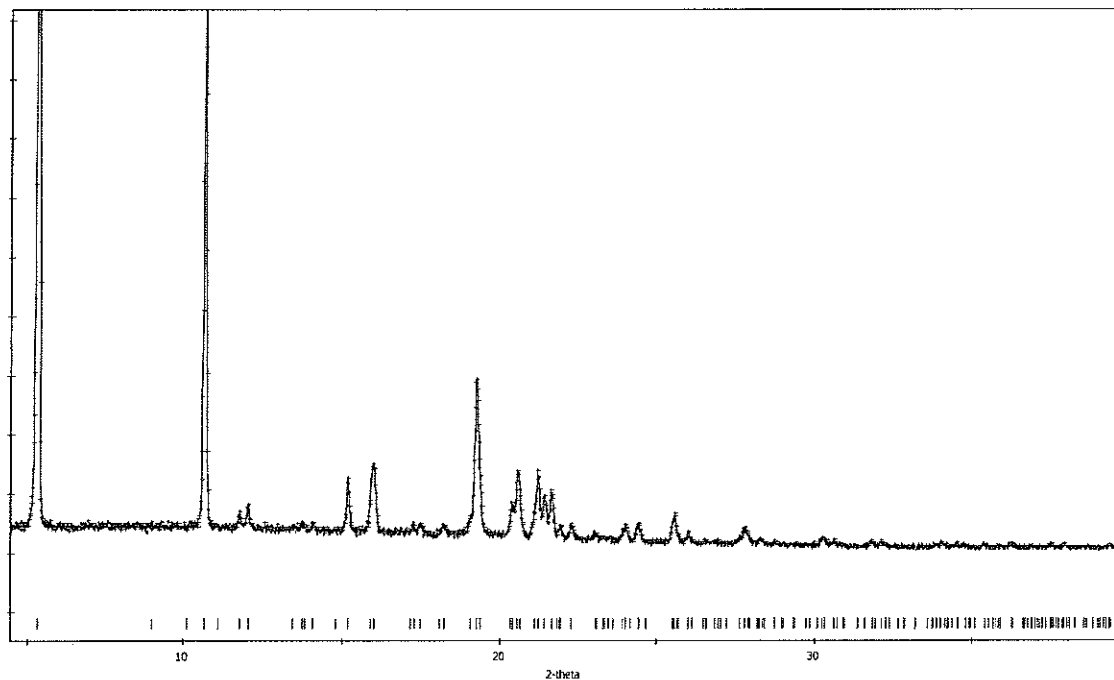


Figure 28 - Pawley refinement of the high-resolution XRPD diagram (λ Cu K α 1) of AVE3183A form C



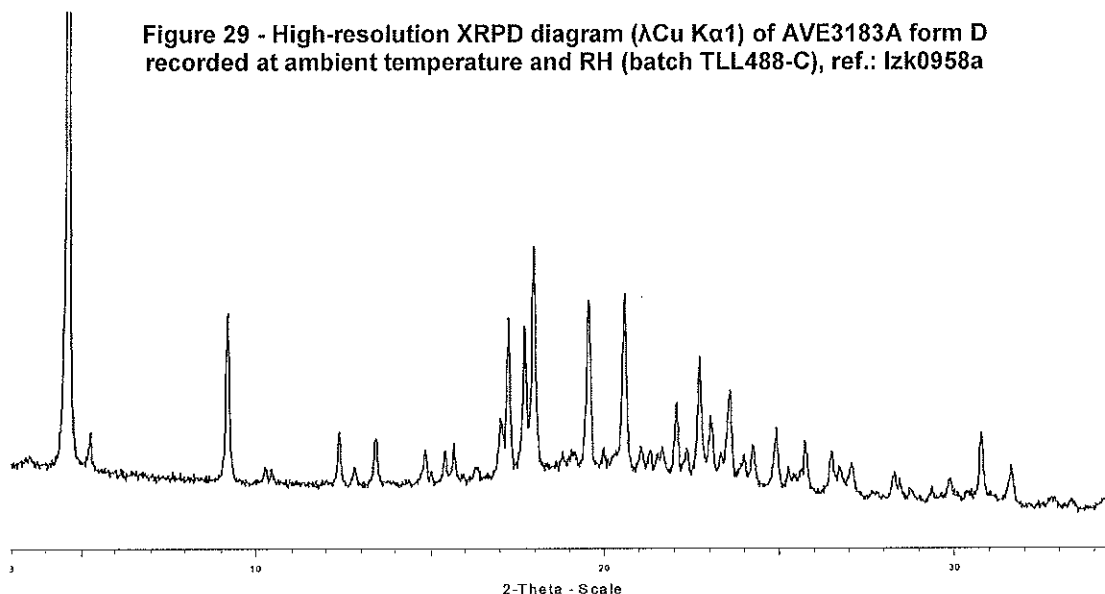
3.5 FORM D

During the AVE3183A polymorphism screening (*Cf.* section 3.1), form A (di-hydrate) was isolated pure under many different conditions, or in a mixture with anhydrous forms B and C. Surprisingly, when the solvent transfer method based on redissolution in azeotropic mixtures was used (*Cf.* Table 1), a new form denoted D appeared. Form D is always obtained in mixture with forms A, B or C in various proportions. To physically characterize this new form, batch TLL488-C was used. This batch was previously identified with low resolution XRPD equipment as unmixed form D.

3.5.1 X-ray powder diffraction (XRPD)

3.5.1.1 Indexing of form D

A high-resolution XRPD Bragg-Brentano diagram of AVE3183A form D (*Cf.* Figure 29) was recorded from batch TLL488-C.



For form D of AVE3183 hemi-galactarate, the indexing result, at $T = 295$ K, is as follows:

the lattice is monoclinic and *conventional* cell parameters are:

$$\begin{aligned}a &= 19.3877(7) \text{ \AA}, & \beta &= 92.632(2)^\circ \\b &= 7.7306(3) \text{ \AA}, & V &= 2840.2(2) \text{ \AA}^3 \\c &= 18.9699(4) \text{ \AA} & Z &= 2 \text{ or } 4 \text{ formula units}\end{aligned}$$

with a final R_{wp} of 4.0% after Pawley refinement (*Cf.* Figure 30).

Study of the systematic extinctions indicates that the space group is either $P2_1$ ($Z = 2$ formula units) or $P2_1/m$ ($Z = 4$ formula units). As AVE3183 is a chiral molecule, it is impossible for it to crystallize in a space group containing either a mirror (or glide) plane or a center of symmetry. Thus, for AVE3183 form D, only the $P2_1$ space group is allowed.

Figure 30 - Pawley refinement of the high-resolution XRPD diagram ($\lambda\text{Cu K}\alpha_1$) of AVE3183A form D
black arrows indicate unindexed XRPD lines

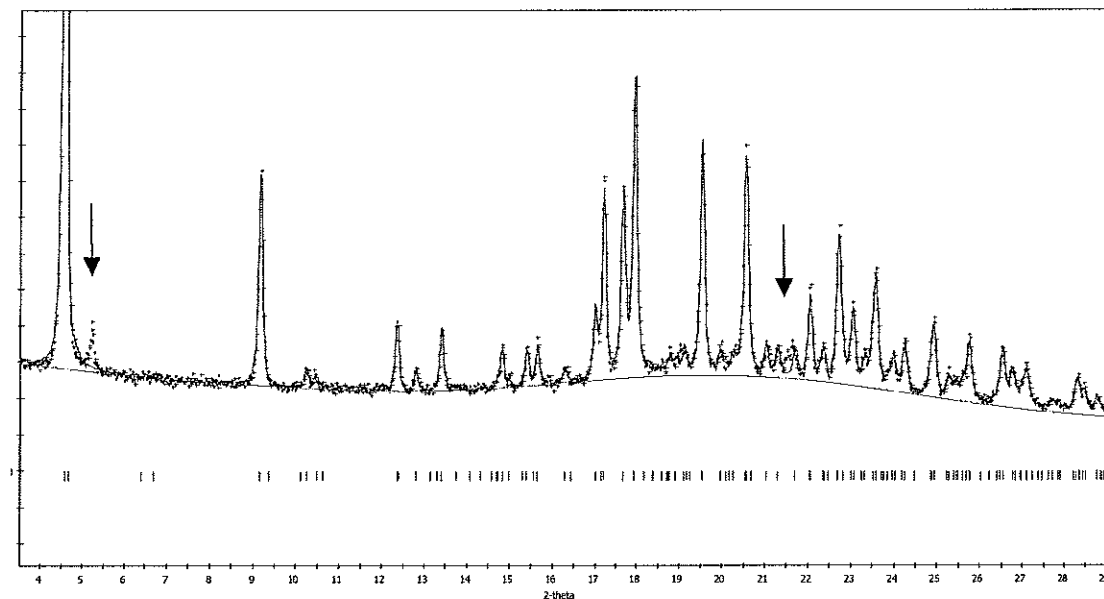
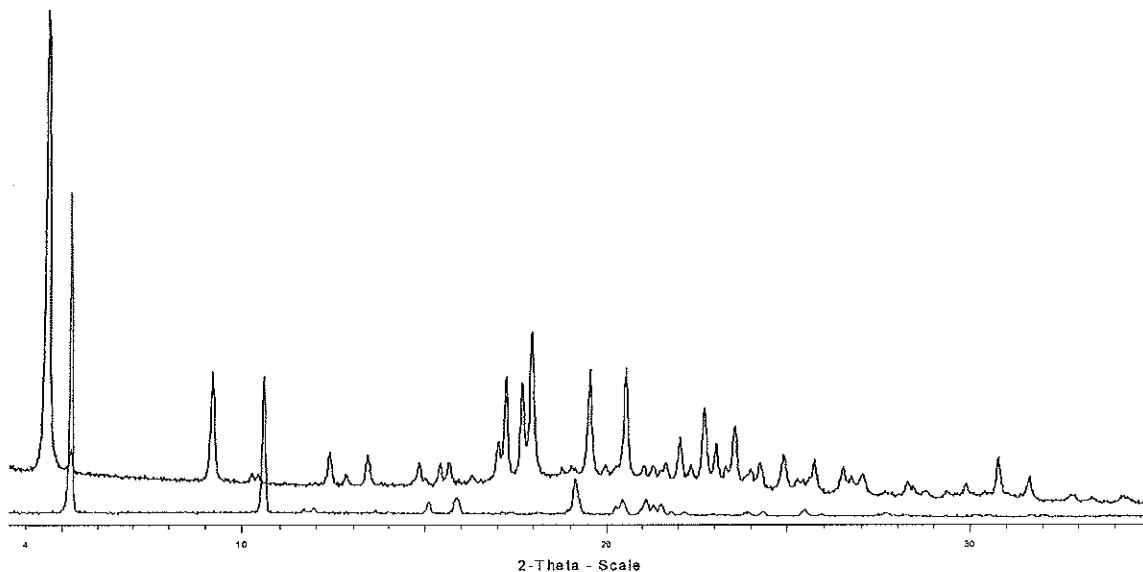


Figure 31 - Superimposition of high-resolution XRPD diagrams ($\lambda\text{Cu K}\alpha_1 = 1,5406 \text{ \AA}$) of form C
recorded at 130°C (batch 03N-0636B) and of form D recorded under ambient conditions
(batch TLL488-C), refs: lzk1159a, lzk0958a



All but two diffraction lines present on the high-resolution XRPD diagram are indexed and correspond to **form D** (batch TLL488-C) (*Cf.* Figure 30). The two unindexed diffraction lines belong to form C, as indicated in Figure 31, where form C and form D high-resolution XRPD diagrams are superimposed.

Complete indexing of AVE3183A form D XRPD lines, given in d-spacings and in 2θ positions $\lambda_{Cu}K\alpha$ and $\lambda_{Co}K\alpha$, is to be found in Appendix 6.

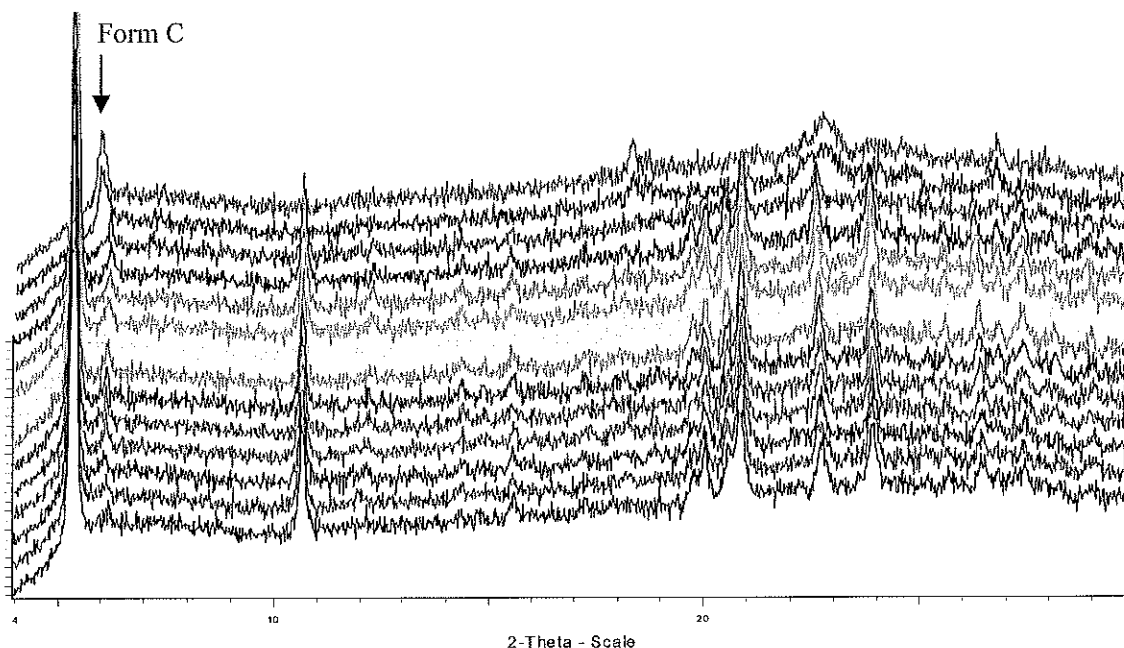
3.5.1.2 Variable temperature T-XRPD of form D

The structural behavior of form D (batch TLL488-C) under heating was investigated in situ by variable T-XRPD in a nitrogen flow (*Cf.* Figure 32). The temperature was raised by 10°C steps from room temperature to 160°C. Up to 130°C no perceptible modification of the XRPD diagram other than a normal heat-induced dilatation of the crystal lattice was observed. Above 130°C a significant and continuous structural evolution is observed, possibly reflecting the start of the thermal degradation of form D prior to its melting at around 150°C.

The constant presence of a distinct diffraction line, apparently attributable to form C, over the whole temperature range (form C melts above 160°C; its Tonset is 177.5°C) is clearly identified on Figure 32. Furthermore, in the high temperature range [130-160°C], the melt begins to recrystallize into form C.

A final XRPD diagram was recorded after cooling the sample back down to room temperature, but nothing new took place; there was no further recrystallization of the melt.

Figure 32 - T-XRPD diagrams ($\lambda_{Co} K\alpha 1\alpha 2$) of AVE3183A form D (batch TLL488-C) recorded from room temperature to 160°C in 10°C steps under a nitrogen flow, ref: zxs246b



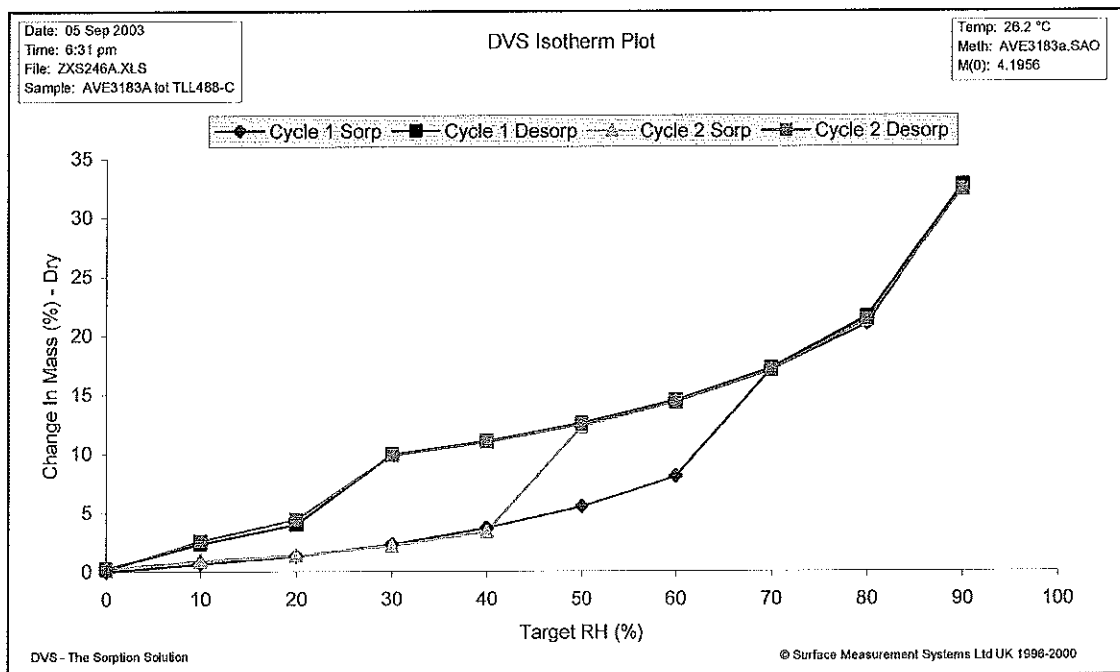
3.5.2 Water activity isotherm DVS of form D

Figure 33 shows two consecutive sorption/desorption cycles recorded at 25°C on form D (batch TLL488-C). The two cycles are different: This is characteristic of a phase transformation upon exposure to moisture.

First cycle: Between 0 and 60% RH, a continuous water uptake is observed, up to 8% (w/w) or about 1.4 mole of water; This can be attributed to water physisorption on crystal surfaces. Between 60 to 70% RH the water uptake increases considerably, reaching the value of 17% (w/w), corresponding to 3 moles of water. Beyond 80% RH, water uptake increases strongly which is a sign of the onset of deliquescence. A huge hysteresis between the sorption and desorption phases is then observed. The water uptake is totally reversible, and before going down at 0% RH, a desorption step takes place between 30 to 20% RH.

Second cycle: It is similar to DVS cycles of form A (*Cf.* section 3.2.3). The water sorption isotherm exhibits a gain in water uptake from 40% RH to a "plateau" around 50% RH, corresponding to 12% w/w. This result is in keeping with a dihydrate form (with a theoretical water uptake of 11.4% by anhydrous compound). Nevertheless, there is a slight difference when compared with form A DVS cycles: There is a weak water uptake (around 2.5% w/w) during the first RH steps of the cycle, which can once again be attributed to water physisorption on crystal surfaces.

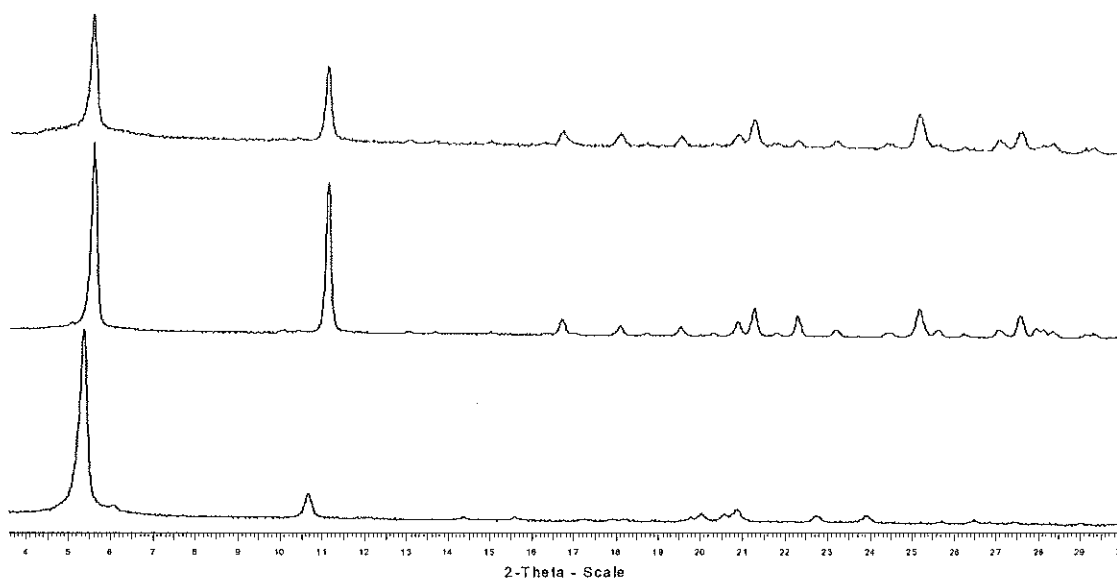
Figure 33 - DVS sorption/desorption water isotherm of AVE3183A form D (batch TLL488-C) recorded at 25°C, ref: zxs246a



After the two DVS cycles, XRPD analysis of the sample confirms that there has been a structural transformation of the sample under exposure to moisture. The XRPD pattern is characteristic of pure form A (Cf. Figure 34) as analyzed by XRPD in laboratory conditions of temperature, pressure and relative humidity ($\approx 50\%$ RH).

Thus, at ambient temperature, form D is an anhydrous compound that is stable from 0 to 60% RH. As the RH rate nears 70% RH, a phase transformation into form A dihydrate occurs. Once exposed to a RH rate greater than 70%, Form D is never reobtained by exposure to low RH rates.

Figure 34 - XRPD (λ_{Co} $K\alpha_1\alpha_2$) diagrams of AVE3183A form D (batch TLL488-C) recorded at room temperature before and after DVS, Form A pattern is given for comparison purpose, refs.: zxs246a, zxs257a, zxs241a

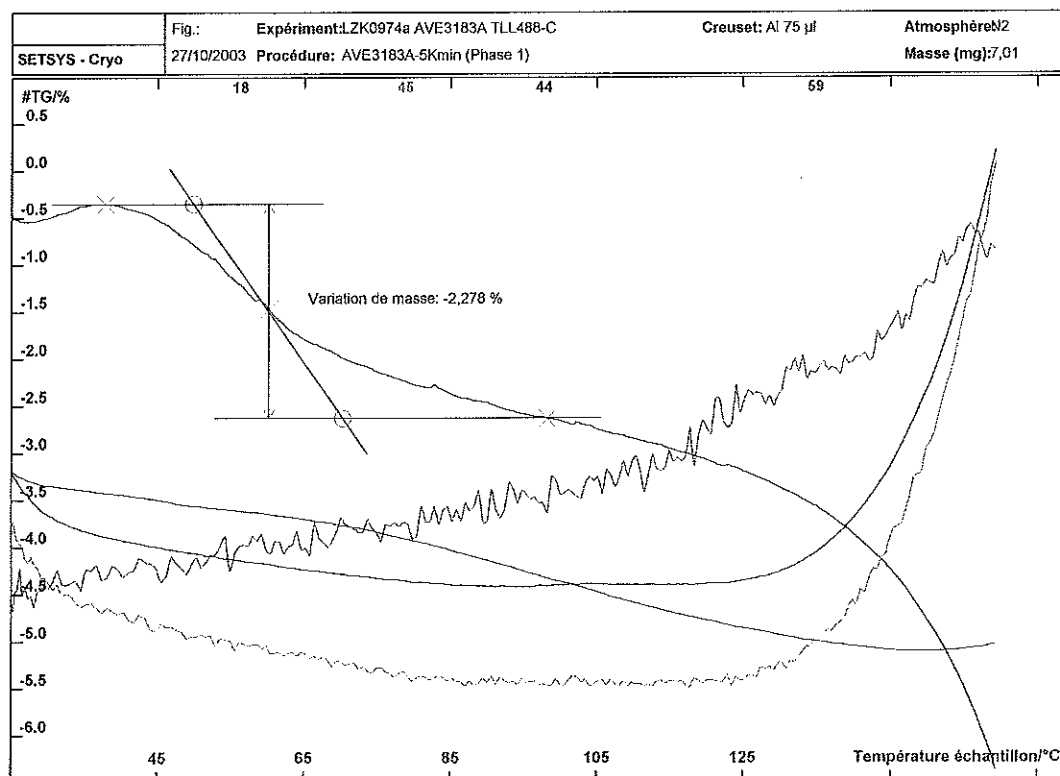


3.5.3 Thermal analysis of form D

3.5.3.1 Simultaneous TGA-MS

Simultaneous TGA-MS analysis of powder drawn from batch TLL488-C is given in Figure 35. The TGA signal (blue curve) indicates a mass loss of 2.3% between 40 and 100°C. The simultaneous MS recording shows that only water is detected at this level of temperature. As shown in the DVS experiment, this corresponds to the removal of physisorbed water molecules on crystal surface. Beyond 130°C a continuous loss of mass, accelerating after 145°C, is recorded. The MS signal then corresponds to the m/e values: « 44 », characteristic of CO_2 emission, « 45 and 59 », characteristic of the solvent propanol in mixture with water, in which form D has been crystallized. Thus form D, which is stable from ambient temperature, begins to melt and decompose at 130°C.

Figure 35 - Simultaneous TGA-MS analysis of AVE3183A form D (batch TLL488-C), ref: lzk0974a
For easier readability "m/e" signals are represented on different scales



3.5.3.2 DSC

The DSC recording is given in Figure 36. Three endotherms are detected with onsets measured at 13.6°C, 78.4°C and 152.8°C. The first endotherm corresponds to the departure of adsorbed water; it begins close to 10°C, confirming that the departure of water molecules occurs rapidly when the sample is under DSC measurement conditions.

The second endotherm is very tiny (Cf. Figure 37) and could possibly be attributed to the non-equilibrium transformation of form C traces into form B. No other DSC experiments can be performed at this time due to the unavailability of form D.

The last endothermic peak is massive and corresponds to the melting/decomposition of form D.

Figure 36 - DSC curve of AVE3183A form D (batch TLL488-C), ref: zxs246a

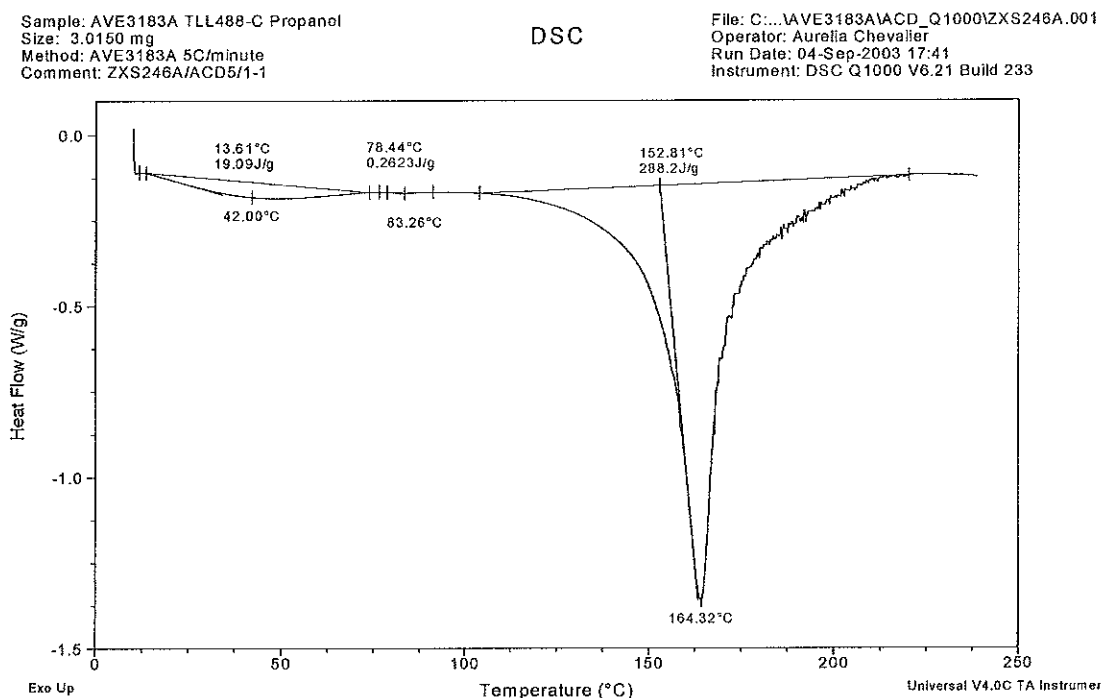
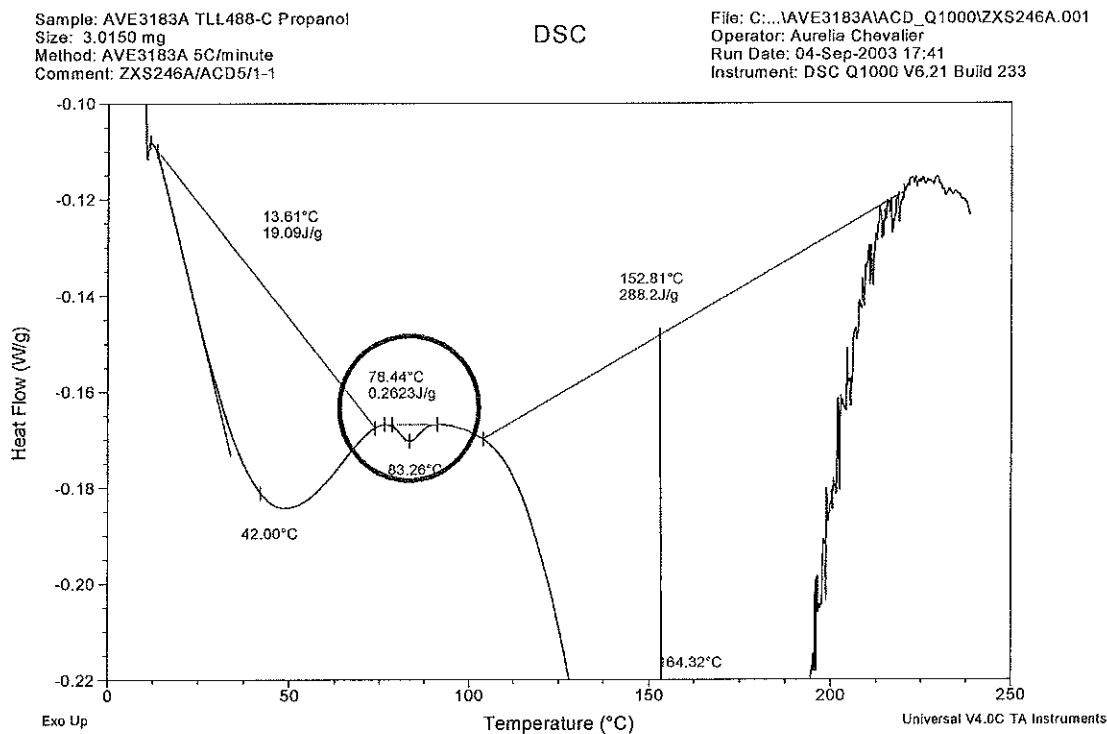


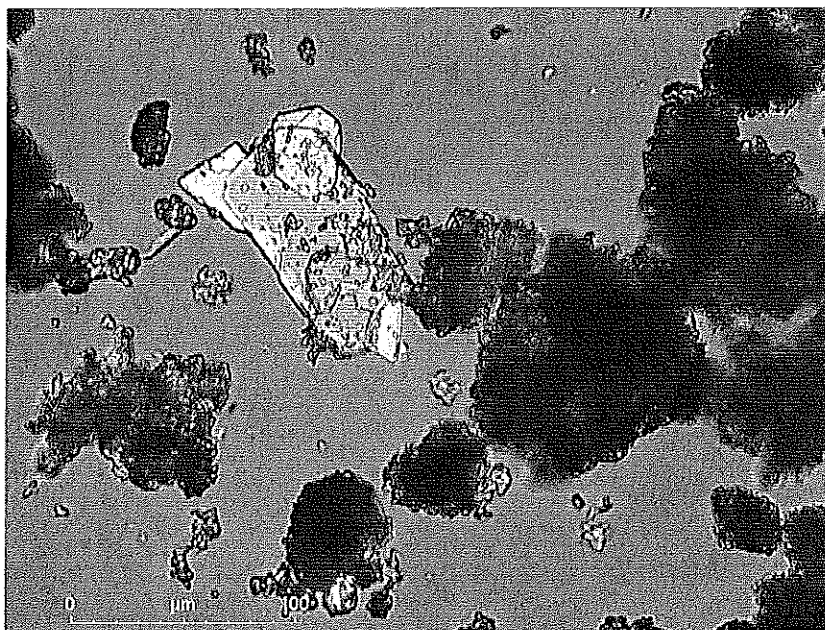
Figure 37 - Expanded DSC curve of AVE3183A form D (batch TLL488-C), ref: zxs246a



3.5.3.3 Optical microscopy

When observed under polarized light, the crystals of batch TLL488-C (identified as physically pure form D with a slight amount of form C) are bi-refringent (*Cf.* Figure 38), which is a sign of crystallinity. The particles, composed of small irregular crystals with particle sizes ranging between 15 x 15 μm and 35 x 80 μm , agglomerate very easily.

Figure 38 - Optical microscopy of AVE3183A form D (batch TLL488-C) under polarized light, ref: lzk0994a



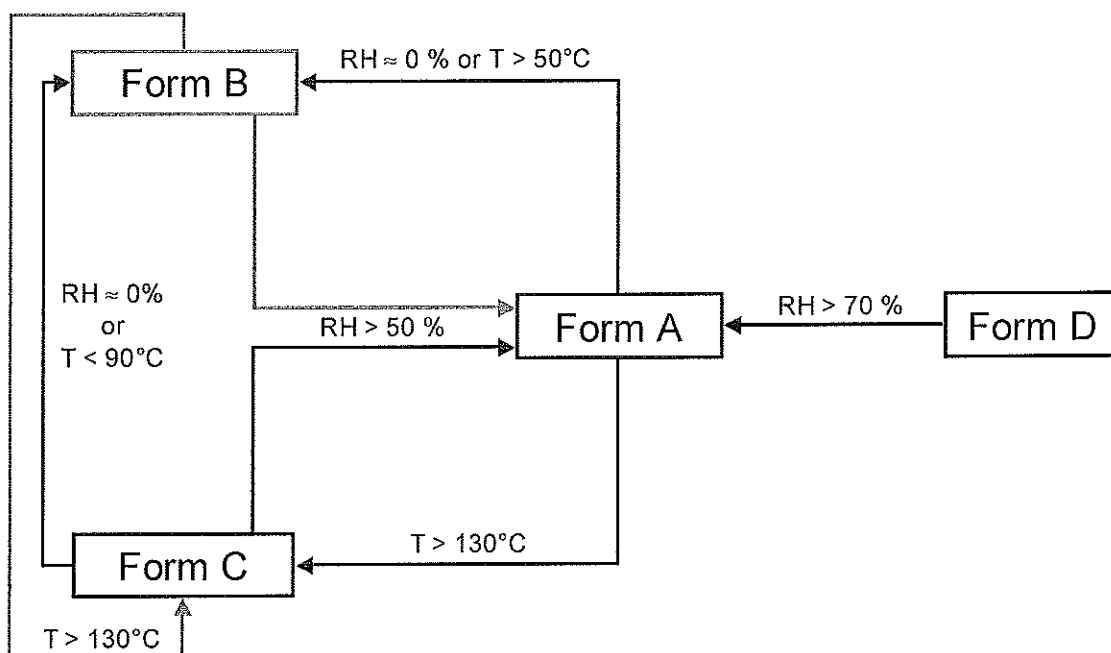
3.5.4 Physical characterization of form D - Conclusion

Form D crystallizes in a monoclinic system and its XRPD diagram was indexed. It is **an anhydrous compound that is stable up to melting, and at room temperature from 0 to 60% RH**. As soon as the RH rate goes beyond 70% RH, a phase transformation into form A dihydrate occurs. Once exposed to RH rate greater than 70%, Form D is never reobtained by exposure to low RH rates.

3.6 POLYMORPHISM SCREENING SUMMARY

In Figure 39 are summarized the solid forms of AVE3183A hemi-galactarate identified so far under the experimental conditions allowing passage from one form to another.

Figure 39 - AVE3183A solid forms identified so far under the experimental conditions allowing passage from one form to another



3.7 OPTIMIZATION OF STORAGE CONDITIONS OF FORM A

The physical characterization of AVE3183A form A shows that this solid form is highly sensitive to the ambient conditions of temperature and relative humidity. With a view to investigating the impact of temperature on the RH stability domain of AVE3183A form A (dihydrate) so as to optimize the storage conditions of form A batches, water sorption/desorption (DVS) experiments were performed at different temperatures.

Taken into account the limitations of the DVS apparatus, the temperature range chosen was 10 to 40°C²¹. The DVS results are given in Figure 40, Figure 41 and Figure 42 respectively for the three temperatures tested: 10, 25 and 40°C.

²¹ The water condensation generated at 4°C can damage the DVS measurement probes.

No significant modification was detected at any of the temperatures used. The DVS cycles recorded at 10°C are very similar to those acquired at 25°C. These cycles have been described in detail in 3.2.3. The only slight difference at 40°C, compared to the recordings made at the two lower temperatures, is a 5% shift of the desorption curve toward the higher RH rates.

These observations suggest that decreasing storage temperature does not extend the RH stability range of form A. Thus, batches of AVE3183A form A can be confidently stored at 25°C, if the relative humidity rate is greater than 50%.

Figure 40 - DVS sorption/desorption water isotherm of form A (batch 03N-0636B) recorded at 10°C, ref: lzk1034b

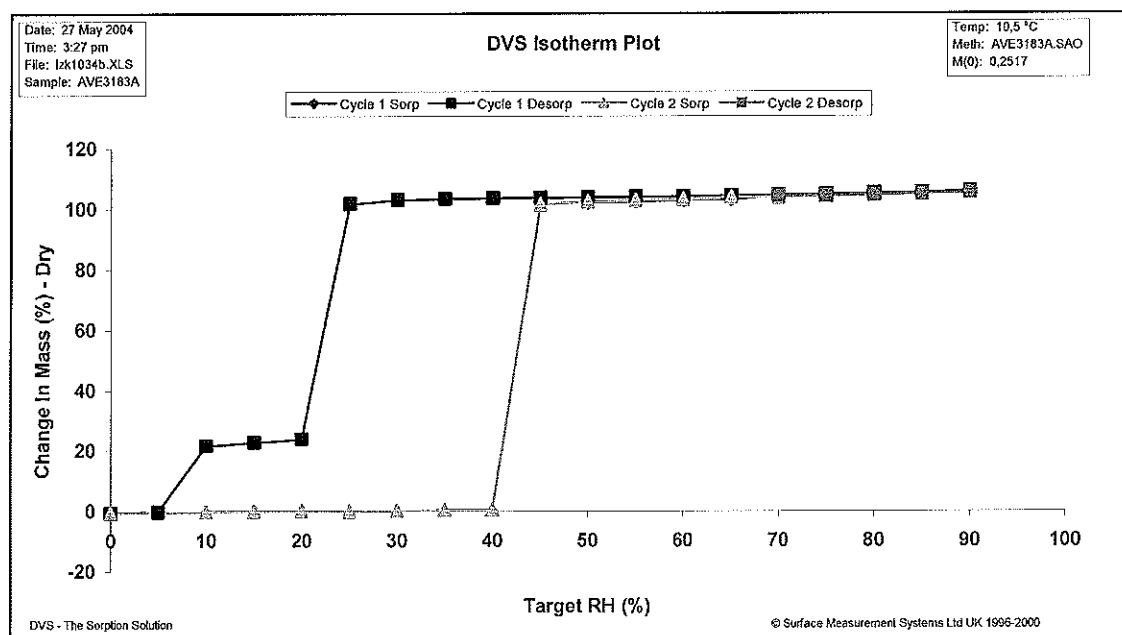


Figure 41 - DVS sorption/desorption water isotherm of form A (batch TLL489-D) recorded at 25°C, ref: lzk0982a

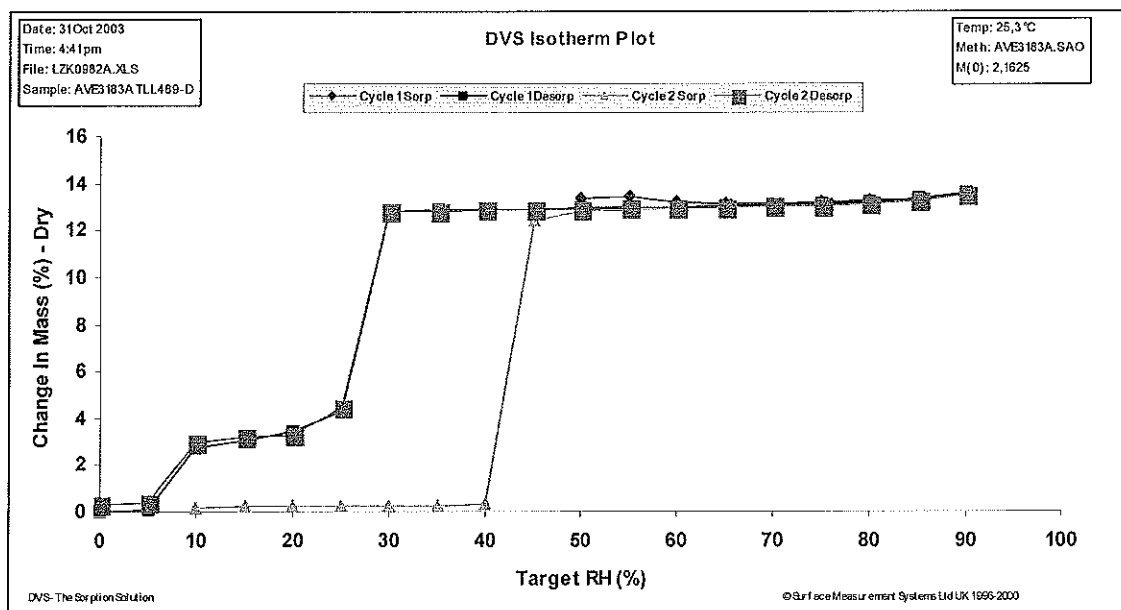
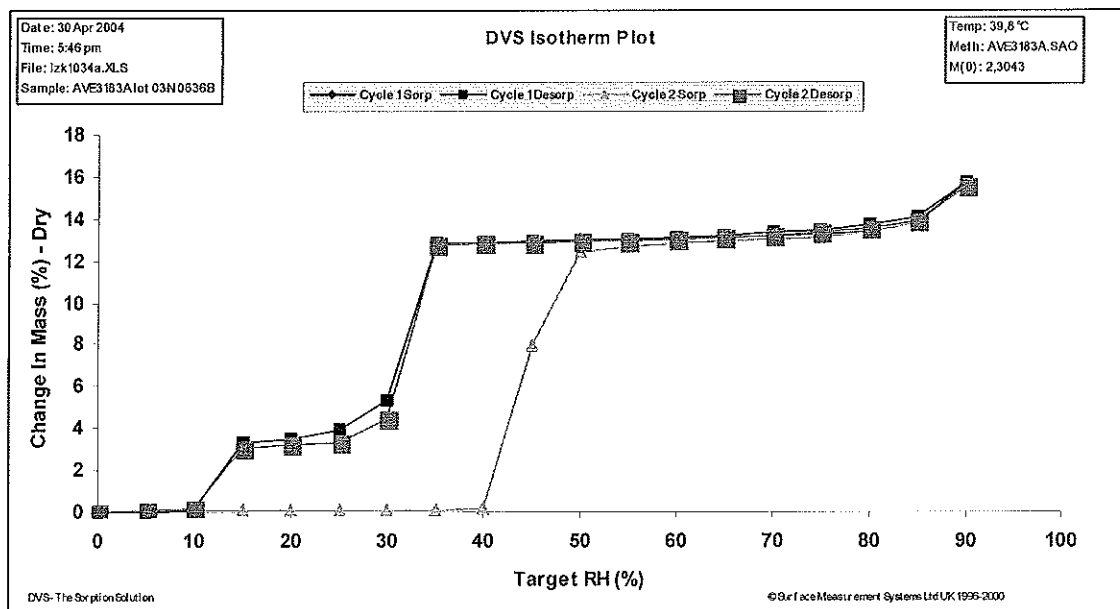


Figure 42 - DVS sorption/desorption water isotherm of form A (batch 03N-0636B) recorded at 40°C, ref: lzk1034a



4 CONCLUSION

To date, four crystalline forms of AVE3183A (hemi-galactarate) have been identified. They have been denoted A, B, C and D. Full physical characterization of these four solid forms reveals that:

- **Form A** crystallizes in an orthorhombic system. Its XRPD diagram was indexed and its crystal structure was determined from SCD data. **It is a dihydrate form that is physically unaltered from 50 to 85% RH. When placed in dehydrating conditions, form A transforms** (easily but more or less quickly depending on the strength of dehydration conditions) **into anhydrous form C**, a kinetically favored transitory state in this low temperature range. **Then metastable form C transforms rather promptly into anhydrous form B**, which appears to be a more thermodynamically stable product of form A dehydration in the low temperature range. Above 115°C, form B reversibly transforms into form C. Forms B and C are enantiotropically related.

When form A is subjected to a mechanical strain (e.g. mortar grinding), no structural modification takes place. In addition, since form A is sensitive to the RH rate, its XRPD diagram will be characteristic of pure form A only if the XRPD analysis is effected in the 50 – 80% RH range. If the RH rate is under 50% the XRPD diagram will show a mixture of forms A and B, plus possibly form C. In this case, pure form A can easily be reobtained by simply exposing the sample to a relative humidity rate within the range 50 - 80 for a couple of minutes, as described in report CMC-PA-2004-04429 FR3.0.

- **Form B** crystallizes in an orthorhombic system. Its XRPD diagram was indexed and its crystal structure was determined from XRPD data using the “direct space” method. It is an **anhydrous form**. At room temperature form B transforms into form A under RH rates higher than 50%. Above 115°C, form B reversibly transforms into form C. Forms B and C are enantiotropically related.
- **Form C** crystallizes in an orthorhombic system and its XRPD diagram was indexed. **It is a high-temperature anhydrous form**. Below 115°C, form C reversibly transforms into form B. Forms B and C are enantiotropically related.
- **Form D** crystallizes in a monoclinic system and its XRPD diagram was indexed. **It is an anhydrous form that is stable up to melting, and at room temperature from 0 to 60% RH**. As soon as the RH rate goes beyond 70% RH, a phase transformation into form A dihydrate occurs. Once exposed to a RH rate over 70%, Form D is never reobtained by exposure to low RH rates. Form D has always been obtained in mixture with forms A, B and/or C in various proportions.

Hence, the knowledge acquired so far indicates that **form A (dihydrate) is, from the standpoint of physical stability, the most appropriate form for further development steps (FIM,...).**

Caution should be taken, however, since at RH rates lower than 50%, form A begins to transform into anhydrous forms B and C; nevertheless back-transformation into form A is immediate under exposure to a RH rate higher than 50%.

Appendix 1 – Details of crystal structure refinement of AVE3183A form A (ref.: amp044b)

_cell_measurement_temperature 293 K
_diffn_radiation_wavelength: MoK α ; λ = 0.71073 ; monochromator: graphite
_diffn_reflns_number 10518
_diffn_reflns_av_R_equivalents 0.0260
_diffn_reflns_av_sigmaI/netI 0.0197
_diffn_reflns_number: 20041
h_min: -9 k_min: -11 l_min: -41 θ _min: 1.10
h_max: 9 k_max: 11 l_max: 41 θ _max: 23.27
_reflns_number_total: 4512 _reflns_number_gt >2sigma(I): 4473

_computing_structure_solution_and_refinement: 'SHELXS-97'

Refinement of F² against ALL reflections. The weighted R-factor wR and goodness of fit S are based on F², conventional R-factors R are based on F, with F set to zero for negative F². The threshold expression of F² > 2sigma(F²) is used only for calculating R-factors(gt) etc. and is not relevant to the choice of reflections for refinement. R-factors based on F² are statistically about twice as large as those based on F, and R-factors based on ALL data will be even larger.

_refine_ls_structure_factor_coef: Fsqd
_refine_ls_matrix_type: full _refine_ls_weighting_scheme: calc
_refine_ls_weighting_details 'calc w=1/[s²(Fo²)+(0.0576P)²+0.0000P] where P=(Fo²+2Fc²)/3'
_atom_sites_solution_primary: direct _atom_sites_solution_secondary: difmap
_atom_sites_solution_hydrogens: geom _refine_ls_hydrogen_treatment: mixed
_refine_ls_extinction_method: SHELXL
_refine_ls_extinction_coef 0.016(2)

_refine_ls_number_reflns 4512
_refine_ls_number_parameters 422
_refine_ls_number_restraints 0
_refine_ls_R_factor_all 0.0762
_refine_ls_R_factor_gt 0.0743
_refine_ls_wR_factor_ref 0.2022
_refine_ls_wR_factor_gt 0.1983
_refine_ls_goodness_of_fit_ref 1.267
_refine_ls_restrained_S_all 1.267

_diffn_measured_fraction_theta_max 1.000
_diffn_reflns_theta_full 23.27
_diffn_measured_fraction_theta_full 1.000
_refine_diff_density_max 0.411
_refine_diff_density_min -0.367
_refine_diff_density_rms 0.122

Appendix 2 – Form A crystal structure: Atom's coordinates and displacement parameters

Space group: $P2_12_12_1$

Unit cell parameters:

a 8.2447(7) Å b 10.2493(9) Å c 37.047(3) Å

Atom	x/a	y/b	z/c	Ueq [Å ²]
C1	1.0858 (5)	0.3388 (4)	0.99248 (11)	0.0295 (9)
C2	0.9574 (5)	0.4433 (4)	0.98509 (10)	0.0266 (9)
C3	0.8041 (6)	0.6401 (4)	1.00692 (11)	0.0299 (10)
C4	1.0051 (5)	0.9406 (4)	0.92217 (10)	0.0260 (9)
C5	0.9432 (5)	0.5449 (4)	1.01497 (11)	0.0294 (9)
C6	1.0868 (6)	0.2438 (4)	0.96125 (12)	0.0324 (10)
C7	0.9414 (6)	0.7748 (5)	0.88054 (13)	0.0441 (12)
C8	0.6074 (7)	0.4615 (6)	0.88364 (15)	0.0561 (15)
C9	0.8062 (6)	0.7553 (4)	1.03267 (12)	0.0341 (11)
C10	0.9172 (7)	0.7379 (5)	0.84217 (14)	0.0498 (13)
C11	0.9498 (7)	0.6217 (5)	0.82816 (13)	0.0493 (14)
C12	0.9272 (7)	0.5824 (5)	0.79072 (14)	0.0487 (13)
C13	0.4670 (6)	0.5588 (5)	0.87669 (13)	0.0416 (11)
C14	1.0456 (6)	0.9002 (5)	0.88550 (13)	0.0426 (12)
C15	0.4426 (7)	0.5918 (6)	0.83812 (13)	0.0470 (13)
C16	0.4427 (7)	0.7107 (5)	0.82431 (15)	0.0512 (13)
C17	0.8491 (8)	0.6561 (6)	0.76429 (15)	0.0550 (14)
C18	0.4217 (7)	0.7418 (6)	0.78626 (14)	0.0520 (13)
C19	0.3492 (8)	0.6622 (6)	0.76092 (15)	0.0569 (15)
C20	0.9904 (10)	0.4674 (7)	0.77800 (17)	0.078 (2)
C21	0.3288 (6)	0.4905 (5)	0.89460 (13)	0.0441 (12)
C22	0.7858 (7)	0.8072 (6)	0.90176 (15)	0.0529 (14)
C23	0.3904 (14)	0.8034 (9)	0.7174 (2)	0.103 (3)
C24	0.4710 (12)	0.8610 (7)	0.77264 (19)	0.089 (2)
C25	0.9019 (14)	0.5050 (9)	0.7232 (2)	0.105 (3)
C26	0.4007 (5)	0.4433 (4)	0.92855 (9)	0.0250 (9)
N1	0.3362 (7)	0.6899 (6)	0.72647 (14)	0.0708 (15)
N2	0.4594 (13)	0.8917 (7)	0.73802 (17)	0.118 (3)
N3	0.9800 (11)	0.4274 (7)	0.74401 (16)	0.098 (2)
N4	0.8358 (8)	0.6213 (6)	0.73027 (14)	0.0712 (16)
N5	0.8281 (7)	0.9131 (6)	0.92811 (15)	0.0705 (15)
N6	0.5731 (7)	0.4023 (6)	0.92060 (15)	0.0720 (15)
O1	1.2163 (4)	0.2229 (3)	0.94447 (10)	0.0450 (9)
O2	1.2363 (4)	0.4050 (3)	0.99651 (9)	0.0376 (8)
O3	0.9561 (4)	0.1872 (3)	0.95384 (9)	0.0445 (9)
O4	0.9806 (4)	0.5010 (3)	0.95100 (8)	0.0348 (7)
O5	0.9214 (4)	0.4856 (3)	1.04967 (8)	0.0355 (7)
O6	0.6599 (4)	0.5693 (3)	1.00737 (10)	0.0418 (8)
O7	0.6807 (4)	0.7771 (3)	1.05092 (10)	0.0448 (9)
O8	0.9315 (4)	0.8247 (3)	1.03368 (10)	0.0467 (9)
O9	0.7326 (8)	0.7139 (6)	0.65973 (14)	0.0966 (18)
O10	0.2350 (8)	0.5852 (8)	0.65828 (17)	0.111 (2)
O11	0.0234 (9)	0.7699 (9)	0.62805 (16)	0.119 (2)
O12	0.5305 (7)	0.5321 (7)	0.62610 (13)	0.1011 (18)
H6	0.5884	0.6116	0.9973	0.063
H5	0.8515	0.4289	1.0483	0.053
H4	1.0567	0.5524	0.9520	0.052
H2	1.2853	0.3750	1.0139	0.056
H1	1.0594	0.2925	1.0149	0.035
H2A	0.8528	0.3980	0.9841	0.032
H3	0.8197	0.6738	0.9824	0.036
H4A	1.0268	1.0330	0.9252	0.031
H4B	1.0701	0.8925	0.9395	0.031
H5A	1.0445	0.5949	1.0156	0.035
H29A	0.3391	0.3698	0.9378	0.030
H29B	0.3996	0.5120	0.9466	0.030
H8	0.9965	0.7025	0.8928	0.053
H9A	0.6095	0.3943	0.8652	0.067
H9B	0.7110	0.5065	0.8836	0.067
H11	0.8761	0.8013	0.8267	0.060
H12	0.9911	0.5590	0.8438	0.059
H15	0.4895	0.6394	0.8900	0.050
H16A	1.1604	0.8812	0.8832	0.051
H16B	1.0160	0.9667	0.8681	0.051
H17	0.4258	0.5228	0.8222	0.056

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Atom	x/a	y/b	z/c	Ueq [Å ²]
H18	0.4574	0.7801	0.8402	0.061
H19	0.8033	0.7352	0.7712	0.066
H21	0.3065	0.5833	0.7688	0.068
H22	1.0441	0.4137	0.7943	0.094
H24A	0.2398	0.5501	0.8992	0.053
H24B	0.2898	0.4186	0.8800	0.053
H25A	0.7013	0.8367	0.8854	0.064
H25B	0.7470	0.7306	0.9145	0.064
H26	0.3792	0.8255	0.6932	0.123
H27	0.5141	0.9222	0.7885	0.107
H28	0.8894	0.4761	0.6996	0.126
H40	0.255 (7)	0.625 (6)	0.6786 (18)	0.055 (17)
H41	0.336 (10)	0.626 (8)	0.643 (2)	0.09 (2)
H46	0.667 (6)	0.457 (5)	0.9402 (12)	0.038 (13)
H161	0.784 (8)	0.870 (6)	0.9553 (16)	0.063 (17)
H61	0.108 (7)	0.725 (5)	0.6342 (14)	0.033 (14)

Atom	U(1,1)	U(2,2)	U(3,3)	U(2,3)	U(1,3)	U(1,2)
C1	0.031 (2)	0.027 (2)	0.030 (2)	0.0035 (18)	0.0001 (18)	0.0034 (19)
C2	0.026 (2)	0.030 (2)	0.024 (2)	0.0026 (17)	0.0022 (16)	0.0005 (18)
C3	0.036 (2)	0.024 (2)	0.030 (2)	0.0012 (18)	0.0036 (19)	-0.0008 (19)
C4	0.031 (2)	0.0159 (19)	0.031 (2)	-0.0039 (16)	-0.0105 (18)	-0.0036 (17)
C5	0.026 (2)	0.025 (2)	0.037 (2)	-0.0006 (18)	-0.0046 (18)	-0.0025 (18)
C6	0.037 (3)	0.020 (2)	0.040 (2)	0.0049 (18)	0.009 (2)	0.000 (2)
C7	0.052 (3)	0.037 (3)	0.043 (3)	-0.008 (2)	-0.013 (2)	0.006 (2)
C8	0.040 (3)	0.077 (4)	0.051 (3)	0.018 (3)	0.009 (2)	0.009 (3)
C9	0.037 (3)	0.028 (2)	0.037 (2)	0.0045 (19)	0.010 (2)	0.006 (2)
C10	0.054 (3)	0.045 (3)	0.050 (3)	0.001 (2)	-0.012 (3)	0.007 (3)
C11	0.059 (3)	0.048 (3)	0.040 (3)	-0.006 (2)	-0.005 (2)	0.003 (3)
C12	0.048 (3)	0.047 (3)	0.051 (3)	-0.010 (3)	-0.003 (2)	0.001 (3)
C13	0.047 (3)	0.037 (2)	0.041 (2)	0.000 (2)	0.003 (2)	-0.006 (2)
C14	0.039 (3)	0.042 (3)	0.046 (3)	0.000 (2)	-0.003 (2)	0.000 (2)
C15	0.050 (3)	0.049 (3)	0.042 (3)	-0.003 (2)	0.003 (2)	0.002 (3)
C16	0.055 (3)	0.047 (3)	0.051 (3)	0.001 (3)	0.001 (3)	-0.005 (3)
C17	0.067 (4)	0.049 (3)	0.049 (3)	-0.004 (3)	0.002 (3)	-0.005 (3)
C18	0.055 (3)	0.054 (3)	0.047 (3)	0.010 (3)	0.004 (3)	-0.004 (3)
C19	0.067 (4)	0.061 (4)	0.042 (3)	0.007 (3)	-0.002 (3)	0.003 (3)
C20	0.112 (6)	0.064 (4)	0.059 (4)	-0.012 (3)	-0.014 (4)	0.028 (4)
C21	0.042 (3)	0.040 (3)	0.050 (3)	-0.011 (2)	-0.001 (2)	-0.002 (2)
C22	0.059 (3)	0.048 (3)	0.052 (3)	-0.008 (2)	-0.008 (3)	-0.015 (3)
C23	0.162 (9)	0.095 (6)	0.051 (4)	0.025 (4)	-0.020 (5)	-0.040 (6)
C24	0.131 (7)	0.070 (5)	0.066 (4)	0.017 (4)	-0.008 (5)	-0.023 (5)
C25	0.164 (9)	0.103 (6)	0.048 (4)	-0.025 (4)	-0.005 (5)	0.016 (7)
C26	0.041 (2)	0.0197 (19)	0.0146 (18)	-0.0021 (15)	0.0053 (16)	-0.0077 (18)
N1	0.084 (4)	0.079 (4)	0.049 (3)	0.009 (3)	-0.007 (3)	-0.005 (3)
N2	0.206 (9)	0.088 (5)	0.060 (4)	0.033 (3)	-0.019 (5)	-0.057 (6)
N3	0.150 (7)	0.084 (4)	0.061 (4)	-0.032 (3)	-0.020 (4)	0.044 (5)
N4	0.094 (4)	0.076 (4)	0.044 (3)	-0.010 (3)	-0.005 (3)	0.001 (3)
N5	0.065 (3)	0.078 (4)	0.069 (3)	-0.014 (3)	0.008 (3)	-0.010 (3)
N6	0.063 (3)	0.078 (4)	0.075 (3)	0.005 (3)	0.003 (3)	-0.006 (3)
O1	0.049 (2)	0.0283 (16)	0.058 (2)	-0.0034 (16)	0.0173 (18)	0.0005 (15)
O2	0.0277 (16)	0.0377 (17)	0.047 (2)	-0.0013 (15)	-0.0051 (14)	0.0049 (14)
O3	0.043 (2)	0.0346 (17)	0.056 (2)	-0.0167 (15)	0.0094 (16)	-0.0090 (16)
O4	0.0371 (17)	0.0327 (16)	0.0345 (16)	-0.0017 (13)	-0.0010 (14)	0.0005 (14)
O5	0.0385 (18)	0.0343 (17)	0.0335 (15)	0.0021 (13)	-0.0035 (14)	-0.0052 (14)
O6	0.0271 (16)	0.0396 (18)	0.059 (2)	-0.0051 (16)	-0.0082 (15)	0.0006 (15)
O7	0.049 (2)	0.0301 (17)	0.055 (2)	-0.0034 (16)	0.0224 (18)	0.0048 (15)
O8	0.049 (2)	0.0285 (17)	0.063 (2)	-0.0131 (15)	0.0212 (18)	-0.0105 (17)
O9	0.112 (4)	0.102 (4)	0.076 (3)	0.016 (3)	-0.026 (3)	0.004 (3)
O10	0.091 (4)	0.173 (7)	0.071 (3)	-0.044 (4)	0.000 (3)	-0.017 (4)
O11	0.094 (4)	0.182 (7)	0.081 (4)	-0.002 (4)	-0.014 (3)	-0.004 (5)
O12	0.092 (4)	0.150 (5)	0.061 (3)	-0.006 (3)	0.021 (3)	-0.027 (4)

Appendix 3 – Indexing of AVE3183A form A XRPD Lines

given in d-spacings and in 2θ positions $\lambda_{Cu}K\alpha$ and $\lambda_{Co}K\alpha$

Conventional unit cell parameters (Angstroms and degrees)

<i>a</i>	<i>b</i>	<i>c</i>	alpha	beta	gamma
10.2864	37.1686	8.2651	90.0	90.0	90.0

Volume of unit cell is 3159.4 Å³

Space group $P2_12_12_1$ - Lattice P - Laue class is mmm

Extinction conditions

$h00 : h = 2n, 0k0 : k = 2n, 00l : l = 2n$

h	k	l	multiplicity		d-spacing	2-theta	2-theta
			J			$\lambda_{Cu} K\alpha$ 1.54184 Å	$\lambda_{Co} K\alpha$ 1.79030 Å
0	2	0	2		18.5843	4.7549	5.5217
1	1	0	4		9.9138	8.9200	10.3610
0	4	0	2		9.2921	9.5180	11.0562
1	2	0	4		8.9998	9.8279	11.4166
0	1	1	4		8.0680	10.9662	12.7402
1	3	0	4		7.9142	11.1800	12.9888
0	2	1	4		7.5519	11.7182	13.6149
1	4	0	4		6.8953	12.8386	14.9184
0	3	1	4		6.8756	12.8756	14.9614
1	0	1	4		6.4430	13.7442	15.9724
1	1	1	8		6.3483	13.9502	16.2122
0	6	0	2		6.1948	14.2976	16.6168
0	4	1	4		6.1756	14.3422	16.6686
1	2	1	8		6.0875	14.5509	16.9117
1	5	0	4		6.0251	14.7025	17.0882
1	3	1	8		5.7162	15.5017	18.0190
0	5	1	4		5.5271	16.0356	18.6411
1	6	0	4		5.3067	16.7060	19.4224
1	4	1	8		5.2947	16.7443	19.4670
2	0	0	2		5.1432	17.2412	20.0462
2	1	0	4		5.0947	17.4068	20.2392
0	6	1	4		4.9570	17.8942	20.8075
2	2	0	4		4.9569	17.8945	20.8080
1	5	1	8		4.8687	18.2212	21.1890
2	3	0	4		4.7502	18.6801	21.7242
1	7	0	4		4.7183	18.8075	21.8729
0	8	0	2		4.6461	19.1024	22.2171
2	4	0	4		4.4999	19.7291	22.9485
0	7	1	4		4.4673	19.8743	23.1179
1	6	1	8		4.4655	19.8825	23.1275
2	0	1	4		4.3668	20.3369	23.6581
2	1	1	8		4.3369	20.4783	23.8232
2	2	1	8		4.2510	20.8969	24.3121
1	8	0	4		4.2342	20.9807	24.4099
2	5	0	4		4.2296	21.0040	24.4371
0	0	2	2		4.1326	21.5028	25.0200

h	k	l	multiplicity J	d-spacing	2-theta $\lambda_{Cu} K\alpha$ 1.54184 Å	2-theta $\lambda_{Co} K\alpha$ 1.79030 Å
2	3	1	8	4.1184	21.5774	25.1071
0	1	2	4	4.1072	21.6369	25.1766
1	7	1	8	4.0976	21.6885	25.2369
0	8	1	4	4.0500	21.9463	25.5382
0	2	2	4	4.0340	22.0345	25.6414
2	6	0	4	3.9571	22.4683	26.1484
2	4	1	8	3.9521	22.4971	26.1821
0	3	2	4	3.9202	22.6825	26.3989
1	0	2	4	3.8347	23.1956	26.9990
1	9	0	4	3.8325	23.2088	27.0145
1	1	2	8	3.8144	23.3204	27.1450
0	4	2	4	3.7760	23.5613	27.4268
1	8	1	8	3.7685	23.6088	27.4824
2	5	1	8	3.7652	23.6297	27.5068
1	2	2	8	3.7555	23.6912	27.5789
0	10	0	2	3.7169	23.9414	27.8717
0	9	1	4	3.6943	24.0896	28.0451
2	7	0	4	3.6943	24.0899	28.0454
1	3	2	8	3.6632	24.2973	28.2883
0	5	2	4	3.6119	24.6476	28.6984
2	6	1	8	3.5691	24.9480	29.0501
1	4	2	8	3.5447	25.1228	29.2549
1	10	0	4	3.4957	25.4811	29.6746
1	9	1	8	3.4769	25.6210	29.8384
2	8	0	4	3.4477	25.8419	30.0974
0	6	2	4	3.4378	25.9174	30.1858
3	1	0	4	3.4143	26.0988	30.3985
1	5	2	8	3.4079	26.1483	30.4565
0	10	1	4	3.3899	26.2904	30.6231
2	7	1	8	3.3727	26.4265	30.7826
3	2	0	4	3.3719	26.4330	30.7903
3	3	0	4	3.3046	26.9815	31.4334
0	7	2	4	3.2612	27.3470	31.8623
1	6	2	8	3.2605	27.3531	31.8694
2	0	2	4	3.2215	27.6912	32.2662
2	9	0	4	3.2202	27.7025	32.2794
1	10	1	8	3.2195	27.7082	32.2861
3	4	0	4	3.2168	27.7324	32.3145
1	11	0	4	3.2102	27.7904	32.3826
2	1	2	8	3.2094	27.7971	32.3905
2	8	1	8	3.1819	28.0424	32.6784
2	2	2	8	3.1741	28.1126	32.7608
3	0	1	4	3.1671	28.1765	32.8359
3	1	1	8	3.1556	28.2808	32.9583
0	11	1	4	3.1277	28.5390	33.2615
3	2	1	8	3.1221	28.5914	33.3230
2	3	2	8	3.1178	28.6313	33.3699
3	5	0	4	3.1136	28.6712	33.4168
1	7	2	8	3.1087	28.7166	33.4702
0	12	0	2	3.0974	28.8241	33.5964
0	8	2	4	3.0878	28.9154	33.7037
3	3	1	8	3.0684	29.1022	33.9232
2	4	2	8	3.0437	29.3434	34.2065
2	10	0	4	3.0125	29.6543	34.5720
2	9	1	8	3.0005	29.7760	34.7151
3	6	0	4	2.9999	29.7819	34.7220
3	4	1	8	2.9977	29.8040	34.7480

h	k	l	multiplicity		d-spacing	2-theta	2-theta
						$\lambda_{Cu} K\alpha$ 1.54184 Å	$\lambda_{Co} K\alpha$ 1.79030 Å
1	11	1	8		2.9924	29.8584	34.8119
1	12	0	4		2.9658	30.1321	35.1339
1	8	2	8		2.9574	30.2198	35.2370
2	5	2	8		2.9559	30.2364	35.2565
0	9	2	4		2.9212	30.6039	35.6888
3	5	1	8		2.9137	30.6849	35.7841
0	12	1	4		2.9004	30.8287	35.9533
3	7	0	4		2.8804	31.0478	36.2111
2	6	2	8		2.8581	31.2965	36.5039
2	10	1	8		2.8304	31.6110	36.8743
2	11	0	4		2.8240	31.6840	36.9603
3	6	1	8		2.8199	31.7314	37.0161
1	9	2	8		2.8101	31.8455	37.1505
1	12	1	8		2.7916	32.0625	37.4061
0	10	2	4		2.7635	32.3965	37.7998
3	8	0	4		2.7588	32.4530	37.8663
1	13	0	4		2.7547	32.5033	37.9257
2	7	2	8		2.7542	32.5092	37.9325
0	1	3	4		2.7475	32.5908	38.0288
0	2	3	4		2.7253	32.8644	38.3513
3	7	1	8		2.7200	32.9298	38.4284
0	13	1	4		2.7020	33.1550	38.6941
0	3	3	4		2.6893	33.3159	38.8838
2	11	1	8		2.6723	33.5340	39.1412
1	10	2	8		2.6689	33.5787	39.1939
1	0	3	4		2.6612	33.6782	39.3113
0	14	0	2		2.6549	33.7610	39.4090
1	1	3	8		2.6544	33.7670	39.4161
2	12	0	4		2.6534	33.7810	39.4326
2	8	2	8		2.6473	33.8602	39.5261
0	4	3	4		2.6414	33.9390	39.6191
3	0	2	4		2.6388	33.9735	39.6598
3	9	0	4		2.6381	33.9828	39.6708
1	2	3	8		2.6344	34.0322	39.7291
3	1	2	8		2.6322	34.0616	39.7638
3	8	1	8		2.6169	34.2661	40.0053

Appendix 4 – Indexing of AVE3183A form B XRPD Lines

given in d-spacings and in 2 θ positions $\lambda_{Cu}K\alpha$ and $\lambda_{Co}K\alpha$

Conventional unit cell parameters (Angstroms and degrees)

<i>a</i>	<i>b</i>	<i>c</i>	alpha	beta	gamma
9.7542	35.0138	8.0243	90.0	90.0	90.0

Volume of unit cell is 2740.6 Å³

Space group *P*2₁2₁2₁ - Lattice *P* - Laue class is mmm

Extinction conditions

*h*00 : *h* = 2*n*, 0*k*0 : *k* = 2*n*, 00*l* : *l* = 2*n*

<i>h</i>	<i>k</i>	<i>l</i>	multiplicity <i>J</i>	d-spacing	2-theta $\lambda_{Cu}K\alpha$ 1.54184 Å	2-theta $\lambda_{Co}K\alpha$ 1.79030 Å
0	2	0	2	17.5069	5.0477	5.8618
1	1	0	4	9.3964	9.4122	10.9332
0	4	0	2	8.7535	10.1052	11.7389
1	2	0	4	8.5209	10.3818	12.0605
0	1	1	4	7.8215	11.3130	13.1435
1	3	0	4	7.4845	11.8242	13.7381
0	2	1	4	7.2946	12.1332	14.0976
0	3	1	4	6.6123	13.3906	15.5608
1	4	0	4	6.5148	13.5919	15.7952
1	0	1	4	6.1969	14.2928	16.6111
1	1	1	8	6.1020	14.5161	16.8711
0	4	1	4	5.9150	14.9776	17.4085
1	2	1	8	5.8417	15.1667	17.6288
0	6	0	2	5.8356	15.1826	17.6473
1	5	0	4	5.6886	15.5775	18.1073
1	3	1	8	5.4732	16.1944	18.8261
0	5	1	4	5.2761	16.8036	19.5361
1	4	1	8	5.0577	17.5348	20.3885
1	6	0	4	5.0078	17.7110	20.5939
2	0	0	2	4.8771	18.1897	21.1522
2	1	0	4	4.8305	18.3668	21.3588
0	6	1	4	4.7195	18.8023	21.8669
2	2	0	4	4.6982	18.8886	21.9675
1	5	1	8	4.6407	19.1246	22.2429
2	3	0	4	4.5000	19.7286	22.9478
1	7	0	4	4.4509	19.9486	23.2047
0	8	0	2	4.3767	20.2901	23.6034
2	4	0	4	4.2604	20.8500	24.2573
1	6	1	8	4.2484	20.9099	24.3272
0	7	1	4	4.2448	20.9277	24.3480
2	0	1	4	4.1677	21.3194	24.8057
2	1	1	8	4.1385	21.4717	24.9836
2	2	1	8	4.0544	21.9225	25.5104
0	0	2	2	4.0121	22.1561	25.7835
2	5	0	4	4.0021	22.2123	25.8492
1	8	0	4	3.9932	22.2628	25.9082

multiplicity					2-theta	2-theta
h	k	l	J	d-spacing	$\lambda_{Cu} K\alpha$ 1.54184 Å	$\lambda_{Co} K\alpha$ 1.79030 Å
0	1	2	4	3.9861	22.3030	25.9552
2	3	1	8	3.9249	22.6548	26.3665
0	2	2	4	3.9108	22.7381	26.4639
1	7	1	8	3.8922	22.8479	26.5923
0	8	1	4	3.8423	23.1486	26.9440
0	3	2	4	3.7942	23.4463	27.2923
2	4	1	8	3.7629	23.6440	27.5236
2	6	0	4	3.7423	23.7766	27.6788
1	0	2	4	3.7105	23.9829	27.9202
1	1	2	8	3.6899	24.1192	28.0798
0	4	2	4	3.6473	24.4051	28.4144
1	2	2	8	3.6299	24.5239	28.5535
1	9	0	4	3.6136	24.6361	28.6849
2	5	1	8	3.5814	24.8611	28.9484
1	8	1	8	3.5750	24.9066	29.0016
1	3	2	8	3.5361	25.1847	29.3274
0	10	0	2	3.5014	25.4387	29.6250
0	9	1	4	3.5007	25.4439	29.6310
2	7	0	4	3.4919	25.5087	29.7069
0	5	2	4	3.4813	25.5883	29.8002
1	4	2	8	3.4163	26.0836	30.3806
2	6	1	8	3.3916	26.2770	30.6073
0	6	2	4	3.3061	26.9685	31.4183
1	10	0	4	3.2955	27.0573	31.5224
1	9	1	8	3.2949	27.0622	31.5281
1	5	2	8	3.2787	27.1986	31.6881
2	8	0	4	3.2574	27.3799	31.9008
3	1	0	4	3.2375	27.5517	32.1025
0	10	1	4	3.2092	27.7995	32.3933
2	7	1	8	3.2019	27.8639	32.4689
3	2	0	4	3.1967	27.9099	32.5228
3	3	0	4	3.1321	28.4976	33.2128
1	6	2	8	3.1312	28.5065	33.2234
0	7	2	4	3.1297	28.5199	33.2390
2	0	2	4	3.0984	28.8141	33.5847
2	1	2	8	3.0864	28.9292	33.7198
2	2	2	8	3.0510	29.2719	34.1225
1	10	1	8	3.0484	29.2973	34.1524
3	4	0	4	3.0479	29.3022	34.1581
2	9	0	4	3.0413	29.3672	34.2345
1	11	0	4	3.0260	29.5191	34.4131
2	8	1	8	3.0182	29.5975	34.5052
3	0	1	4	3.0134	29.6454	34.5615
3	1	1	8	3.0023	29.7575	34.6933
2	3	2	8	2.9947	29.8350	34.7844
1	7	2	8	2.9801	29.9847	34.9605
3	2	1	8	2.9697	30.0916	35.0862
0	11	1	4	2.9588	30.2057	35.2204
0	8	2	4	2.9575	30.2190	35.2360
3	5	0	4	2.9490	30.3081	35.3408
2	4	2	8	2.9209	30.6076	35.6931
0	12	0	2	2.9178	30.6402	35.7315
3	3	1	8	2.9177	30.6410	35.7325
3	4	1	8	2.8493	31.3956	36.6207
2	10	0	4	2.8443	31.4525	36.6876
2	9	1	8	2.8439	31.4567	36.6926
3	6	0	4	2.8403	31.4979	36.7411

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h	k	l	multiplicity J	d-spacing	2-theta $\lambda_{Cu} K\alpha$ 1.54184 Å	2-theta $\lambda_{Co} K\alpha$ 1.79030 Å
2	5	2	8	2.8335	31.5757	36.8327
1	11	1	8	2.8314	31.5995	36.8608
1	8	2	8	2.8303	31.6122	36.8757
1	12	0	4	2.7954	32.0169	37.3524
0	9	2	4	2.7930	32.0456	37.3862
3	5	1	8	2.7680	32.3426	37.7362
0	12	1	4	2.7422	32.6561	38.1057
2	6	2	8	2.7366	32.7241	38.1858
3	7	0	4	2.7261	32.8540	38.3391
1	9	2	8	2.6851	33.3703	38.9480
2	10	1	8	2.6809	33.4244	39.0119
3	6	1	8	2.6775	33.4674	39.0626
0	1	3	4	2.6670	33.6033	39.2229
2	11	0	4	2.6656	33.6216	39.2445
0	2	3	4	2.6441	33.9032	39.5769
1	12	1	8	2.6398	33.9596	39.6434
0	10	2	4	2.6381	33.9829	39.6709
2	7	2	8	2.6340	34.0366	39.7344
3	8	0	4	2.6100	34.3596	40.1157

**Appendix 5 – Form B crystal structure: Atom's coordinates
 from XRPD crystal structure determination**

Space group: $P2_12_12_1$

Unit cell parameters:

a 9.7542 Å
 b 35.0138 Å
 c 8.0243 Å

Atom	x/a	y/b	z/c
N13	0.36067	0.33106	0.70402
H13	0.45165	0.31865	0.68222
C17	0.29028	0.39328	0.76506
H17	0.19494	0.38831	0.73160
C20	0.34190	0.33156	0.88983
H20A	0.26402	0.31592	0.92233
H20B	0.42357	0.32203	0.94583
C19	0.38443	0.37116	0.65079
H19A	0.47967	0.37852	0.66687
H19B	0.35976	0.37489	0.53430
C11	0.31338	0.43591	0.76640
H11	0.38508	0.44535	0.83147
C12	0.24004	0.46032	0.68313
H12	0.16962	0.45074	0.61622
C14	0.36456	0.52066	0.77466
H14	0.42806	0.50559	0.83160
C18	0.31672	0.37360	0.93417
H18A	0.23766	0.37623	1.00736
H18B	0.39645	0.38459	0.98914
C13	0.26037	0.50251	0.68666
N11	0.37822	0.55845	0.78177
C15	0.17312	0.52694	0.60145
H15	0.10408	0.51640	0.53552
C16	0.28838	0.57762	0.69616
H16	0.29984	0.60407	0.69588
N12	0.18337	0.56470	0.60977
N3	0.58205	-0.15106	0.58168
H3N	0.60525	-0.16050	0.48399
C7	0.52673	-0.10290	0.77686
H7	0.46018	-0.11765	0.84299
C1	0.50370	-0.06109	0.80931
H1	0.42975	-0.05432	0.87687
C9	0.50922	-0.11373	0.59038
H9A	0.55163	-0.09484	0.51774
H9B	0.41296	-0.11638	0.56081
C3	0.56098	0.00771	0.77841
C2	0.58056	-0.03338	0.74918
H2	0.65433	-0.04051	0.68188
C10	0.70409	-0.14805	0.69231
H10A	0.72114	-0.17231	0.74811
H10B	0.78503	-0.14129	0.62779
N1	0.61309	0.07388	0.72627
C4	0.63600	0.03690	0.70074
H4	0.70592	0.02998	0.62713
C5	0.45878	0.02071	0.88350
H5	0.40594	0.00268	0.94029
N2	0.43108	0.05805	0.90854
C8	0.67205	-0.11712	0.81930
H8A	0.67486	-0.12741	0.93194
H8B	0.73793	-0.09633	0.81113
C6	0.50994	0.08139	0.82957
H6	0.49209	0.10723	0.84746
O24	0.21998	0.30511	0.31650
H24	0.17351	0.30270	0.23149
O25	0.27500	0.20085	0.32977
H25	0.32927	0.20051	0.40827
O21	0.56442	0.31752	0.14959
O23	0.36131	0.26937	0.06278
H23	0.42137	0.26383	-0.00524
O22	0.58011	0.29371	0.40739

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Atom	x/a	y/b	z/c
H22O	0.65981	0.30140	0.40296
O28	-0.00867	0.17337	0.53860
H28	-0.00453	0.18186	0.63403
O27	-0.07610	0.20107	0.30499
C24	0.19831	0.23543	0.33393
H24A	0.14873	0.23749	0.22750
C25	0.09065	0.23418	0.47421
H25A	0.03792	0.25812	0.47441
C26	-0.00639	0.20025	0.43709
O26	0.16036	0.23020	0.62755
H26	0.12394	0.24388	0.69815
C22	0.41413	0.26614	0.22470
H22	0.45254	0.24038	0.23767
C21	0.52741	0.29511	0.26409
C23	0.29310	0.27039	0.34804
H23A	0.33020	0.27137	0.46196

Appendix 6 – Indexing of AVE3183A form D XRPD Lines

given in d-spacings and in 2 θ positions $\lambda_{Cu}K\alpha$ and $\lambda_{Co}K\alpha$

Conventional unit cell parameters (Angstroms and degrees)

<i>a</i>	<i>b</i>	<i>c</i>	alpha	beta	gamma
19.3877	7.7306	18.9699	90	92.632	90

Volume of unit cell is 2840.2 Å³

Space group $P2_1$ - Lattice P - Laue class is 2/m

Extinction conditions

0k0 : k = 2n

h	k	l	multiplicity J	d-spacing	2-theta $\lambda_{Cu}K\alpha$ 1.54184 Å	2-theta $\lambda_{Co}K\alpha$ 1.79030 Å
1	0	0	2	19.3672	4.563	5.298
0	0	1	2	18.9499	4.663	5.415
-1	0	1	2	13.8668	6.374	7.402
1	0	1	2	13.2441	6.674	7.751
2	0	0	2	9.6836	9.132	10.608
0	0	2	2	9.4749	9.334	10.842
-2	0	1	2	8.7881	10.065	11.693
-1	0	2	2	8.6696	10.203	11.853
2	0	1	2	8.4669	10.448	12.138
1	0	2	2	8.3608	10.581	12.292
1	1	0	4	7.1798	12.328	14.324
0	1	1	4	7.1579	12.366	14.368
-2	0	2	2	6.9334	12.768	14.836
-1	1	1	4	6.7522	13.112	15.236
1	1	1	4	6.6765	13.261	15.410
2	0	2	2	6.6221	13.371	15.538
3	0	0	2	6.4557	13.717	15.941
0	0	3	2	6.3166	14.020	16.294
-3	0	1	2	6.1984	14.289	16.607
-1	0	3	2	6.0883	14.549	16.910
2	1	0	4	6.0415	14.662	17.041
3	0	1	2	6.0270	14.698	17.083
0	1	2	4	5.9898	14.789	17.189
1	0	3	2	5.9256	14.951	17.377
-2	1	1	4	5.8044	15.265	17.743
-1	1	2	4	5.7699	15.357	17.850
2	1	1	4	5.7089	15.522	18.042
1	1	2	4	5.6761	15.612	18.147
-3	0	2	2	5.4529	16.255	18.897
-2	0	3	2	5.4054	16.399	19.065
3	0	2	2	5.2246	16.971	19.731
2	0	3	2	5.1828	17.109	19.892
-2	1	2	4	5.1616	17.179	19.974
2	1	2	4	5.0292	17.635	20.506
3	1	0	4	4.9552	17.901	20.815
0	1	3	4	4.8914	18.136	21.090

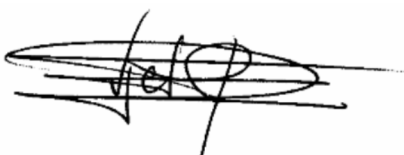
h	k	l	multiplicity J	d-spacing	2-theta $\lambda_{Cu} K\alpha$ 1.54184 Å	2-theta $\lambda_{Co} K\alpha$ 1.79030 Å
4	0	0	2	4.8418	18.323	21.308
-3	1	1	4	4.8359	18.346	21.335
-1	1	3	4	4.7830	18.551	21.573
3	1	1	4	4.7531	18.668	21.710
-4	0	1	2	4.7436	18.706	21.754
0	0	4	2	4.7375	18.731	21.783
1	1	3	4	4.7030	18.869	21.945
-1	0	4	2	4.6514	19.081	22.191
4	0	1	2	4.6403	19.127	22.245
-3	0	3	2	4.6223	19.202	22.333
1	0	4	2	4.5538	19.493	22.673
-3	1	2	4	4.4559	19.926	23.178
-2	1	3	4	4.4299	20.044	23.316
3	0	3	2	4.4147	20.114	23.397
-4	0	2	2	4.3940	20.209	23.509
-2	0	4	2	4.3348	20.488	23.835
3	1	2	4	4.3287	20.518	23.869
2	1	3	4	4.3049	20.633	24.003
4	0	2	2	4.2334	20.985	24.414
2	0	4	2	4.1804	21.254	24.729
4	1	0	4	4.1034	21.657	25.200
-4	1	1	4	4.0431	21.984	25.582
0	1	4	4	4.0393	22.005	25.607
-1	1	4	4	3.9855	22.306	25.959
4	1	1	4	3.9786	22.346	26.005
-3	1	3	4	3.9672	22.410	26.081
-4	0	3	2	3.9309	22.620	26.326
1	1	4	4	3.9236	22.662	26.375
-3	0	4	2	3.9059	22.767	26.497
5	0	0	2	3.8734	22.960	26.724
0	2	0	2	3.8653	23.009	26.781
3	1	3	4	3.8336	23.202	27.006
-5	0	1	2	3.8296	23.226	27.035
-4	1	2	4	3.8201	23.285	27.104
1	2	0	4	3.7905	23.469	27.319
0	0	5	2	3.7900	23.473	27.323
0	2	1	4	3.7873	23.490	27.343
-2	1	4	4	3.7810	23.530	27.390
5	0	1	2	3.7612	23.655	27.536
4	0	3	2	3.7603	23.661	27.543
-1	0	5	2	3.7520	23.714	27.605
3	0	4	2	3.7384	23.802	27.708
-1	2	1	4	3.7234	23.899	27.822
4	1	2	4	3.7131	23.966	27.900
1	2	1	4	3.7105	23.983	27.920
1	0	5	2	3.6877	24.134	28.097
2	1	4	4	3.6772	24.204	28.179
-5	0	2	2	3.6445	24.424	28.436
2	2	0	4	3.5899	24.801	28.879
-2	0	5	2	3.5856	24.831	28.914
0	2	2	4	3.5789	24.879	28.969
-2	2	1	4	3.5382	25.170	29.310
-1	2	2	4	3.5303	25.227	29.377
5	0	2	2	3.5291	25.236	29.387
2	2	1	4	3.5162	25.330	29.497
1	2	2	4	3.5085	25.386	29.563
-4	1	3	4	3.5039	25.420	29.603

h	k	l	multiplicity		d-spacing	2-theta	2-theta
						$\lambda_{Cu} K\alpha$ 1.54184 Å	$\lambda_{Co} K\alpha$ 1.79030 Å
-3	1	4	4		3.4862	25.551	29.757
2	0	5	2		3.4755	25.631	29.850
-4	0	4	2		3.4667	25.698	29.928
5	1	0	4		3.4631	25.725	29.960
-5	1	1	4		3.4316	25.965	30.241
0	1	5	4		3.4030	26.187	30.502
5	1	1	4		3.3822	26.351	30.694
4	1	3	4		3.3815	26.357	30.701
-2	2	2	4		3.3761	26.399	30.751
-1	1	5	4		3.3755	26.404	30.757
-5	0	3	2		3.3718	26.434	30.791
3	1	4	4		3.3655	26.484	30.850
2	2	2	4		3.3382	26.704	31.109
-3	0	5	2		3.3359	26.723	31.130
1	1	5	4		3.3284	26.785	31.203
3	2	0	4		3.3163	26.884	31.319
4	0	4	2		3.3110	26.928	31.371
0	2	3	4		3.2970	27.045	31.508
-5	1	2	4		3.2965	27.049	31.512
-3	2	1	4		3.2798	27.189	31.677
-1	2	3	4		3.2632	27.330	31.843
3	2	1	4		3.2537	27.412	31.939
-2	1	5	4		3.2528	27.420	31.947
1	2	3	4		3.2374	27.552	32.103
5	0	3	2		3.2365	27.560	32.113
6	0	0	2		3.2279	27.635	32.200
5	1	2	4		3.2104	27.789	32.381
-6	0	1	2		3.2065	27.823	32.421
3	0	5	2		3.2048	27.839	32.439
2	1	5	4		3.1699	28.151	32.806
-4	1	4	4		3.1632	28.212	32.877
0	0	6	2		3.1583	28.256	32.930
6	0	1	2		3.1581	28.258	32.932
-3	2	2	4		3.1534	28.301	32.982
-2	2	3	4		3.1441	28.386	33.082
-1	0	6	2		3.1401	28.423	33.126
3	2	2	4		3.1073	28.730	33.486
-6	0	2	2		3.0992	28.807	33.576
2	2	3	4		3.0985	28.814	33.584
1	0	6	2		3.0946	28.850	33.627
-5	1	3	4		3.0906	28.889	33.672
-5	0	4	2		3.0686	29.101	33.922
-3	1	5	4		3.0629	29.156	33.986
-4	0	5	2		3.0532	29.250	34.097
-2	0	6	2		3.0441	29.339	34.202
4	1	4	4		3.0436	29.345	34.208
4	2	0	4		3.0208	29.572	34.475
6	0	2	2		3.0135	29.645	34.561
-4	2	1	4		2.9965	29.817	34.763
0	2	4	4		2.9949	29.833	34.782
5	1	3	4		2.9854	29.930	34.896
6	1	0	4		2.9786	30.000	34.978
-1	2	4	4		2.9728	30.060	35.049
4	2	1	4		2.9699	30.090	35.084
-3	2	3	4		2.9652	30.139	35.142
2	0	6	2		2.9628	30.164	35.171
-6	1	1	4		2.9618	30.174	35.183

h	k	l	multiplicity J	d-spacing	2-theta $\lambda_{Cu} K\alpha$ 1.54184 Å	2-theta $\lambda_{Co} K\alpha$ 1.79030 Å
3	1	5	4	2.9605	30.188	35.200
1	2	4	4	2.9469	30.331	35.368
5	0	4	2	2.9334	30.473	35.535
-6	0	3	2	2.9294	30.517	35.586
0	1	6	4	2.9237	30.577	35.657
6	1	1	4	2.9236	30.578	35.659
4	0	5	2	2.9200	30.616	35.704
-1	1	6	4	2.9093	30.732	35.840
3	2	3	4	2.9081	30.745	35.854
-4	2	2	4	2.9022	30.809	35.930
-3	0	6	2	2.8899	30.944	36.089
-2	2	4	4	2.8849	30.998	36.153
-6	1	2	4	2.8766	31.090	36.261
1	1	6	4	2.8730	31.130	36.308
4	2	2	4	2.8545	31.337	36.552
-5	1	4	4	2.8521	31.364	36.584
-4	1	5	4	2.8398	31.504	36.748
2	2	4	4	2.8380	31.524	36.771
-2	1	6	4	2.8324	31.587	36.846
6	0	3	2	2.8223	31.704	36.984
6	1	2	4	2.8077	31.873	37.183
3	0	6	2	2.7869	32.117	37.470
-5	0	5	2	2.7734	32.279	37.661
7	0	0	2	2.7667	32.358	37.754
2	1	6	4	2.7666	32.360	37.757
-4	2	3	4	2.7561	32.486	37.906
-7	0	1	2	2.7559	32.489	37.909
-3	2	4	4	2.7474	32.592	38.030
5	1	4	4	2.7426	32.651	38.099
-6	1	3	4	2.7393	32.691	38.147
5	2	0	4	2.7361	32.731	38.194
4	1	5	4	2.7317	32.785	38.258
-6	0	4	2	2.7264	32.850	38.334
-5	2	1	4	2.7205	32.924	38.421
7	0	1	2	2.7199	32.931	38.429
0	0	7	2	2.7071	33.091	38.618
-3	1	6	4	2.7069	33.093	38.621
0	2	5	4	2.7062	33.103	38.633
-4	0	6	2	2.7027	33.147	38.684
-1	0	7	2	2.6981	33.205	38.753
5	2	1	4	2.6956	33.236	38.789
4	2	3	4	2.6953	33.240	38.795
-1	2	5	4	2.6922	33.279	38.840
-7	0	2	2	2.6893	33.317	38.885
3	2	4	4	2.6872	33.343	38.916
1	2	5	4	2.6682	33.588	39.205
1	0	7	2	2.6643	33.638	39.264
-5	2	2	4	2.6517	33.803	39.459
6	1	3	4	2.6511	33.810	39.467
5	0	5	2	2.6488	33.841	39.503
-2	0	7	2	2.6388	33.974	39.660
-2	2	5	4	2.6287	34.107	39.818
7	0	2	2	2.6236	34.176	39.899
3	1	6	4	2.6218	34.201	39.928
6	0	4	2	2.6123	34.328	40.079

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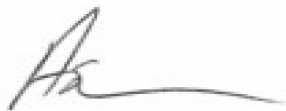
Salt Selection Study on TC-6499 for Targacept

Signature: 

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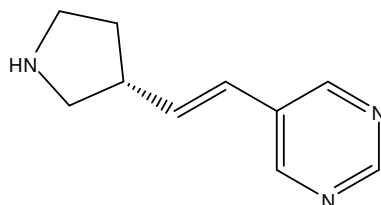
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Executive Summary

Targacept is developing TC-6499, a novel small molecule for the treatment of neuropathic pain. TC-6499, which is a modulator of the $\alpha 4\beta 2$ NNR, is currently in Phase I clinical development. In preclinical studies, TC-6499 demonstrated analgesic activity in multiple models of neuropathic pain. TC-6499 requires a stable solid form. Pharmorphix have been requested to perform a salt selection study, in order to identify a suitable salt for this purpose.



TC-6499

Targacept supplied TC-6499 as a hemi-galactaric acid salt, which was fully characterised. Crystallinity was assessed by XRPD, and thermal analyses were carried out (TGA, DSC, VT-XRPD and hot stage microscopy). A number of events were identified for this material (dehydration, solid phase transformations, etc). DVS experiments also showed a fully reversible dehydration-hydration process.

Experimental pKa values were determined for TC-6499, and based on these, 23 acids were selected to carry out a preliminary mono salt screen. Out of these counter ions, 12 of them were also used to prepare hemi salts. These acids were all Class 1 or Class 2 acids, and to our knowledge, no toxicity issues had been previously reported for them. Very lypophilic acids ($\log P > 5$) were also discarded, as well as potentially very hygroscopic acids (with several hydroxy and amino groups).

Crystallinity of the salts was assessed by XRPD, and stability at 40°C and 75%RH was established by storage of the salts in the humidity chamber for a week. With these criteria, a number of salts were identified as interesting candidates to scale up for further investigation: mono-citrate, orotate, and mono-di-*p*-toluoyl-D-tartrate. Maleate and fumarate were not stable in the humidity chamber, but were also investigated as back-up salts.

After full characterisation of the salts, we identified three salts that exhibited good solid form properties:

- **Orotate:** the best of the three salts, from a solid form point of view; no precedent of orotates as marketed drugs (although some mineral supplements are available as orotates).
- **Mono-citrate:** two forms were identified; it is an acceptable candidate, but a full polymorphism programme on this salt would be recommended.
- **Mono-maleate (Form 2):** two forms were identified, but only Form 2 had acceptable solid form properties. There are also indications of a possible third form, but to establish this point, a full polymorphism programme would be required. A single crystal structure was collected for Form 2 of the mono-maleate.

1 Introduction

Targacept is developing TC-6499, a novel small molecule for the treatment of neuropathic pain. TC-6499, which is a modulator of the $\alpha 4\beta 2$ NNR, is currently in Phase I clinical development. In preclinical studies, TC-6499 demonstrated analgesic activity in multiple models of neuropathic pain. TC-6499 requires a stable solid form. Pharmorphix have been requested to carry out a salt selection study, in order to identify a suitable salt for this purpose.

2 Objectives

This study was focused on trying to provide Targacept with a salt(s) form to improve the physical properties of TC-6499. An outline of the proposal is shown below:

Phase I: Characterisation of the supplied batch of TC-6499-12

Phase II: Salt selection studies and preliminary characterisation on salts of TC-6499

Phase III: Scale-up and characterisation of selected salts

3 Materials

Table 1 Details of supplied samples

Name of Compound	Pharmorphix Tracking Number	Customer Batch / Lot Number	Quantity Supplied
TC-6499-12	E00118	GMD-011671-2	6.94 g
TC-6499-12	E00478	GMD-011671-2	2.50 g

4 Safety Information

An MSDS was supplied by Targacept with the material received, which included some toxicological information.

Pharmorphix uses the Merck 5 category system to evaluate the safety of the compounds investigated at their facilities. This has been modified to increase its relevance for the techniques and scale that are worked on. This proposal assumes the compounds to be Class III cytotoxics and standard Pharmorphix safety protocols will be used.

5 Instrument and Methodology Details

5.1. X-Ray Powder Diffraction (XRPD): Bruker AXS C2 GADDS

X-Ray Powder Diffraction patterns were collected on a Bruker AXS C2 GADDS diffractometer using Cu K α radiation (40 kV, 40 mA), automated XYZ stage, laser video microscope for auto-sample positioning and a HiStar 2-dimensional area detector. X-ray optics consists of a single Göbel multilayer mirror coupled with a pinhole collimator of 0.3 mm.

The beam divergence, i.e. the effective size of the X-ray beam on the sample, was approximately 4 mm. A θ - θ continuous scan mode was employed with a sample - detector distance of 20 cm which gives an effective 2θ range of 3.2 ° – 29.7 °. Typically the sample would be exposed to the X-ray beam for 120 seconds. The software used for data collection was GADDS for WNT 4.1.16 and the data were analysed and presented using *Diffraction Plus* EVA v 9.0.0.2 or v 13.0.0.2.

Ambient conditions

Samples run under ambient conditions were prepared as flat plate specimens using powder as received without grinding. Approximately 1-2 mg of the sample was lightly pressed on a glass slide to obtain a flat surface.

Non-ambient conditions

Samples run under non-ambient conditions were mounted on a silicon wafer with heat-conducting compound. The sample was then heated to the appropriate temperature at ca. 20 °C.min⁻¹ and subsequently held isothermally for ca 1 minute before data collection was initiated.

5.2. Single Crystal XRD (SXD): Bruker AXS 1K SMART CCD

Data were collected on a Bruker AXS 1K SMART CCD diffractometer equipped with an Oxford Cryosystems Cryostream cooling device. Structures were solved using either the SHELXS or SHELXD programs and refined with the SHELXL program as part of the Bruker AXS SHELXTL suite. Unless otherwise stated, hydrogen atoms attached to carbon were placed geometrically and allowed to refine with a riding isotropic displacement parameter. Hydrogen atoms attached to a heteroatom were located in a difference Fourier synthesis and were allowed to refine freely with an isotropic displacement parameter.

5.3. ¹H-NMR

NMR spectra were collected on a Bruker 400MHz instrument equipped with an auto-sampler and controlled by a DRX400 console. Automated experiments were acquired using ICON-NMR v4.0.4 (build 1) running with Topspin v 1.3 (patch level 8) using the standard Bruker loaded experiments. For non-routine spectroscopy, data were acquired through the use of Topspin alone.

Samples were prepared in *d*₆-DMSO, unless otherwise stated. Off-line analysis was carried out using ACD SpecManager v 9.09 (build 7703).

5.4. Differential Scanning Calorimetry (DSC)

5.4.1. TA Instruments Q2000

DSC data were collected on a TA Instruments Q2000 equipped with a 50 position auto-sampler. The instrument was calibrated for energy and temperature calibration using certified indium. Typically 0.5-3 mg of each sample, in a pin-holed aluminium pan (Unless otherwise specified), was heated at 10 °C.min⁻¹ from 25 °C to 350 °C. A nitrogen purge at 50 ml.min⁻¹ was maintained over the sample.

The instrument control software was Advantage for Q Series v2.8.0.392 and Thermal Advantage v4.8.3 and the data were analysed using Universal Analysis v4.3A.

5.4.2. Mettler DSC 823e

DSC data were collected on a Mettler DSC 823e equipped with a 50 position auto-sampler. The instrument was calibrated for energy and temperature using certified indium. Typically 0.5-3 mg of each sample, in a pin-holed aluminium pan, was heated at 10 °C.min⁻¹ from 25 °C to 350 °C. A nitrogen purge at 50 ml.min⁻¹ was maintained over the sample.

The instrument control and data analysis software was STARe v9.10.

5.5. Thermo-Gravimetric Analysis (TGA)

5.5.1. TA Instruments Q500

TGA data were collected on a TA Instruments Q500 TGA, equipped with a 16 position auto-sampler. The instrument was temperature calibrated using certified Alumel. Typically 5-30 mg of each sample was loaded onto a pre-tared platinum crucible and aluminium DSC pan, and was heated at 10 °C.min⁻¹ from ambient temperature to 350 °C. A nitrogen purge at 60 ml.min⁻¹ was maintained over the sample.

The instrument control software was Advantage for Q Series v2.8.0.392 and Thermal Advantage v4.8.3

5.5.2. Mettler TGA/SDTA 851e

TGA data were collected on a Mettler TGA/SDTA 851e equipped with a 34 position auto-sampler. The instrument was temperature calibrated using certified indium. Typically 5-30 mg of each sample was loaded onto a pre-weighed aluminium crucible and was heated at 10 °C.min⁻¹ from ambient temperature to 350 °C. A nitrogen purge at 50 ml.min⁻¹ was maintained over the sample.

The instrument control and data analysis software was STARe v9.10.

5.6. Polarised Light Microscopy (PLM)

Samples were studied on a Leica LM/DM polarised light microscope with a digital video camera for image capture. A small amount of each sample was placed on a glass slide, mounted in immersion oil and covered with a glass slip, the individual particles being separated as well as possible. The sample was viewed with appropriate magnification and partially polarised light, coupled to a λ false-colour filter.

5.7. Hot Stage Microscopy (HSM)

Hot Stage Microscopy was carried out using a Leica LM/DM polarised light microscope combined with a Mettler-Toledo MTFP82HT hot-stage and a digital video camera for image capture. A small amount of each sample was placed onto a glass slide with individual particles separated as well as possible. The sample was viewed with appropriate magnification and partially polarised light, coupled to a λ false-colour filter, whilst being heated from ambient temperature typically at $10\text{-}20^{\circ}\text{C.min}^{-1}$.

5.8. Dynamic Vapour Sorption (DVS): SMS DVS Intrinsic

Sorption isotherms were obtained using a SMS DVS Intrinsic moisture sorption analyser, controlled by SMS Analysis Suite software. The sample temperature was maintained at 25°C by the instrument controls. The humidity was controlled by mixing streams of dry and wet nitrogen, with a total flow rate of 200 ml.min^{-1} . The relative humidity was measured by a calibrated Rotronic probe (dynamic range of $1.0\text{-}100\%$ RH), located near the sample. The weight change, (mass relaxation) of the sample as a function of %RH was constantly monitored by the microbalance (accuracy $\pm 0.005\text{ mg}$).

Typically $5\text{-}20\text{ mg}$ of sample was placed in a tared mesh stainless steel basket under ambient conditions. The sample was loaded and unloaded at 40% RH and 25°C (typical room conditions). A moisture sorption isotherm was performed as outlined below (2 scans giving 1 complete cycle). The standard isotherm was performed at 25°C at 10% RH intervals over a $0\text{-}90\%$ RH range.

Table 2 DVS method parameters

Parameters	Values
Adsorption - Scan 1	40 - 90
Desorption / Adsorption - Scan 2	90 - Dry, Dry - 40
Intervals (%RH)	10
Number of Scans	2
Flow rate (ml.min^{-1})	200
Temperature ($^{\circ}\text{C}$)	25
Stability ($^{\circ}\text{C.min}^{-1}$)	0.2
Sorption Time (hours)	6 hour time out

The sample was recovered after completion of the isotherm and re-analysed by XRPD.

5.9. Water Determination by Karl Fischer (KF)

The water content of each sample was measured on a Mettler Toledo DL39 Coulometer using Hydranal Coulomat AG reagent and an argon purge. Weighed solid samples were introduced into the vessel on a platinum TGA pan which was connected to a subseal to avoid water ingress. Approx 10 mg of sample was used per titration and duplicate determinations were made.

5.10. Thermodynamic Aqueous Solubility by HPLC

Aqueous solubility was determined by suspending sufficient compound in water to give a maximum final concentration of $\geq 10 \text{ mg.ml}^{-1}$ of the parent free-form of the compound. The suspension was equilibrated at 25 °C for 24 hours then the pH was measured. The suspension was then filtered through a glass fibre C filter into a 96 well plate. The filtrate was then diluted by a factor of 101. Quantitation was by HPLC with reference to a standard solution of approximately 0.1 mg.ml^{-1} in DMSO. Different volumes of the standard, diluted and undiluted sample solutions were injected. The solubility was calculated using the peak areas determined by integration of the peak found at the same retention time as the principal peak in the standard injection.

If there was sufficient solid in the filter plate, the XRPD was collected.

Table 3 HPLC method parameters for solubility measurements

Type of method:	Reverse phase with gradient elution		
Column:	Phenomenex Luna, C18 (2) 5µm 50 x 4.6 mm		
Column Temperature (°C):	25		
Standard Injections (µl):	1, 2, 3, 5, 7, 10		
Test Injections (µl):	1, 2, 3, 10, 20, 50		
Detection: Wavelength, Bandwidth (nm) :	260,80		
Flow Rate (ml.min ⁻¹):	2		
Phase A:	0.1% TFA in water		
Phase B:	0.085% TFA in acetonitrile		
Timetable:	Time (min)	% Phase A	% Phase B
	0.0	95	5
	1.0	80	20
	2.3	5	95
	3.3	5	95
	3.5	95	5
	4.4	95	5

Analysis was performed on an Agilent HP1100 series system equipped with a diode array detector and using ChemStation software vB.02.01-SR1.

5.11. Chemical Purity Determination by HPLC

Purity analysis was performed on an Agilent HP1100 series system equipped with a diode array detector and using ChemStation software vB.02.01-SR1.

Table 4 HPLC method parameters for chemical purity determinations

Sample Preparation:	0.5mg/ml in acetonitrile : water 1:1 v/v		
Column:	Phenomenex Luna C18 (2), 150x4.6mm, 5µm		
Column Temperature (°C):	25		
Injection (µl):	5		
Detection:	255, 90		
Wavelength, Bandwidth(nm):			
Flow Rate (ml.min ⁻¹):	1		
Phase A:	0.1%TFA in water		
Phase B:	0.085% TFA in acetonitrile		
Timetable:	Time (min)	% Phase A	% Phase B
	0	95	5
	25	5	95
	25.2	95	5
	30	95	5

5.12. Ion Chromatography (IC)

Data were collected on a Metrohm 761 Compact IC (for cations) and a Metrohm 861 Advanced Compact IC (for anions) using IC Net software v2.3. Samples were prepared as 1000 ppm stocks in DMSO. Samples were diluted to 100 ppm with DMSO prior to testing. Quantification was achieved by comparison with standard solutions of known concentration of the ion being analysed.

Table 5 HPLC method parameters for anion chromatography

Type of method	Anion exchange
Column:	Metrosep A Supp 5 – 250 (4.0 x 250 mm)
Column Temperature (°C):	Ambient
Injection (µl):	20
Detection:	Conductivity detector
Flow Rate (ml.min ⁻¹):	0.7
Eluent:	3.2 mM sodium carbonate, 1.0 mM sodium hydrogen carbonate in water

Table 6 HPLC method parameters for cation chromatography

Type of method	Cation exchange
Column:	Metrosep C 2 – 250 (4.0 x 250 mm)
Column Temperature (°C):	Ambient
Injection (µl):	20
Detection:	Conductivity detector
Flow Rate (ml.min ⁻¹):	1.0
Eluent:	4.0 mM Tartaric acid, 0.75 mM Dipicolinic acid in water

5.13. pKa Determination and Prediction

5.13.1. Determination

Data were collected on a Sirius GlpKa instrument with a D-PAS attachment. Measurements were made at 25 °C in aqueous solution by UV and in methanol water mixtures by potentiometry. The titration media was ionic-strength adjusted (ISA) with 0.15 M KCl (aq). The values found in the methanol water mixtures were corrected to 0% co-solvent via a Yasuda-Shedlovsky extrapolation. The data were refined using Refinement Pro software v1.0.

5.13.2. Prediction

Prediction of pKa values was made using ACD pKa prediction software v9.

5.14. Log P Determination

Data were collected by potentiometric titration on a Sirius GlpKa instrument using three ratios of octanol : ionic-strength adjusted (ISA) water to generate Log P, Log Pion, and Log D values. The data were refined using Refinement Pro software v1.0.

Prediction of Log P values was made using ACD v9 and Syracuse KOWWIN v1.67 software.

6 Batch Characterisation of E00118

The Table below is a summary of the batch characterisation of E00118. The experimental data can be found in the Data Section 1 at the end of this report.

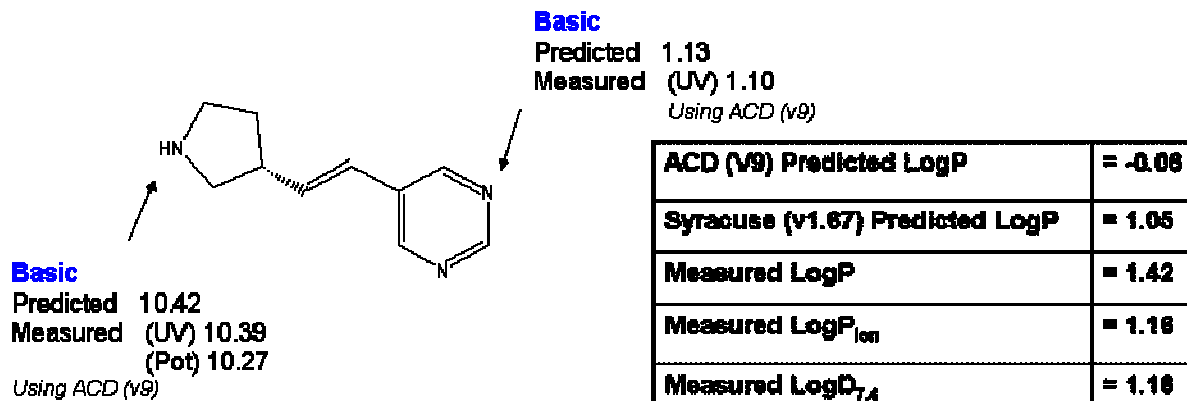
Table 7 Batch characterisation of TC-6499-12 (E00118)

Experiment	Comments
¹ H-NMR (400 MHz, D ₂ O)	¹ H-NMR spectrum was consistent with proposed structure. No impurities were identified by NMR. Stoichiometry was confirmed by integration of the signals of the counter ion.
HPLC Purity	99.99% (using Pharmorphix generic method)
XRPD Stability	The material supplied was crystalline. No changes were observed in the humidity chamber after the sample was stored at 40°C and 75%RH for one week (maybe a slightly improvement in the crystallinity). When the sample was stored at 60°C for one week, a new pattern was observed (Pattern 2 – see VT-XRPD).
VT-XRPD	A new crystalline pattern was observed when the sample was heated to 50°C (probably a mixture of patterns). A new XRPD pattern was observed at temperatures between 80 and 120°C (Pattern 2). A new change in the pattern occurred when the sample was heated to 130°C and it remained unchanged until the material melted (Pattern 3). When the sample was cooled down back to room temperature, it showed Pattern 2.
Optical Microscopy	Blade shape plates of up to 120 µm (length) and 60 µm (width). Birefringence was clearly observed with polarised light.
Hot Stage Microscopy	The material was initially very crystalline. At 55-60°C, some colour changes were observed, leading to completely colourless material at around 75-80°C. At 115-120°C colour appeared again, which indicated that a recrystallisation was occurring. Between 175-180°C the material melted and started decomposing.
TGA/DSC	A weight loss of 10.9% was observed between room temperature and 90°C (Karl Fisher analysis showed 11.1% of water in the sample, which corresponded to two molecules of water per molecule of API). Decomposition started at ~190°C. The DSC showed two endotherms at low temperature, related to the weight loss (sequential dehydration of two molecules of water). Another small endotherm occurred at 116°C (possibly a solid phase change). The material melted with decomposition at 190°C. To obtain the melting point of the di-hydrate, a hermetic pan was used, which prevented the loss of water. This melting point was estimated at 96°C.
DVS	The di-hydrate formed at relative humidity above 40%, when the sample took up to ~12% of water. On reducing the humidity levels, the two molecules of water were lost sequentially when humidity reached levels below 30% and 10%. When the experiment finished, and the sample was re-analysed by XRPD, a mixture of di-hydrate and anhydrous form was observed (Patterns 1 and 2).
Thermodynamic Solubility	> 10 mg/ml (pH of unfiltered solution 8.06)

7 Salt Selection Study

7.1. pKa and logP Determination

Figure 1 Calculated and experimental pKa values for E00118



The pKa values for TC-6499 were calculated and measured, and the correlation between both values was in good agreement. The prediction of the logP value is known to vary depending on which method was used.

Taking in consideration these pKa values, a number of acids were selected to carry out the salt screen. These acids are shown in the Table 8.

Table 8 Acids selected for salt screen

Acid	Class	pKa		
		pKa1	pKa2	pKa3
Hydrochloric acid	1	-6.10		
Sulphuric acid	1	-3.00	1.92	
Methane sulphonic acid	2	-1.20		
Maleic acid	1	1.92	6.23	
Phosphoric acid	1	1.96	7.12	12.32
1-Hydroxy-2-Naphthoic acid	2	2.70	13.50	
Ketoglutaric acid	2	2.70	4.50	
Malonic acid	2	2.83	5.70	
L-Tartaric acid	1	3.02	4.36	
Fumaric acid	1	3.03	4.38	
Citric acid	1	3.13	4.76	6.40
L-Malic acid	1	3.46	5.10	
Hippuric acid	1	3.55		
L-Lactic acid	1	3.86		
Benzoic acid	2	4.19		
Succinic acid	1	4.21	5.64	
Adipic acid	1	4.44	5.44	
Acetic acid	1	4.76		
Nicotinic acid	2	4.85		
Propionic acid	2	4.87		
Orotic acid	2	5.85	8.95	
4-Hydroxybenzoic acid	n/a	4.58	9.23	
di-p-Toluoyl-D-tartaric acid	n/a			

These acids were
suggested by
Targacept

All acids were used in the preliminary mono-salt screen. The acids highlighted in blue were also used in the hemi-salt screen.

7.2. Preparation of the free base

The material supplied was a hemi-galactarate of TC-6499. In order to carry out the salt screen, batches of free base were prepared by treatment of the starting material with sodium hydroxide as required.

E00118 (200 mg, 0.63 mmol) was suspended in dichloromethane (1 ml) and treated with an excess of aqueous sodium hydroxide (1M, 1.26 ml, 1.26 mmol). The mixture was shaken at room temperature for 1-2 h, and the two phases were then separated. The aqueous phase was washed with dichloromethane (3 x 1 ml) and the combined organic extracts were washed with water (1 ml), dried with magnesium sulphate and concentrated. The residue (colourless oil, 105.5 mg, 0.60 mmol, 95.5%) was analysed by ¹H-NMR, and used in the preparation of salts without further purification.

7.3. Preliminary Hydrochloride screen

An initial hydrochloride screen was carried out with the first batch of free base prepared, by splitting the residue in three lots, and dissolving each one of them in either, IPAc, THF or MIBK, and treating these solutions with 1 eq. of HCl (1M in Et₂O). The mixtures were incubated at 50°C/r.t. (4 h cycles) for 24 h. The preparations in THF and MIBK yielded an insoluble gum, which was not further investigated. The experiment in IPAc yielded a crystalline solid (for which an XRPD pattern was collected), but this material deliquesced within minutes at ambient conditions. ¹H-NMR of this solid confirmed the protonation of the basic nitrogen.

7.4. Salt Screen: Mono salts

Two solvents were chosen to carry out the salt screen: IPAc and MeCN. IPAc produced interesting results in the preliminary hydrochloride screening. THF and MIBK had previously failed to dissolve the free base, which resulted in a poor choice of solvent to obtain the salts. MeCN was chosen based in the higher solubility of the free base in this solvent. IPA and THF were also chosen to mature the gums and oils obtained in the preparation of the salts, if no crystalline solid was obtained initially.

7.4.1. Procedure

The free base (10 mg, 0.057 mmol) was dissolved in either IPAc or MeCN. The solutions were then treated with 1 eq. of the corresponding acid (see Table 8), warmed up to 50°C and cooled down slowly to room temperature overnight.

The solvent was then evaporated under vacuum without heating, and the residues analysed by XRPD. The solids were then stored in the humidity chamber at 40°C and 75%RH for a week, and re-analysed by XRPD.

In the cases where the experiment did not yield a crystalline solid, these samples were matured in THF and IPA, and where a solid was obtained, this was analysed by XRPD and stored in the humidity chamber for a week to assess stability.

7.4.2. Results

Table 9 Summary mono salt screen

Acid	IPAc				MeCN			
	Initial Observations	After Evaporation of solvent	Maturation THF	Humidity Chamber	Initial Observations	After Evaporation of solvent	Maturation IPA	Humidity Chamber
Hydrochloric acid		<i>It will be investigated separately</i>				<i>It will be investigated separately</i>		
Sulphuric acid	Sticky solid	Sample deliquesces	Yellow sticky solid/deliquesces		Sticky solid	Sample deliquesces	Partially Crystalline – XRPD 1	
Methane sulphonic acid	Solid	Crystalline – XRPD 1	N/A	Sample deliquesces	Solid	Sample deliquesces	N/A	
Maleic acid	Solid	Crystalline – XRPD 1	N/A	Sample deliquesces	Solid	Crystalline – XRPD 1	N/A	Sample deliquesces
Phosphoric acid	Sticky solid	Sample deliquesces	Yellow sticky solid		Sticky solid	Oil	Oil	
1-Hydroxy-2-Naphthoic acid	No solid produced	Dark oil	Dark oil		No solid produced	Dark oil	Dark glass/oil	
Ketoglutaric acid	Sticky solid	Sticky solid	Sticky foam		No solid produced	Dark oil	Glass	
Malonic acid	Oil	Yellow oil	Yellow oil		No solid produced	Yellow oil	Oil	
L-Tartaric acid	Sticky solid	Sample deliquesces	Yellow sticky solid		Sticky solid	Sample deliquesces	Sticky solid	
Fumaric acid	Sticky solid	Crystalline – XRPD 1	N/A	Sample deliquesces	Sticky solid	Sticky solid	N/A	
Citric acid	Sticky solid	Sample deliquesces	Crystalline – XRPD 1	XRPD1	Sticky solid	Glass	Crystalline – XRPD 1	XRPD1
L-Malic acid	Oil	Yellow oil	Dark oil		No solid produced	Yellow oil	Glass/oil	
Hippuric acid	No solid produced	Dark oil	Dark oil		No solid produced	Dark oil	Dark oil	
L-Lactic acid	Oil	Dark oil	Dark oil		No solid produced	Dark oil	Oil	
Benzoic acid	No solid produced	Dark oil	Dark oil		No solid produced	Dark oil	Oil	
Succinic acid	No solid produced	Dark oil	Dark oil		No solid produced	Dark oil	Oil	
Adipic acid	Oil	Dark oil	Dark oil		Oil	Dark oil	Glass/oil	
Acetic acid	No solid produced	Dark sticky solid	Dark oil		No solid produced	Dark oil	Dark oil	
Nicotinic acid	Oil	Dark oil	Dark sticky solid		No solid produced	Dark oil	Glass/oil	
Propionic acid	No solid produced	Dark oil	Dark oil		No solid produced	Dark oil	Dark oil	
Orotic acid	Solid	Mostly amorphous – XRPD 1	N/A	XRPD1	Solid	Crystalline – XRPD 1	N/A	XRPD 1
4-Hydroxybenzoic acid	Solid	XRPD 1	N/A	Sample deliquesces	Solid	XRPD 1	N/A	Sample deliquesces
Di-p-toluyyl-D-tartaric acid	Solid	XRPD 1	N/A	XRPD 1	Solid	XRPD 1	N/A	XRPD 1

7.4.3. Discussion & Conclusions

All solids were analysed by XRPD and ¹H-NMR. In all cases, the NMR spectra were consistent with the proposed structures, and a 1:1 stoichiometry was confirmed by integration of the signals in the NMR spectra or by ion chromatography.

The most promising results were obtained for the citrate, orotate and di-ptoluoyl-D-tartrate. These salts produced crystalline solids that were stable after a week in the humidity chamber at 40°C and 75%RH. These salts were, therefore, selected for scale up to carry out a more detailed investigation of their solid form properties.

Although the 4-hydroxybenzoate, maleate and fumarate produced crystalline solids, these deliquesced in the humidity chamber. Nevertheless, they were also selected to perform a more detailed investigation, with the aim of identifying new, more stable, solid forms of these salts.

7.5. Salt Screen: Hemi salts

7.5.1. Procedure

For the preparation of the hemi salts, we followed a similar procedure as described for the mono salt screen, using 0.5 eq. of acid instead of 1 eq. of acid. The solids obtained were treated and analysed in the same manner.

7.5.2. Results

Table 10 Summary hemi salt screen

Acid	IPAc				MeCN			
	Initial Observations	After Evaporation of solvent	Maturation THF	Humidity Chamber	Initial Observations	After Evaporation of solvent	Maturation IPA	Humidity Chamber
Sulphuric acid	Oil	Yellow oil	Yellow oil		Oil	Oil	Oil	
Maleic acid	Oil	Dark sticky solid	Dark sticky solid		Oil	Oil	Oil	
Phosphoric acid	Oil	Dark sticky solid	Dark sticky solid		Oil	Dark sticky solid	Dark sticky solid	
Ketoglutaric acid	Oil	Oil	Oil		Oil	Oil	Oil	
Malonic acid	Oil	Dark oil	Dark oil		Oil	Yellow oil	Dark oil	
L-Tartaric acid	Solid	Solid/deliquesces	Dark oil		Solid	Solid/deliquesces	Solid/deliquesces	Sample deliquesces
Fumaric acid	Oil	Dark glass/oil	Solid/deliquesces Mostly amorphous	Sample deliquesces	Oil	Solid/deliquesces	Mostly amorphous	Sample deliquesces
Citric acid	Oil	Dark sticky foam	XRPD 1	Sample partially deliquesces	Oil	Solid/deliquesces	Oil	
L-Malic acid	Oil	Dark oil	Dark oil		Oil	Oil	Oil	
Succinic acid	Oil	Dark oil	Dark oil		Oil	Oil	Oil	
Adipic acid	Oil	Dark oil	Dark oil		Oil	Oil	Oil	
Di-p-toluy-D-tartaric acid	Solid	XRPD 1	XRPD 1	XRPD 1	Solid	XRPD 1	XRPD 1	

7.5.3. Discussion & Conclusions

Only the hemi-citrate and hemi di-*p*-toluoyl-D-tartrate produced crystalline solids, although the hemi-citrate deliquesced in the humidity chamber. ¹H-NMR spectra of these two salts were consistent with the proposed structures and their stoichiometries were also confirmed by integration of the signals of the counter ions.

The batch of free base had been prepared 2-3 weeks prior to the hemi salt screen, and had been stored under nitrogen in the fridge. However, an intense red colour had appeared in the sample. This material was then analysed by NMR, and it was observed that extensive transformation of the material had taken place, possibly by polymerisation, although there was still some remaining free base. It was concluded that the free base must be prepared immediately prior to its use, to prevent decomposition. Decomposition of the free base may have affected the yield of the prepared salt, however in the cases where a crystalline solid(s) were isolated; the XRPD pattern for those salts matched their scaled up counterparts.

7.6. Hydrochloride Screening

7.6.1. Procedure

When the preliminary hydrochloride screening was carried out, a crystalline solid was initially isolated, which deliquesced within minutes **at ambient conditions**. It was felt that a more thorough investigation was necessary for this salt. A separate salt screening was then planned, using fresh free base. Four different solvents and five different sources of HCl were used for this purpose.

7.6.2. Results

Table 11 Summary hydrochloride screen

HCl source	IPAc	MeCN	THF	IPA
1M HCl (aq)	No solid produced	No solid produced	No solid produced	No solid produced
1M HCl in AcOH	No solid produced	No solid produced	No solid produced	No solid produced
5M HCl in IPA	Sticky Solid	White Solid XRPD 2	Gum	White Solid XRPD 2
4M HCl in dioxane	Sticky Solid	White Solid	Gum	White Solid
1M HCl in Et ₂ O	Sticky Solid	White Solid	Gum	White Solid XRPD 3

7.6.3. Discussion & Conclusions

A number of preparations produced solids. In other cases a gum or sticky solid, difficult to work with, was obtained, but no XRPD patterns were collected for them. When a solid was isolated by filtration, most of them deliquesced instantly. Some solids were stable enough to allow collection of XRPD pattern (XRPD 2/3), but deliquesced after 3 days in humidity chamber at 40°C/75%RH.

In sight of these results, no further work was done with the hydrochloride salt.

7.7. Scale-up of Selected Salts

Based on the results obtained in the preliminary salt screenings, a number of them were chosen to scale-up to ~200 mg and to carry out a more detailed investigation:

- Mono Salts:
 - o Citrate
 - o Orotate
 - o 4-Hydroxybenzoate
 - o Di-p-Toluoyl-D-tartrate
 - o Maleate
 - o Fumarate
- Hemi Salts:
 - o Citrate
 - o Di-p-Toluoyl-D-tartrate
 - o Maleate
 - o Fumarate

7.7.1. Scale-up Procedure

The free base (189 mg, 1.077 mmol, freshly prepared following the procedure described in section 7.2) was dissolved in MeCN. The solutions were then treated with 1.1 eq. of the corresponding acid for the preparation of the mono salts, and 0.5 eq. for the preparation of the hemi salts. The mixtures were warmed up to 50°C and cooled down slowly to room temperature overnight.

The solids obtained were filtered and dried under suction before being analysed by XRPD, and ¹H-NMR. TGA experiments were performed to determine content of water or other solvents, and DSC experiments were run to establish stability of the isolated forms and the possibility of new forms for each salt. DVS experiments were used to assess hygroscopicity of the salts. HPLC purity and thermodynamic solubility were also measured for each salt.

7.7.2. Attenuated Polymorph screen Procedure

An attenuated polymorphism screen was also performed in 15 different solvents for the most promising salts. No new forms were identified for the mono-citrate or the orotate. Some new forms were identified for the mono- and hemi-fumarates, but these forms were of no interest, as they deliquesced rapidly in the humidity chamber. A new form was also identified for the mono-maleate (Form 2), which was stable in the humidity chamber for a week. This is the same form of the crystals obtained for this salt after Form 1 was stored in the humidity chamber.

Table 12 Polymorphism screen summary

Solvent	Mono-Citrate		Orotate		Mono-Maleate		Mono-Fumarate		Hemi-Fumarate	
	XRPD	Humidity Chamber	XRPD	Humidity Chamber	XRPD	Humidity Chamber	XRPD	Humidity Chamber	XRPD	Humidity Chamber
Water		Dissolves completely; amorphous glass on evaporation of water	XRPD 1	Dissolves completely; produces a crystalline material on evaporation	Film on the walls of the vial		Film on the walls of the vial		Film on the walls of the vial	
MIBK	XRPD 1		XRPD 1		XRPD 1		XRPD 1		XRPD 1	
EtOH	XRPD 1		XRPD 1		XRPD 2 No changes in XRPD pattern		Film on the walls of the vial		Film on the walls of the vial	
IPA	XRPD 1		XRPD 1				Sticky solid		Film on the walls of the vial	
EtOAc	XRPD 1		XRPD 1		XRPD 1		XRPD 2	Deliquesces within 1 h	XRPD 1	
Acetone	XRPD 1		XRPD 1		Very dark oil isolated		Very dark oil isolated		XRPD 1	
MeCN	XRPD 1		XRPD 1		XRPD 1		XRPD 3	Deliquesces within 1 h	XRPD 1	
TBME	XRPD 1		XRPD 1		XRPD 1		XRPD 2	Deliquesces within 1 h	XRPD 1	
THF	XRPD 1		XRPD 1		XRPD 1		XRPD 2	Deliquesces within 1 h	XRPD 1	
DCM	XRPD 1		XRPD 1		XRPD 1		Sticky solid		Sticky solid	
Toluene	XRPD 1		XRPD 1		XRPD 1		XRPD 2	Deliquesces within 1 h	XRPD 2 (impure XRPD 1)	Deliquesces within 1 h
NMP		Dissolves completely; a mixture of oil and crystals after evaporation	XRPD 1	Dissolves completely; produces a crystalline material on evaporation	Oil		Oil		Oil	
DMSO		Dissolves completely; produces an oil and after evaporation	XRPD 1	Dissolves completely; produces a crystalline material on evaporation	Oil		Oil		Oil	
n-Heptane	XRPD 1		XRPD 1		XRPD 1		XRPD 2	Deliquesces within 1 h	XRPD 1	
IPAc	XRPD 1		XRPD 1		XRPD 1		XRPD 2	Deliquesces within 1 h	XRPD 2 (impure XRPD 1)	Deliquesces within 1 h

7.7.3. Results

Table 13 Solid form studies on scale up salts

Salt	Preparation	Stability (40°C/75%RH)	Thermal Analysis	DVS	Solubility (pH of unfiltered solution)
Mono-citrate	Two different crystalline solids isolated from subsequent crystallisations: XRPD 1 , XRPD 2	Both crops produced XRPD 2 (with increased crystallinity)	No weight loss for either Form until decomposition occurs at ~170°C. Form 1 melts at 149°C, Form 2 melts at 157°C.	Both Forms behave similarly. Less than 1.5% of water taken below 70%RH, it goes up to 4% at 90%RH. No major changes are observed in the XRPD of either Form before and after the DVS experiment.	>10 mg/ml (3.82 Form 1; 4.77 Form 2)
Mono-oratate	White crystalline solid: XRPD 1	No changes were observed	No weight loss until decomposition occurs at ~240°C. The sample melts at 224°C.	Less than 1% of water taken below 70%RH, it goes up to 1.8% at 90%RH. No major changes are observed in the XRPD before and after the DVS experiment, maybe some increase in crystallinity.	>10 mg/ml (3.23)
Mono-4-hydroxybenzoate	White crystalline solid: XRPD 1	Sample deliquesces partially. Same pattern with increased amorphous content	No weight loss until decomposition occurs at ~180°C. The sample melts at 158°C.	No further investigation was carried out	
Mono-di- <i>p</i> -tolouyl-D-tartrate	White crystalline solid; not very soluble in cold D ₂ O: XRPD 1	No changes were observed	No weight loss until decomposition occurs at ~190°C. The sample melts at 186°C.	Up to only 1.2% of water taken at 90%RH. No changes are observed in the XRPD before and after the DVS experiment.	0.91 mg/ml (3.64)
Mono-maleate	Off-white solid: XRPD 1	Deliquesces after 3 days in humidity chamber. New crystalline form appears after 7 days in humidity chamber (XRPD 2)	Form 2: No weight loss until decomposition occurs at ~170°C. The sample melts at 133°C and a recrystallisation follows before degradation starts (possible Form 3).	Form 2: Very little uptake of water up to 80%RH (< 5%). Above this level, the uptake of water goes up to 60% at 90%RH. Reversible process, dehydration occurs at 60%RH. No changes in XRPD pattern.	>10 mg/ml (3.28 Form 2)
Mono-fumarate	White crystalline solid: XRPD 1	Deliquesces after 3 days in humidity chamber	No further investigation was carried out		
Hemi-citrate*	Pink-ish crystalline solid (matches the second batch for mono-citrate); low yield	Sample deliquesces partially. Matches XRPD 2 for mono salt	Small weight loss of 0.6% at low temperature. Material decomposes at ~180°C. Endotherm associated to the weight loss and another endotherm at ~135°C (melt of solid phase change).	The hemi-citrate is very hygroscopic, taking more than 30% of water at 90%RH. The sample had deliquesced when it was removed from the system, and no XRPD pattern was collected.	No further investigation was carried out
Hemi-di- <i>p</i> -tolouyl-D-tartrate*	White crystalline solid; Some common peaks with mono-salt; not very soluble in cold D ₂ O	Sample deliquesces partially. Matches XRPD 1 for mono salt	No weight loss until decomposition occurs at ~190°C. The sample melts at 173°C.	No further investigation was carried out	
Hemi-maleate	Sticky glass/gum	No further investigation was carried out			
Hemi-fumarate	White crystalline solid: XRPD 1	Deliquesces after 3 days in humidity chamber	No further investigation was carried out		

* Possibly a mixture of mono- and hemi- salts.

7.7.4. Discussion & Conclusions

All the solids isolated were analysed by ^1H -NMR, and in most cases, the spectra were consistent with the proposed structure and stoichiometry. In the case of the mono-citrate, the solid that crystallised initially was filtered out of the solution (crop 1 – Form 1). The solution was left standing in the fumehood, and a second crop spontaneously crystallised. This second crop of material was filtered and kept separated from the first one. ^1H -NMR spectra of both crops were identical, and in both cases the stoichiometry was confirmed to be 1:1. When the hemi salt was analysed by NMR, the integral of the signals corresponding with the citric acid moiety were bigger than expected, which led us to believe we may have a mixture of mono and hemi salts. This fact seemed to be supported for the XRPD and stability data for the hemi-citrate.

A few samples were stable for a week in the humidity chamber at 40°C and 75%RH (mono-citrate, mono-orotate and mono-di-*p*-toluoyl-D-tartrate). The rest of the samples deliquesced, and were discarded. A particularly interesting case was the mono-maleate. The form initially prepared, deliquesced within a couple of days in the humidity chamber. However, a few days later (the sample was checked again after a week), some big crystals had appeared, which were isolated and investigated. The crystalline structure was determined by single crystal diffraction.

DVS experiments were carried out to assess hygroscopicity and TGA and DSC were run to establish the thermal stability of these salts.

HPLC purity of the salts was measured, and in all cases this was higher than 99% (using Pharmorphix generic method). Thermodynamic solubility was also measured.

An attenuated polymorphism screen was also performed in 15 different solvents for the most promising salts. No new forms were identified for the mono-citrate or the orotate. Some new forms were identified for the mono- and hemi-fumarates, but these forms were of no interest, as they deliquesced rapidly in the humidity chamber. A new form was also identified for the mono-maleate (Form 2), which was stable in the humidity chamber for a week. This is the same form of the crystals obtained for this salt after Form 1 was stored in the humidity chamber.

8 Single Crystal Experiments

A sample was submitted for single crystal structure determination as shown in Table 14.

Table 14 Samples submitted for single crystal X-ray diffraction studies.

Batch Number	Form	Experimental Reference
VDP-340-37-01w	Form 2 (maleate)	PHX-07-111

When the mono-maleate was stored in the humidity chamber at 40°C and 75%RH, the sample deliquesced almost immediately. However, after a few days in these conditions, some crystals appeared (see Figure below), which were submitted for single crystal X-ray diffraction.

Figure 2 Crystals of mono-maleate obtained at 40°C and 75%RH



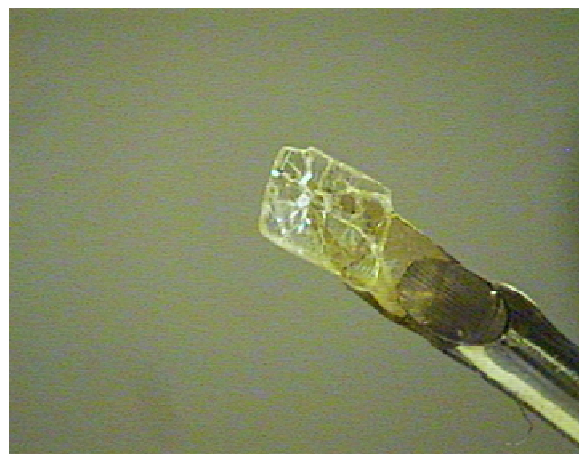
Mono-maleate, as it was obtained



Same sample, after one week in humidity chamber



r.t.



120K

When the crystal was cooled down to 120K in the single crystal diffractometer to collect the structure, a change in the crystal structure occurred, as we can see from the cracks that appeared in the crystal. This new form was not further investigated, as we cannot reproduce these conditions in the lab. The structure was finally collected at room temperature.

The structure solution was obtained by direct methods, full-matrix least-squares refinement on F^2 with weighting $w^{-1} = \sigma^2(F_o^2) + (0.0795P)^2 + (0.0400P)$, where $P = (F_o^2 + 2F_c^2)/3$, anisotropic displacement parameters, no absorption correction. Final $wR^2 = \{\Sigma[w(F_o^2 -$

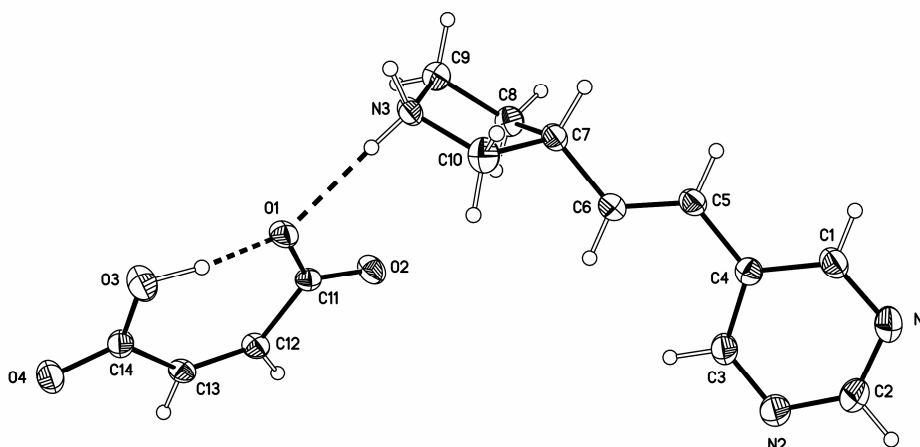
$F_c^2)/\Sigma[w(F_o^2)^{1/2}] = 0.1086$ for all data, conventional $R_1 = 0.0372$ on F values of 2699 reflections with $F_o > 4\sigma(F_o)$, $S = 1.004$ for all data and 202 parameters. Final $\Delta/\sigma(\text{max})$ 0.000, $\Delta/\sigma(\text{mean})$, 0.000. Final difference map between +0.131 and -0.149 e $\text{\AA}^{-3}$.

Table 15 Single crystal structure of Form 2 (maleate)

Molecular formula	$\text{C}_{14}\text{H}_{17}\text{N}_3\text{O}_4$				
Molecular weight	291.31				
Crystal system	Monoclinic				
Space group	$P2(1)$	a	5.657(3) $\text{\AA}$,	α	90°,
		b	6.840(4) $\text{\AA}$,	β	96.019(16)°,
		c	19.164(10) $\text{\AA}$,	γ	90°
V	737.4(7) $\text{\AA}^3$				
Z	2				
D_c	1.312 g.cm $^{-1}$				
μ	0.098 mm $^{-1}$				
Source, λ	Mo-K(alpha), 0.71073 $\text{\AA}$				
$F(000)$	308				
T	298(1)K				
Crystal	Colourless plate, 0.82 x 0.48 x 0.2mm				
Data truncated to	0.80 $\text{\AA}$				
θ_{max}	26.37°				
Completeness	99.5%				
Reflections	6392				
Unique reflections	2979				
R_{int}	0.0128				

Figure 3 shows a view of a molecule of Form 2 (maleate) from the crystal structure showing the numbering scheme employed.

Figure 3 Molecular configuration of Form 2 (maleate)



Anisotropic atomic displacement ellipsoids for the non-hydrogen atoms are shown at the 50% probability level. The inter-molecular hydrogen bonds are shown as dashed lines. Hydrogen atoms are displayed with an arbitrarily small radius.

Figure 4 shows a view of part of the crystal packing of Form 2 (maleate) in the unit cell looking along the *b*-axis of the unit cell. The inter-molecular hydrogen bonds are shown as dashed lines. For clarity all hydrogen atoms other than the O-H and N-H have been removed.

Figure 4 Crystal packing of Form 2 (maleate)

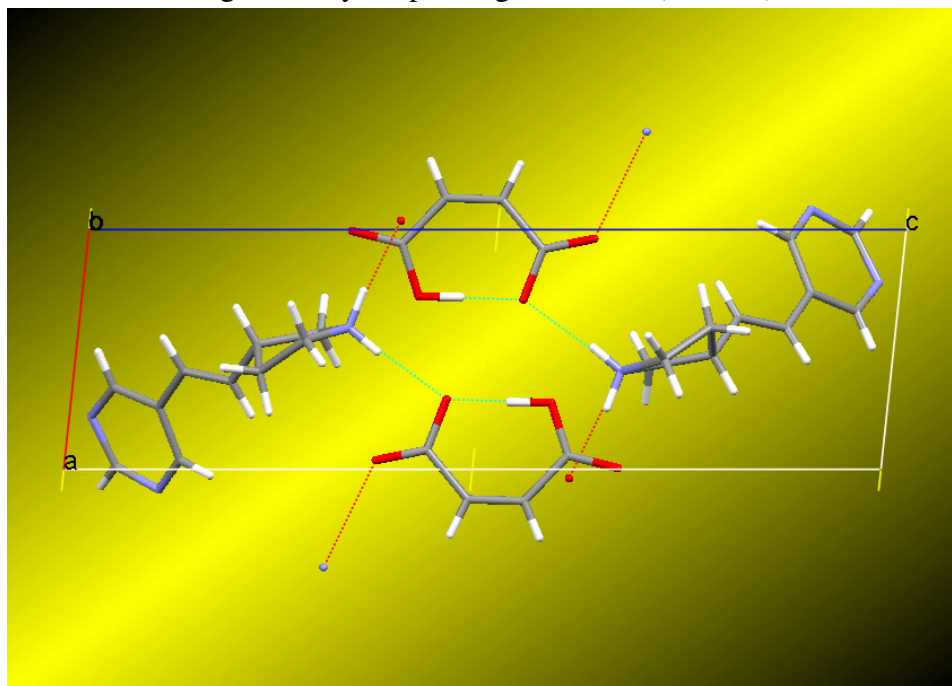
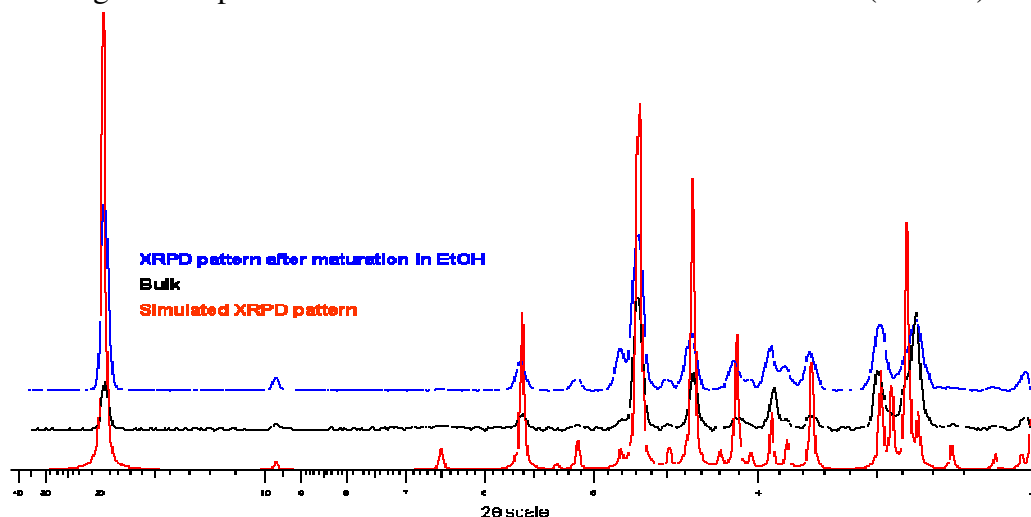


Figure 5 shows the experimental and calculated XRPD patterns of Form 2 (maleate). The experimental pattern from the bulk sample is shown in black and the pattern calculated from the single crystal X-ray structure in red. The XRPD pattern in blue corresponds with the sample obtained after maturation of the mono-maleate in ethanol.

Figure 5 Experimental and calculated XRPD traces of Form 2 (maleate)



9 Recommendations

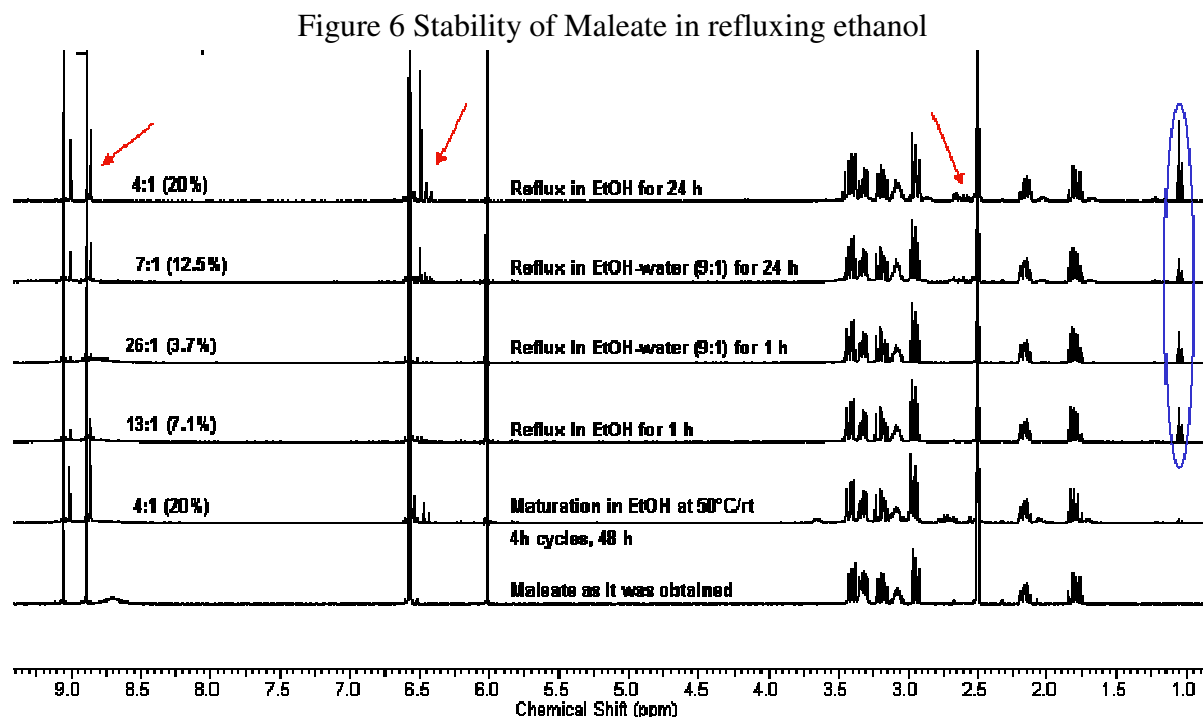
On the basis of these results, our recommendation would be to go with one of the following salts:

- **Mono-citrate:** very popular non-toxic counter ion; acceptable solid form properties; two forms were identified, may require a full polymorph programme.
- **Mono-orotate:** to our knowledge, there are no precedents of orotates as marketed drugs, although there are some of them available as mineral supplements (lithium and magnesium); we have also found a patent for the use of orotates to increase oral bioavailability of drugs (WO/2006/091575); from a solid form point of view, it seems the most robust salt; no polymorphism issue has been identified, and the sample is stable to humidity and temperature.
- **Mono-maleate (Form 2):** several examples of maleates as marketed drugs, it does not exhibit major toxicity issues; two forms have been identified, and there are indications of at least another form which may be more stable; full polymorphism programme would be advisable.

Appendix: Stability of the Maleate salt in EtOH

Targacept reported that a mono maleate salt of TC-6499 with a di-carboxylic acid as counter ion in refluxing ethanol produced the corresponding amide, by reaction of one of the acid groups of the counter ion with the secondary amine of the free base.

In order to establish the robustness of the maleate salt, a number of experiments were carried out where the salt was refluxed over different periods of time in ethanol with and without the addition of water to the mixture. The results of these experiments are shown in the Figure below.



When the sample was matured in the heat/cool incubator at 50°C/rt 4h-cycle for 48 h, which was the procedure used to obtain Form 2, up to a 20% impurity can be detected by ¹H-NMR, based on the integrals of the signals corresponding to the by-product (possibly amide, indicated with red arrows in Figure 6). A very similar result was obtained when the sample was refluxed for 24 h in neat ethanol. When the sample was refluxed for 1 h, and then kept at room temperature in ethanol for 24 h, only ~7% of the impurity can be detected. When 10% of water was added to the mixtures, the impurity level was reduced by half. These observations are summarised in Table 16. The signals highlighted in blue in the ¹H-NMR spectra correspond with some residual ethanol in the samples.

These results may be explained by the formation of the ethyl ester of one of the carboxylic acid groups of the counter ion in refluxing ethanol, which is being catalysed by the acidic proton of the other group, and subsequent reaction of the ester with the amine of the free base to yield the corresponding amide. The presence of water reduces the formation of the ester, and therefore minimises the formation of the amide.

In the sight of these results, our recommendation to prepare Form 2 of the mono-maleate is to incubate the salt as obtained (Form 1) in ethanol at room temperature for 24 to 48 hours, avoiding heating to prevent formation of the ester and the subsequent formation of the amide.

Table 16 Stability of Maleate in refluxing ethanol

Conditions	Comments
Maleate as it was obtained	¹ H-NMR consistent with proposed structure; no impurities can be identified.
Maturation in EtOH (50°C/rt 4 h cycle, 48 h)	¹ H-NMR of the main component consistent with proposed structure, although a structurally related impurity can be identified (~20%).
Reflux in EtOH (1 h)	¹ H-NMR of the main component consistent with proposed structure, although a structurally related impurity can be identified (~7%); traces of ethanol present in the sample.
Reflux in EtOH (24 h)	¹ H-NMR of the main component consistent with proposed structure, although a structurally related impurity can be identified (~20%); traces of ethanol present in the sample.
Reflux in EtOH-water (9:1, 1 h)	¹ H-NMR of the main component consistent with proposed structure, although a structurally related impurity can be identified (~3%); traces of ethanol present in the sample.
Reflux in EtOH-water (9:1, 24 h)	¹ H-NMR of the main component consistent with proposed structure, although a structurally related impurity can be identified (~12%); traces of ethanol present in the sample.

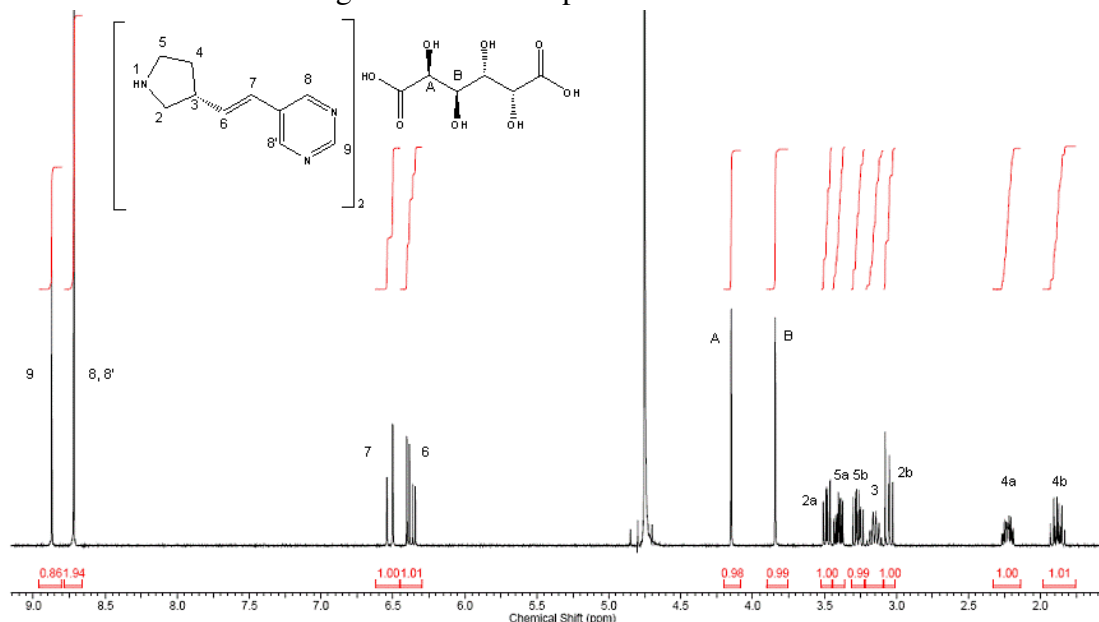
Abbreviations

Table 17 List of abbreviations

DCM	dichloromethane, methylene chloride
DMF	dimethyl formamide
Et ₂ O	di-ethyl ether
EtOAc	ethyl acetate
EtOH	ethanol, ethyl alcohol
H ₂ O	water - distilled or HPLC grade
IPA	iso-propyl alcohol, propan-2-ol
IPAc	iso-propyl acetate
MeCN	methyl cyanide, acetonitrile
MEK	methyl ethyl ketone, butanone
MeOH	methanol, methyl alcohol
MIBK	methyl isobutyl ketone, 2,2-dimethyl butan-3-one
NMP	n-methyl pyrrolidone
RT	room temperature
SM	starting material
TBME	t-butyl methyl ether
THF	tetrahydrofuran

Data Section 1: Batch Characterisation of E00118

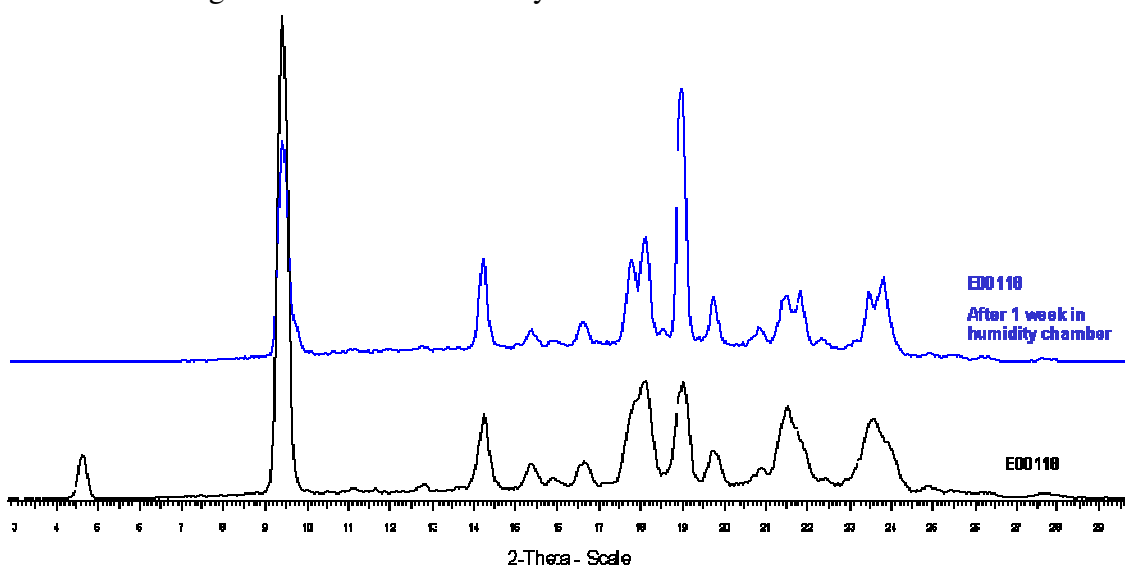
Figure 7 ^1H -NMR spectrum of E00118



^1H -NMR spectrum is consistent with proposed structure. No impurities can be seen by NMR. Stoichiometry confirmed by integration of the signals of the counter ion.

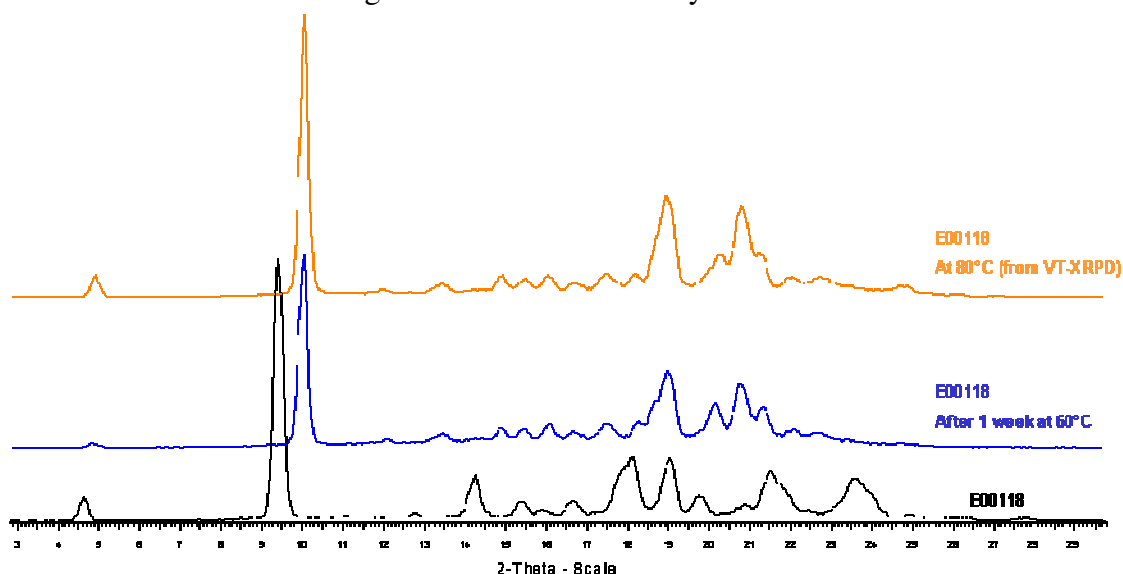
^1H -NMR (400 MHz, $\text{DMSO}-d_6$, 298K): δ , 8.87 (1H, s), 8.72 (2H, s), 6.52 (1H, d, J 16.2), 6.38 (1H, dd, J 16.2, 7.6), 4.15 (1H, s), 3.84 (1H, s), 3.48 (1H, dd, J 11.4, 7.3), 3.40 (1H, ddd, J 12.6, 8.6, 4.3), 3.27 (1H, ddd, J 11.9, 9.4, 7.3), 3.15 (1H, m), 3.05 (1H, dd, J 11.4, 9.4), 2.22 (1H, m) and 1.88 (1H, dq, J 13.1, 9.1).

Figure 8 XRPD and stability at 40°C and 75%RH of E00118



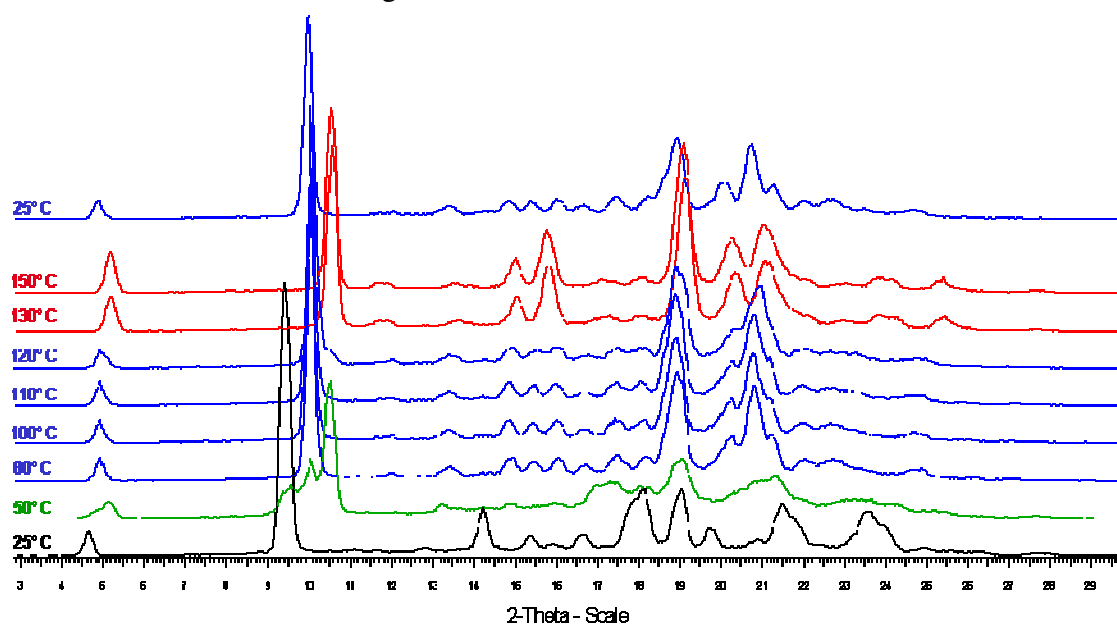
E00118 is crystalline. No major changes were observed in the humidity chamber after the sample was stored at 40°C and 75%RH for one week (only an improvement in the crystallinity).

Figure 9 XRPD and stability at 60°C



When the sample was stored at 60°C for one week, a new pattern was observed. This pattern matches Pattern 2 from the VT-XRPD experiment (see below).

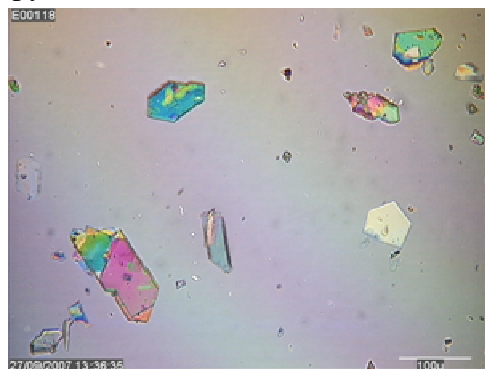
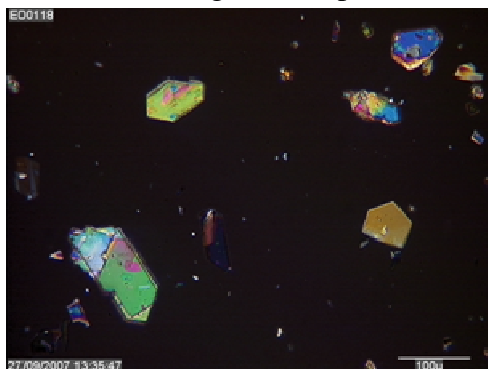
Figure 10 VT-XRPD of E00118



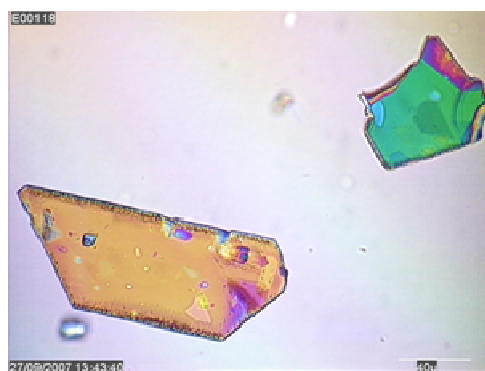
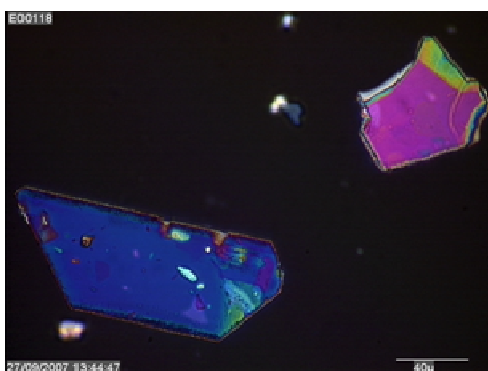
A new crystalline pattern is observed when the sample was heated to 50°C (probably a mixture of patterns). A new XRPD pattern can also be observed at temperatures between 80 and 120°C (Pattern 2 – in blue). A new change in the pattern occurs when the sample is heated to 130°C and it remains unchanged until the material melts (Pattern 3 – in red). When the sample was cooled down back to room temperature, it showed Pattern 2.

Figure 11 Optical microscopy for E00118

x20

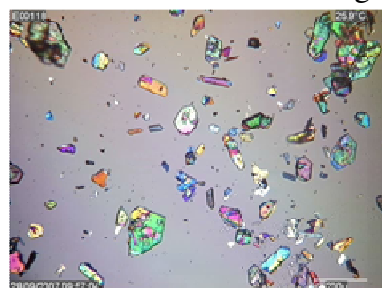


x50

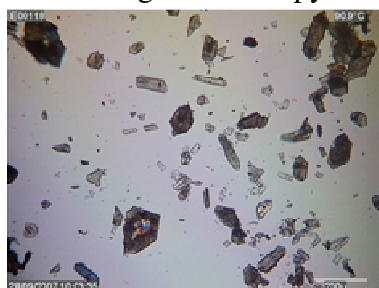


Blade shape plates of up to 120 μm (length) and 60 μm (width). Birefringence is clearly observed with polarised light.

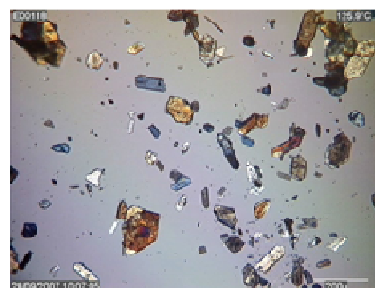
Figure 12 Hot stage microscopy for E00118



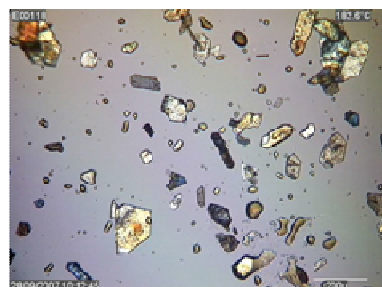
25°C



60°C



125°C



162°C

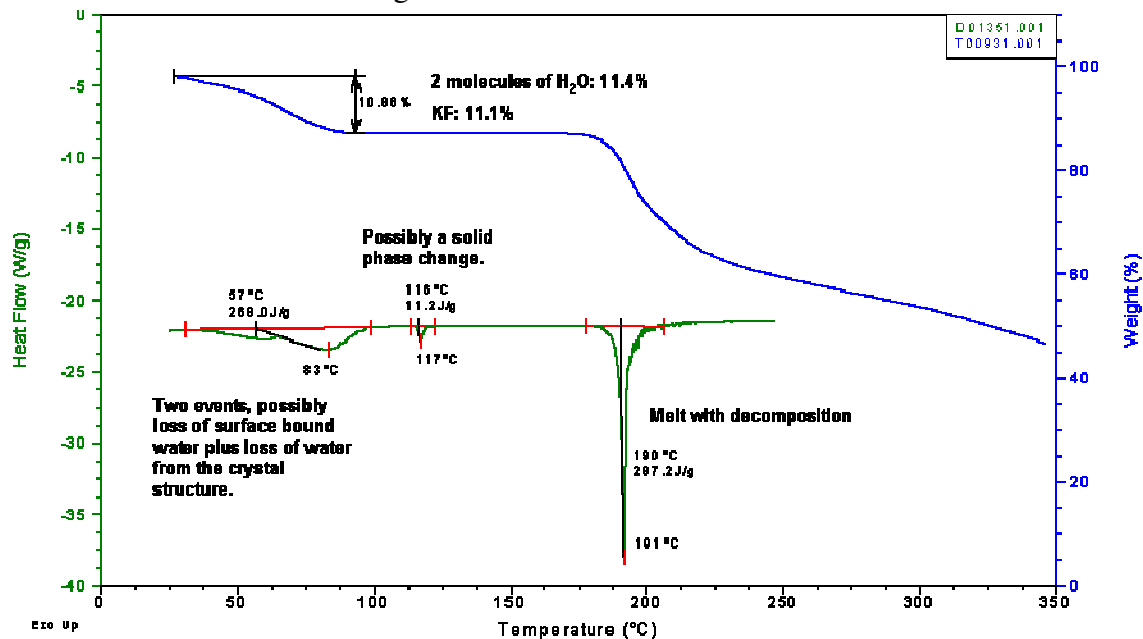


180°C

The material is initially crystalline, showing clear birefringence. At 55-60°C, some color change can be observed, leading to completely transparent material at around 75-80°C. At 115-120°C color appears again, which indicates that a recrystallisation is occurring. Between 175-180°C the material melts and becomes very dark, which indicates decomposition.

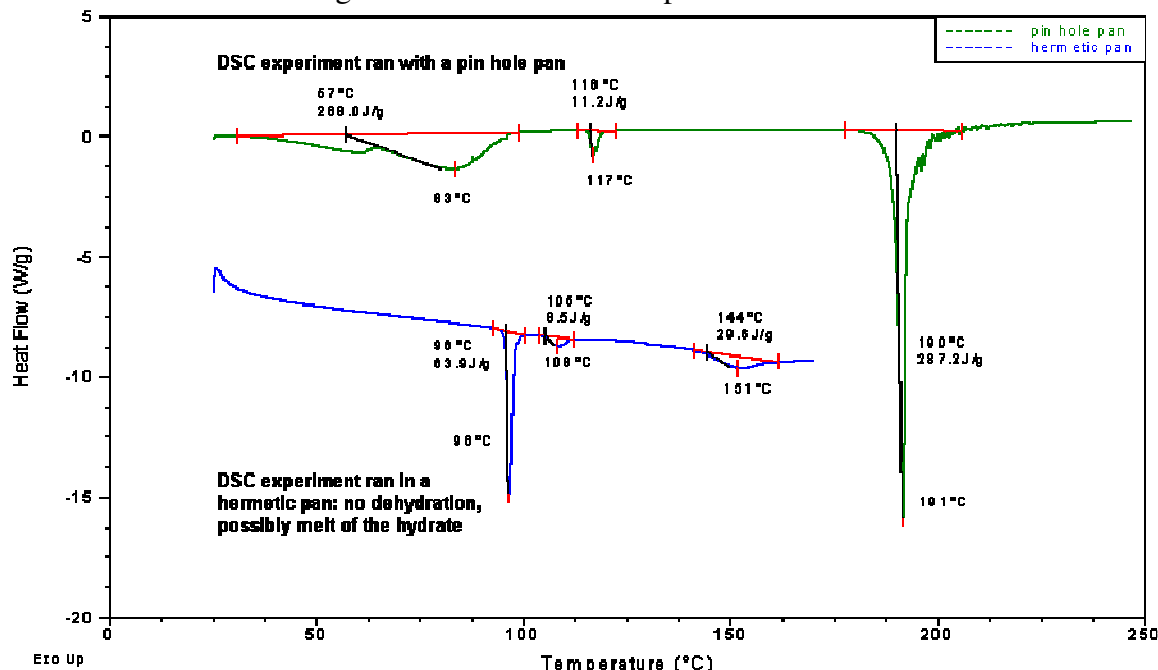
The material was initially very crystalline. When we heated the sample to 55-60°C, some colour changes were observed, leading to completely colourless material at around 75-80°C. At 115-120°C colour appeared again, which indicated that a recrystallisation was occurring. Between 175-180°C the material melted and degradation started.

Figure 13 TGA/DSC for E00118



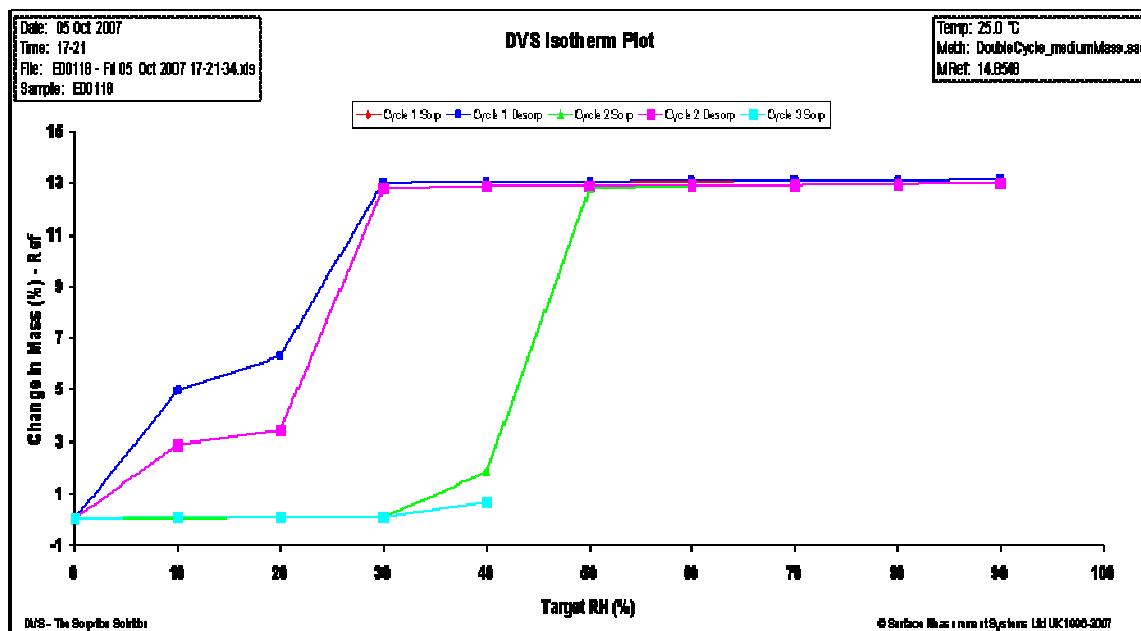
A weight loss of 10.9% is observed between room temperature and 90°C (Karl Fisher analysis showed 11.1% of water in the sample, which corresponds to two molecules of water per molecule of API). Decomposition starts at ~190°C. The DSC shows two endotherms at low temperature, related to the weight loss (sequential dehydration of two molecules of water). Another small endotherm occurs at 116°C (possibly a solid phase change). The material melts with decomposition at 190°C.

Figure 14 DSC in hermetic pan of E00118



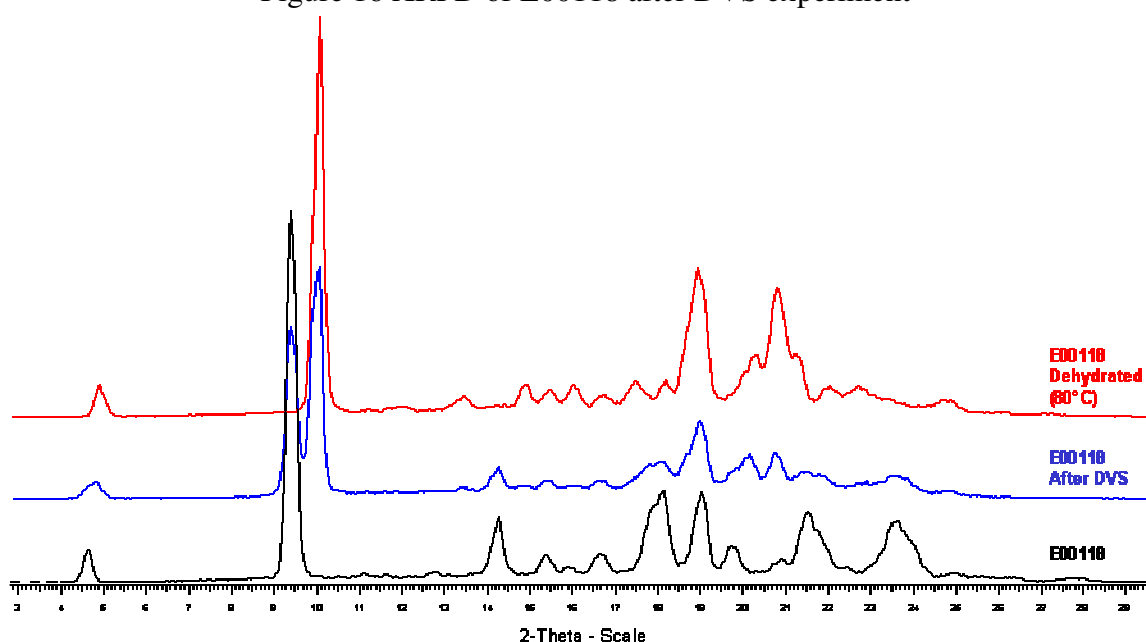
To obtain the melting point of the di-hydrate, a hermetic pan was used, which prevented the loss of water. This melting point was estimated at 96°C.

Figure 15 DVS isotherm of E00118



The DVS isotherm shows that the di-hydrate forms at relative humidity above 40%, when the sample takes up to 12-13% of water. When the humidity level are reduced below 30%, the first molecule of water is lost, while the second one is released from the structure when humidity reaches levels below 10%.

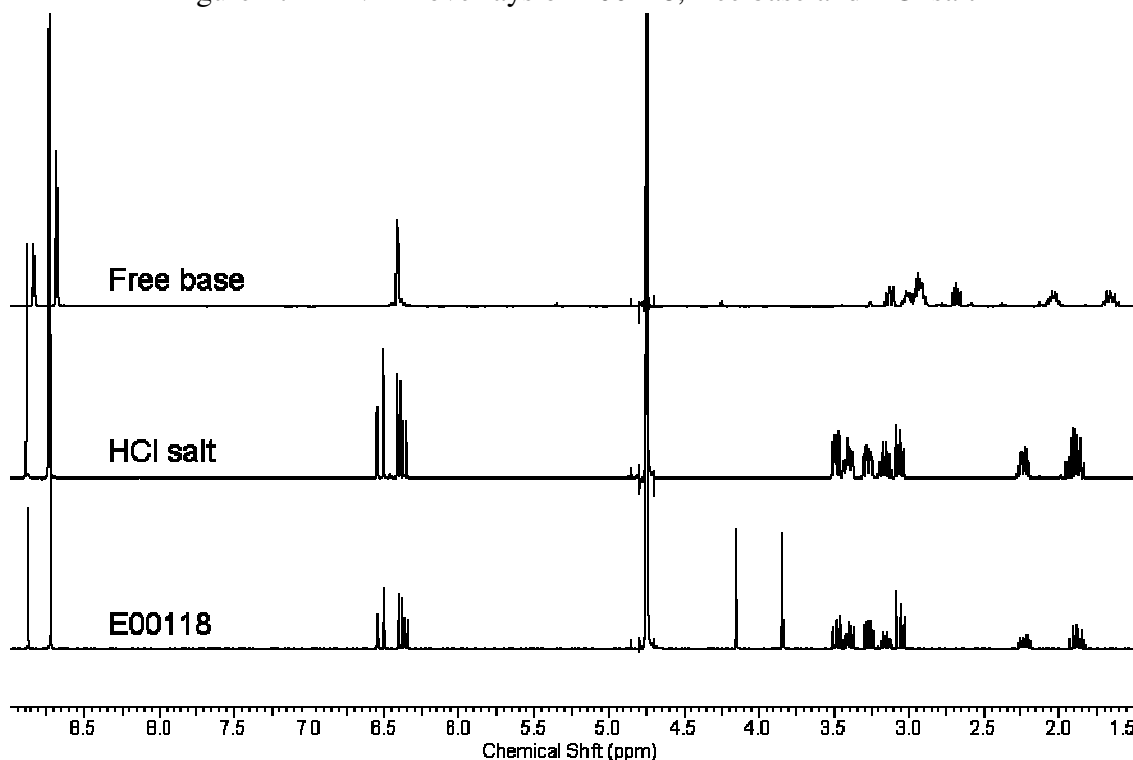
Figure 16 XRPD of E00118 after DVS experiment



When the experiment finished, and the sample was re-analysed by XRPD, a mixture of di-hydrate and anhydrous form was observed (Patterns 1 and 2).

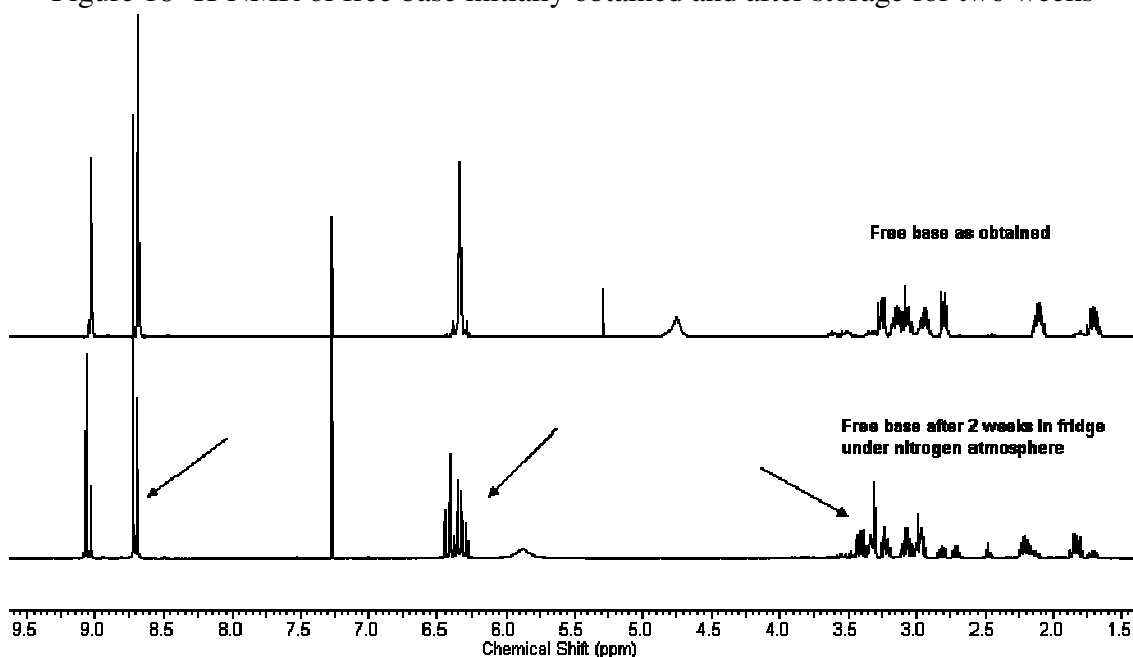
Data Section 2: Salt Screening

Figure 17 ^1H -NMR overlays of E00118, free base and HCl salt

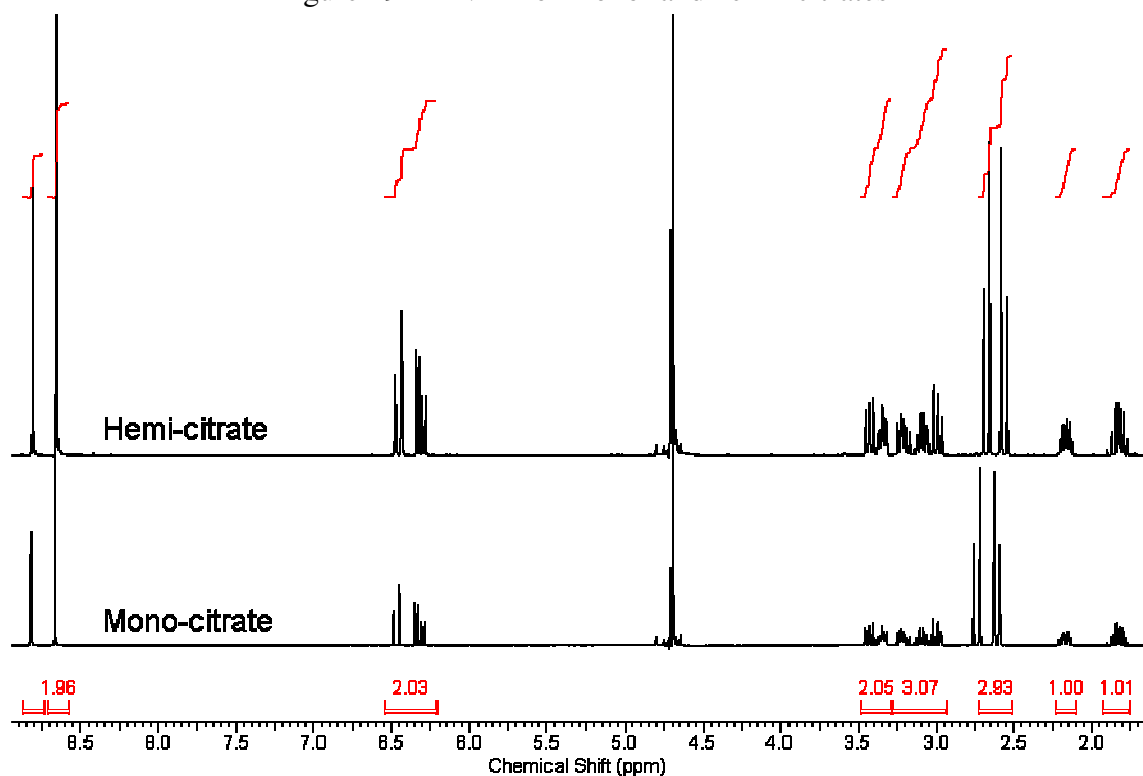


The ^1H -NMR spectrum of the starting material is consistent with the proposed structure, and in it we can identify two singlets corresponding to the galactaric acid. The integral of these signals also confirms the 2:1 stoichiometry (API:counter ion) expected for the hemi salt. The free base shows some changes in the chemical shift of the signals, plus the disappearance of the signals of the counter ion. The HCl salt shows a spectrum consistent with the protonation of the basic nitrogen, as we had seen in E00118, but without the signals for the counter ion.

Figure 18 ^1H -NMR of free base initially obtained and after storage for two weeks

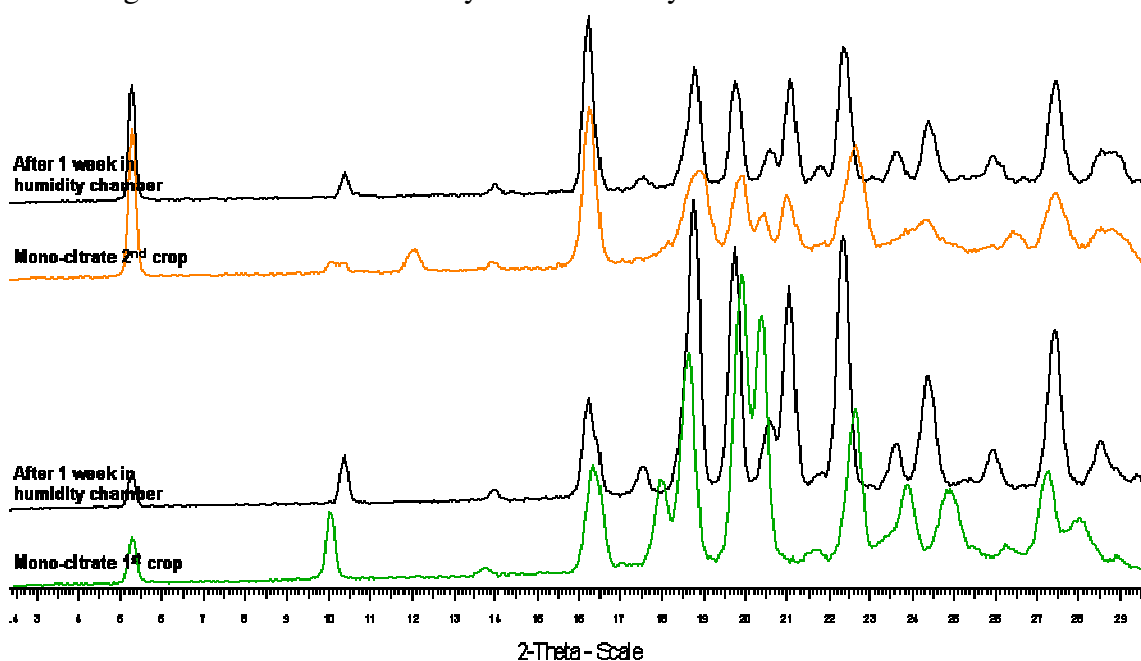


Mono-Citrate

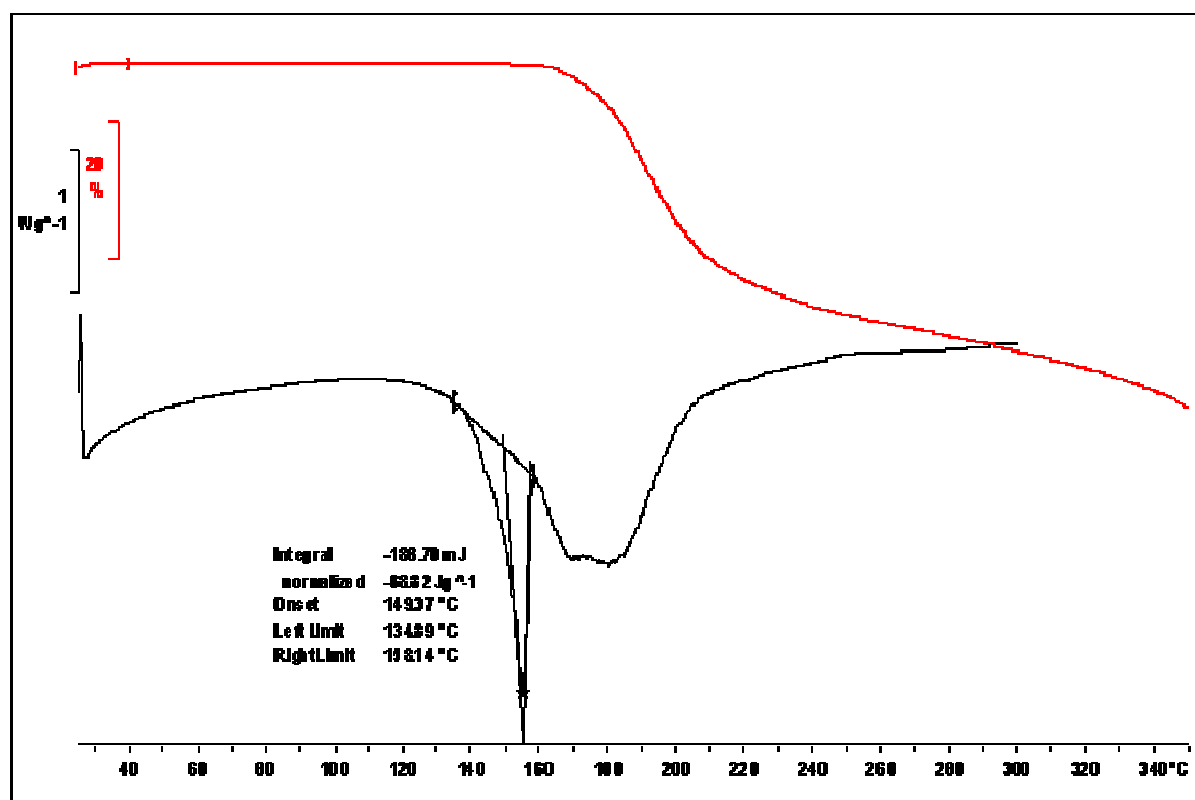
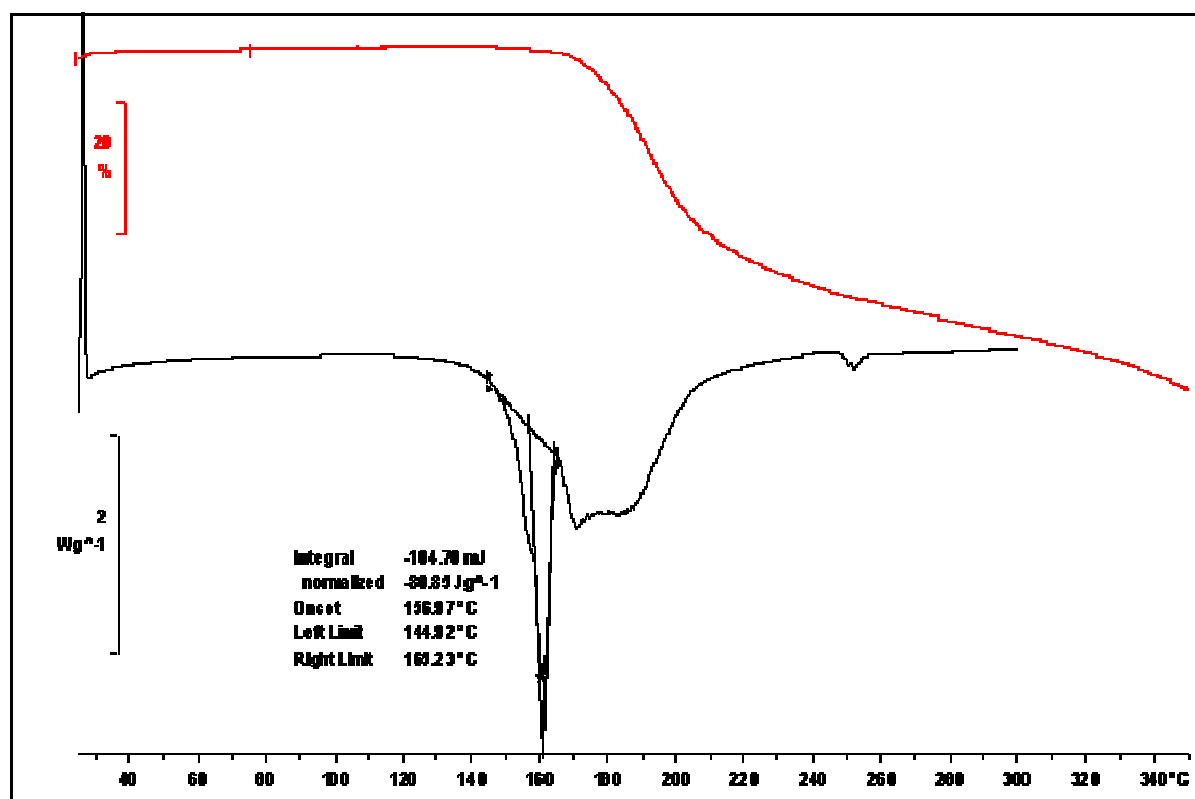
Figure 19 ^1H -NMR of mono- and hemi- citrates

When the hemi-citrate was analysed by NMR, the integral of the signals corresponding with the citric acid moiety were bigger than expected, which led us to believe we may have a mixture of mono and hemi salts. This fact seemed to be supported for the XRPD and stability data for the hemi-citrate.

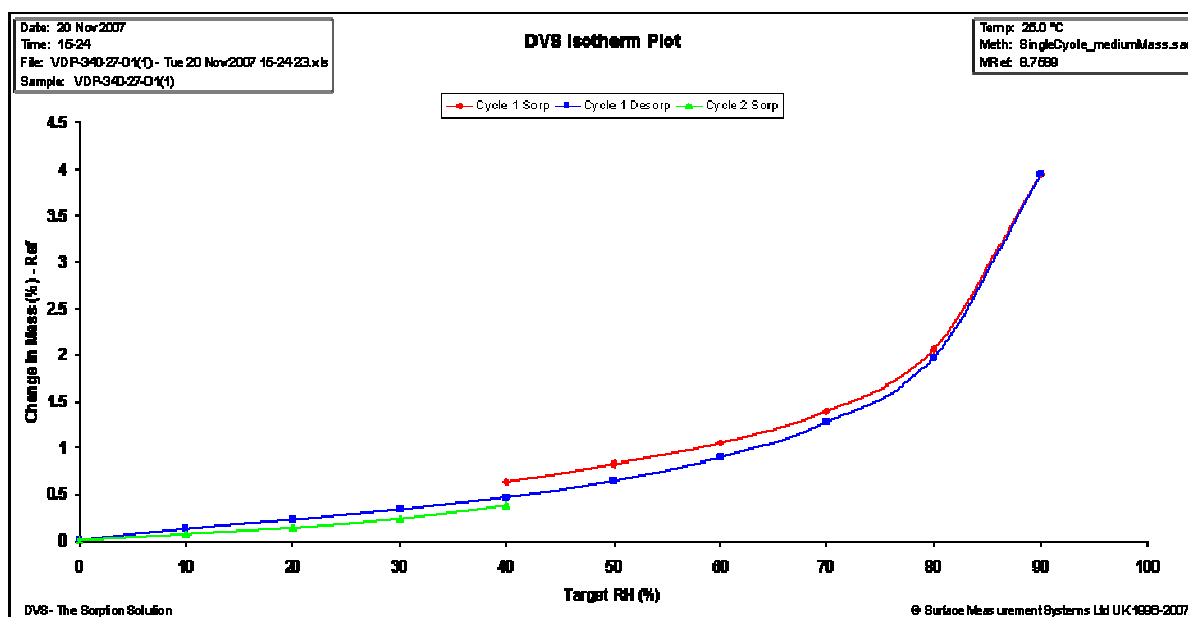
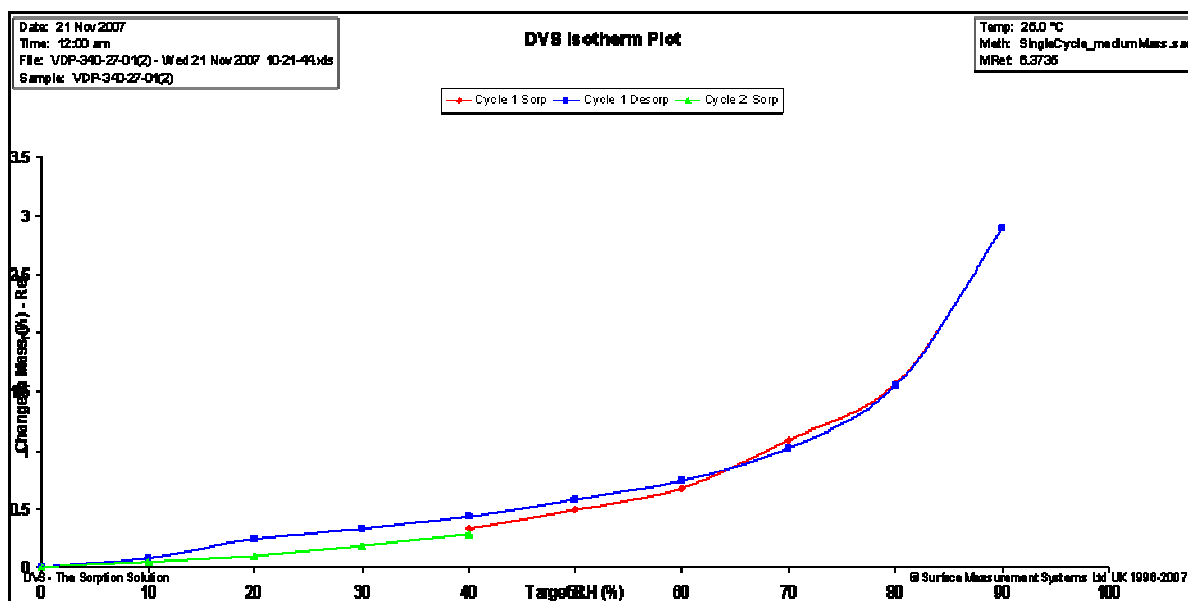
Figure 20 XRPD and stability in the humidity chamber of the mono-citrate



Both form of the mono-citrate produce the same pattern after a week at 40°C and 75%RH, which seems to match Form 2 (2nd crop), with a slight increase in crystallinity.

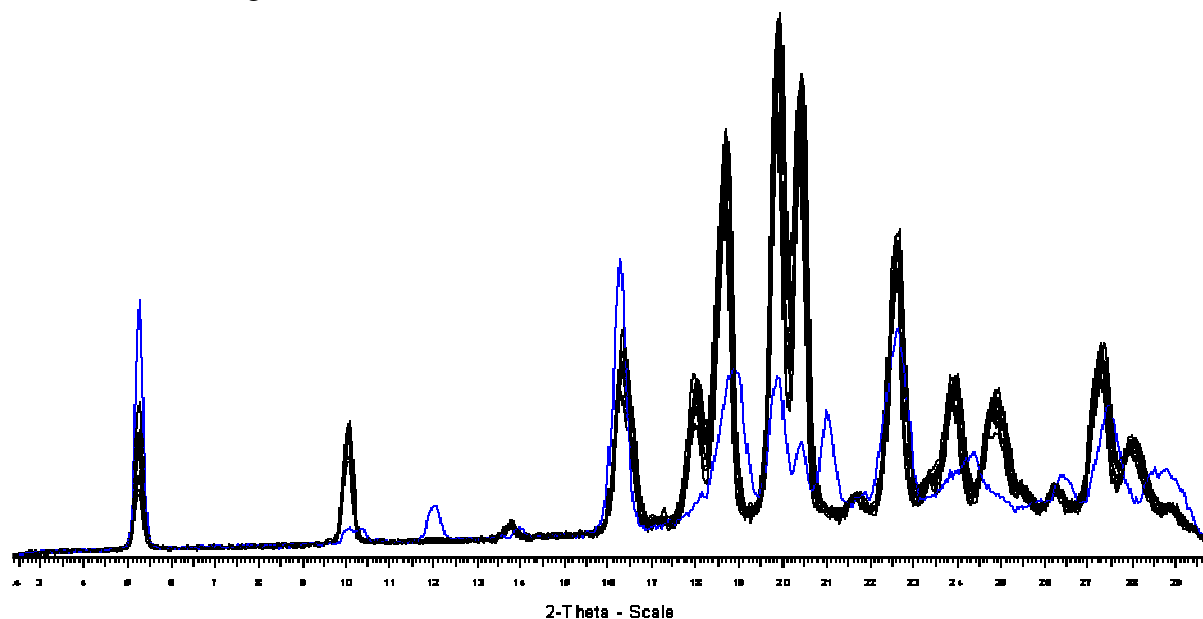
Figure 21 TGA/DSC of Form 1 (1st crop) of mono-citrateFigure 22 TGA/DSC of Form 2 (2nd crop) of mono-citrate

No weight loss for either Form until decomposition occurs at ~170°C. Form 1 melts at 149°C, Form 2 melts at 157°C.

Figure 23 DVS experiment for Form 1 (1st crop) of mono-citrateFigure 24 DVS experiment for Form 2 (2nd crop) of mono-citrate

Both Forms behave similarly. Less than 1.5% of water taken below 70%RH, it goes up to 4% at 90%RH. No major changes are observed in the XRPD of either Form 1 or Form 2 before and after the DVS experiment.

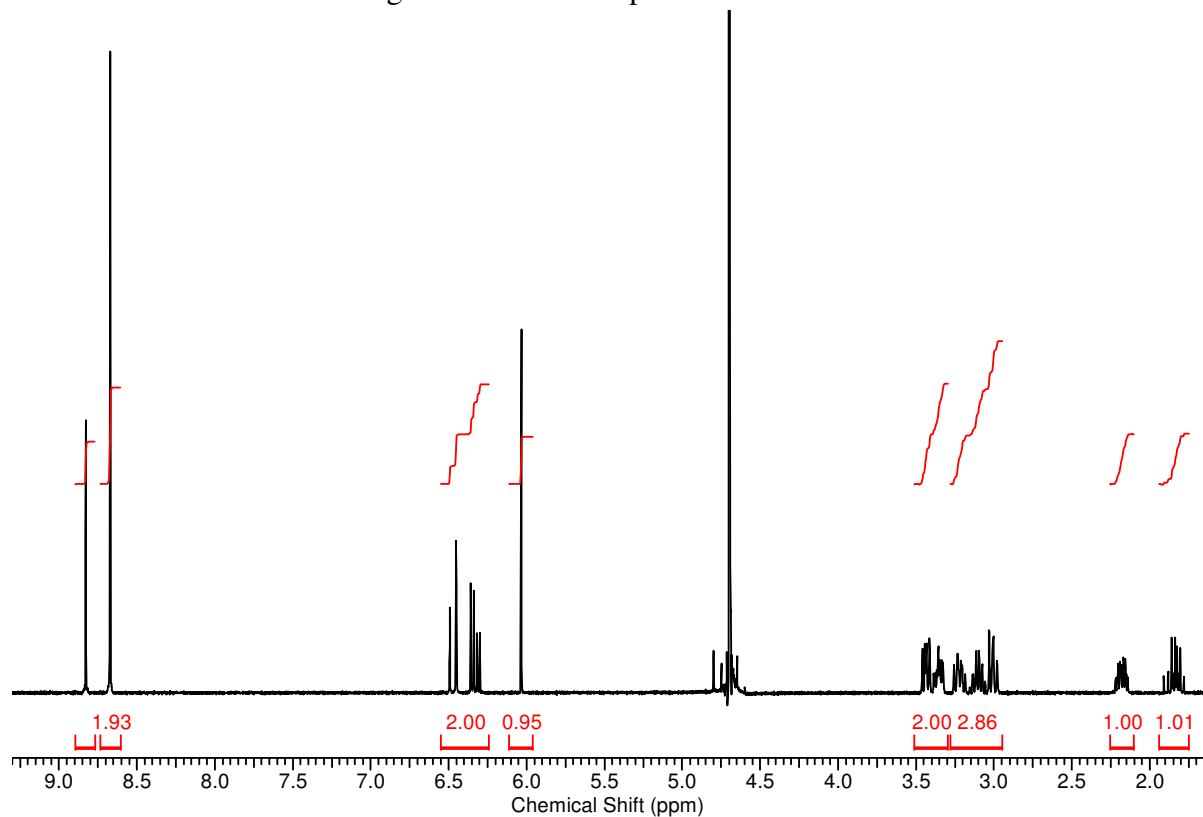
Figure 25 Maturation of mono-citrate in 15 different solvents



Maturation of the mono-citrate in 15 different solvents at 50°C/r.t. (4h cycle) for 24h, only produced Form 1. Form 2 (blue) was obtained when a second crop was crystallised. This is also the form we had previously observed in the preliminary salt screening (possibly thermodynamic vs. kinetic control)

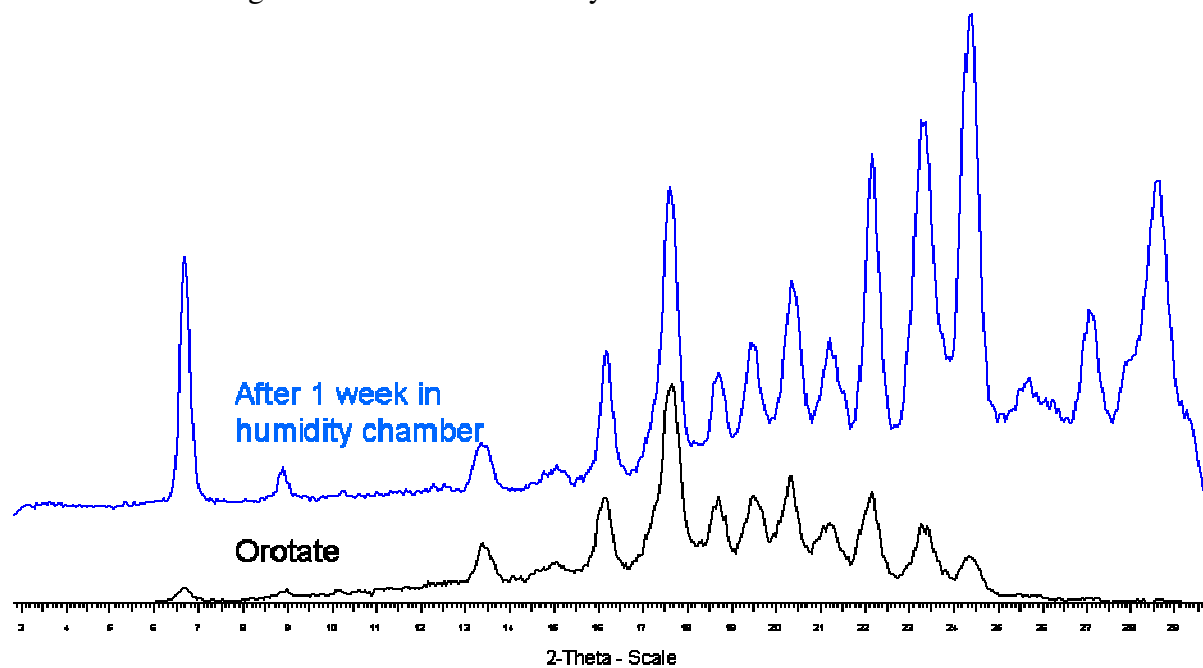
Orotate

Figure 26 ^1H -NMR spectrum of orotate



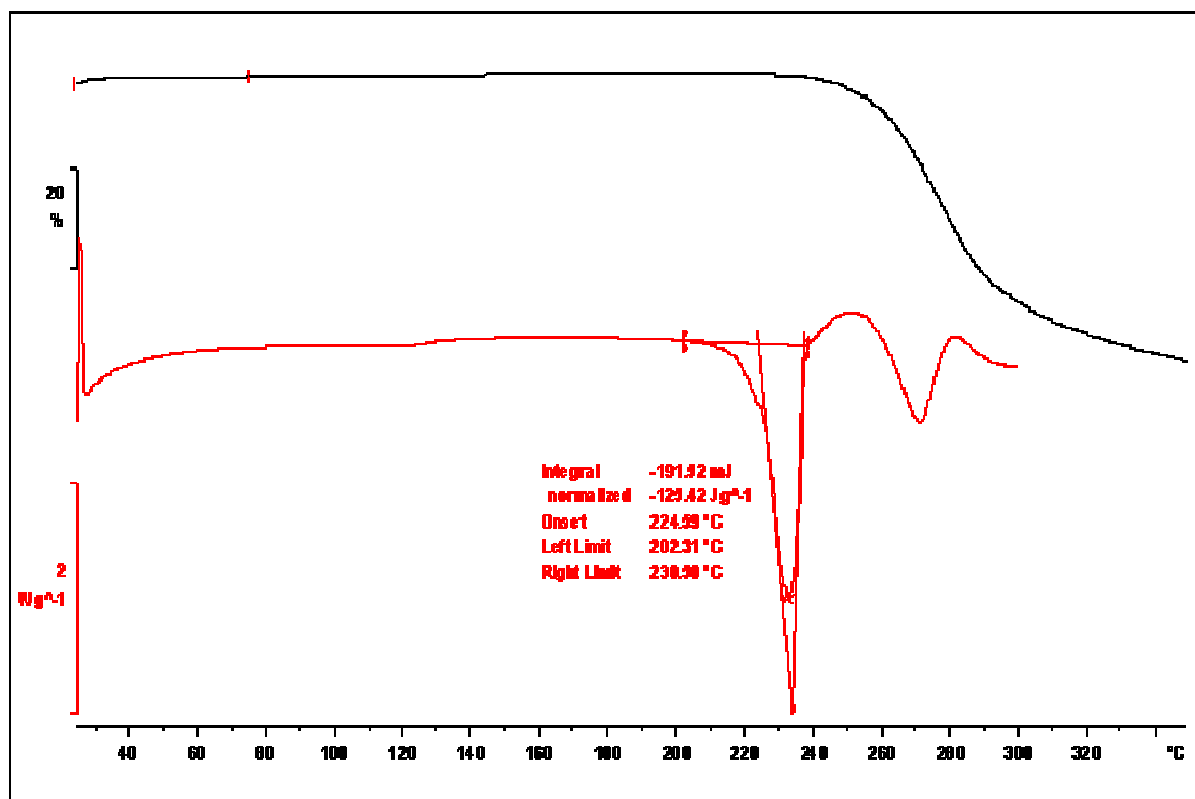
^1H -NMR spectrum is consistent with proposed structure. The stoichiometry has been confirmed by integration of the signals for the counter ion.

Figure 27 XRPD and stability at 40°C and 75%RH of orotate



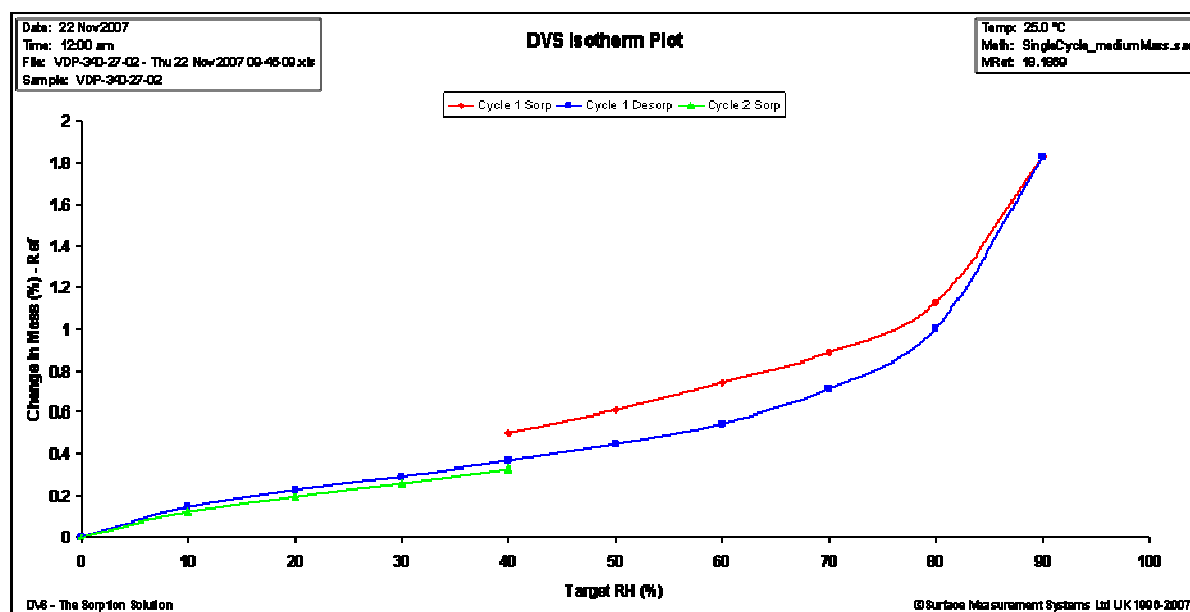
No major changes are observed in the XRPD pattern of the orotate after 1 week at 40°C and 75%RH (the differences on the right hand side of the diffractogram are due to a change of detector in the time passed between the two experiments).

Figure 28 TGA/DSC of orotate



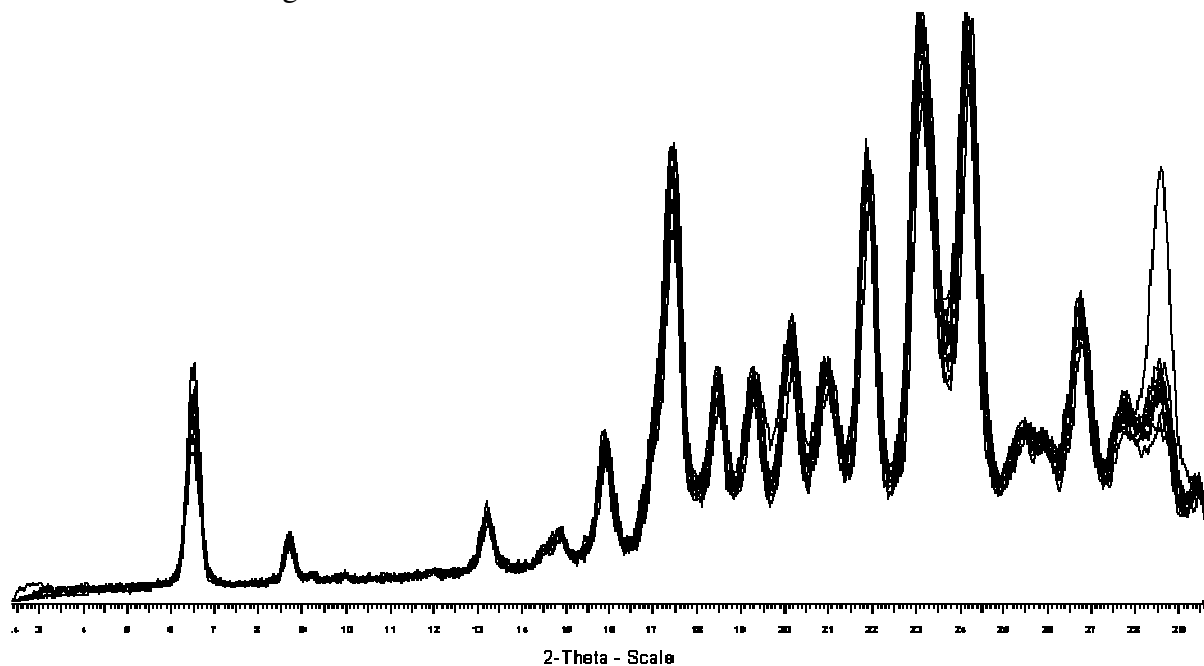
No weight loss until decomposition occurs at ~240°C. The sample melts at 224°C.

Figure 29 DVS experiment for orotate



Less than 1% of water taken below 70%RH, it goes up to 1.8% at 90%RH. No major changes are observed in the XRPD before and after the DVS experiment, maybe some increase in crystallinity.

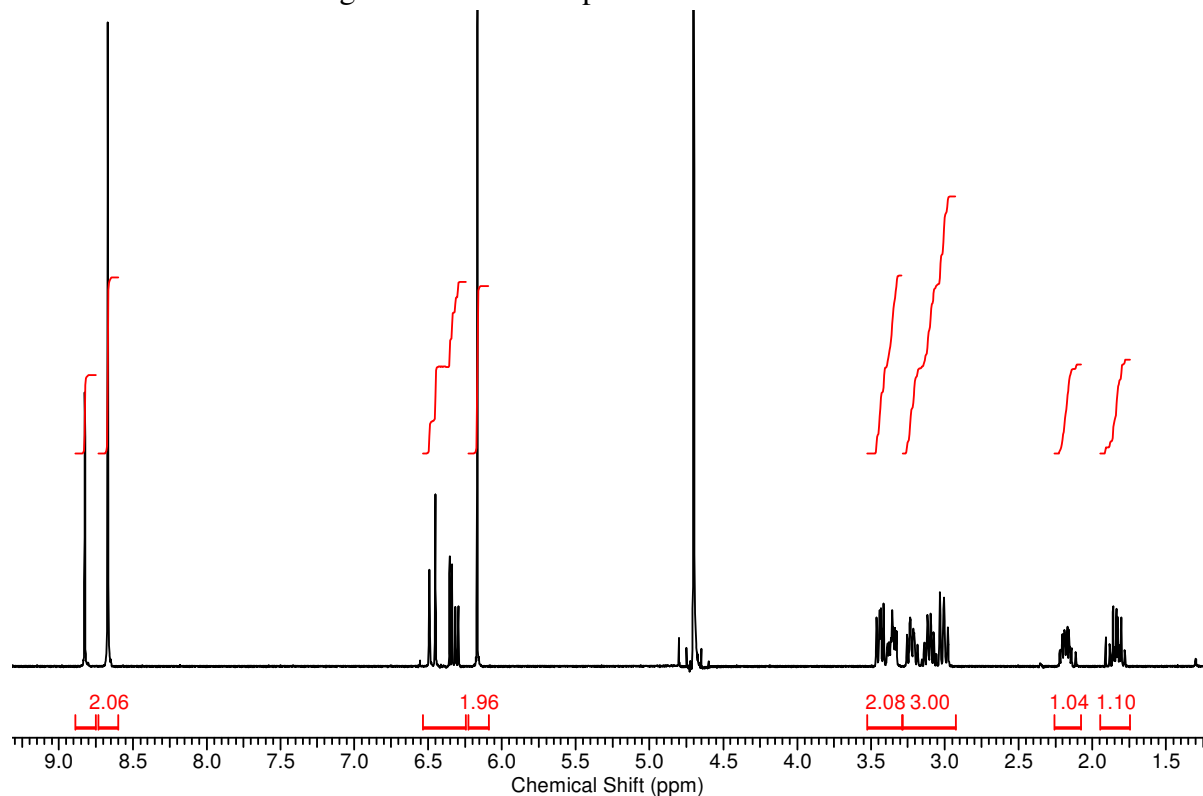
Figure 30 Maturation of orotate in 15 different solvents



Maturation of the orotate in 15 different solvents at 50°C/r.t. (4h cycle) for 24h, only produced Form 1. This form also matches with the form originally identified in the preliminary salt screening.

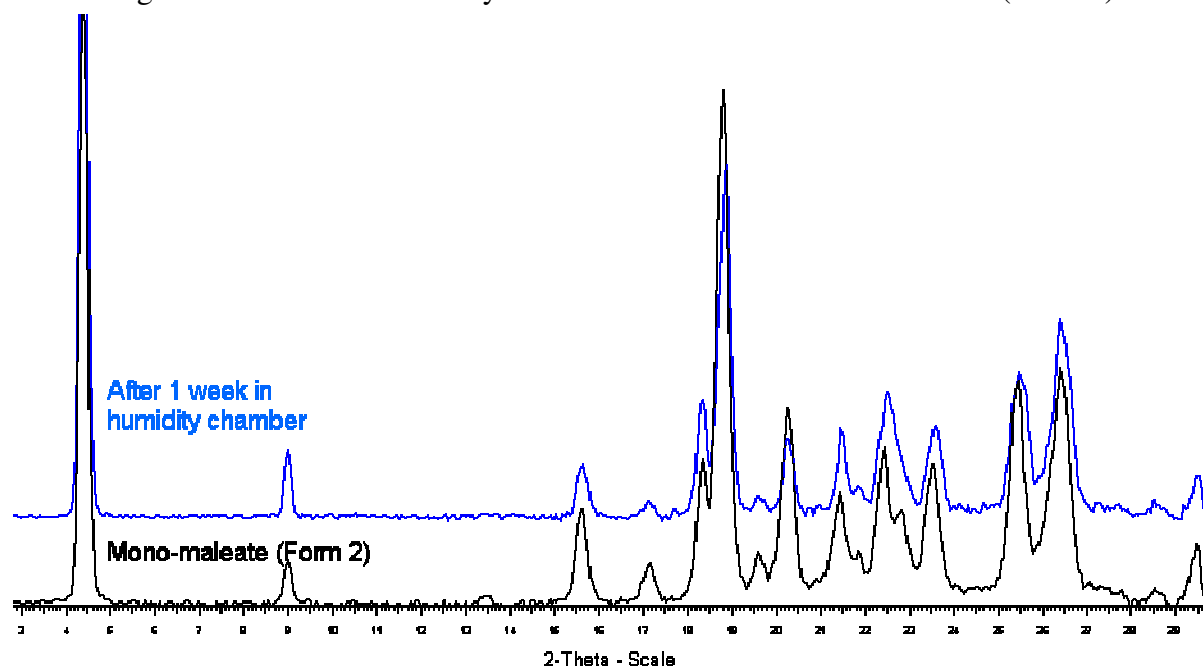
Mono-maleate (Form 2)

Figure 31 ^1H -NMR spectrum of mono-maleate



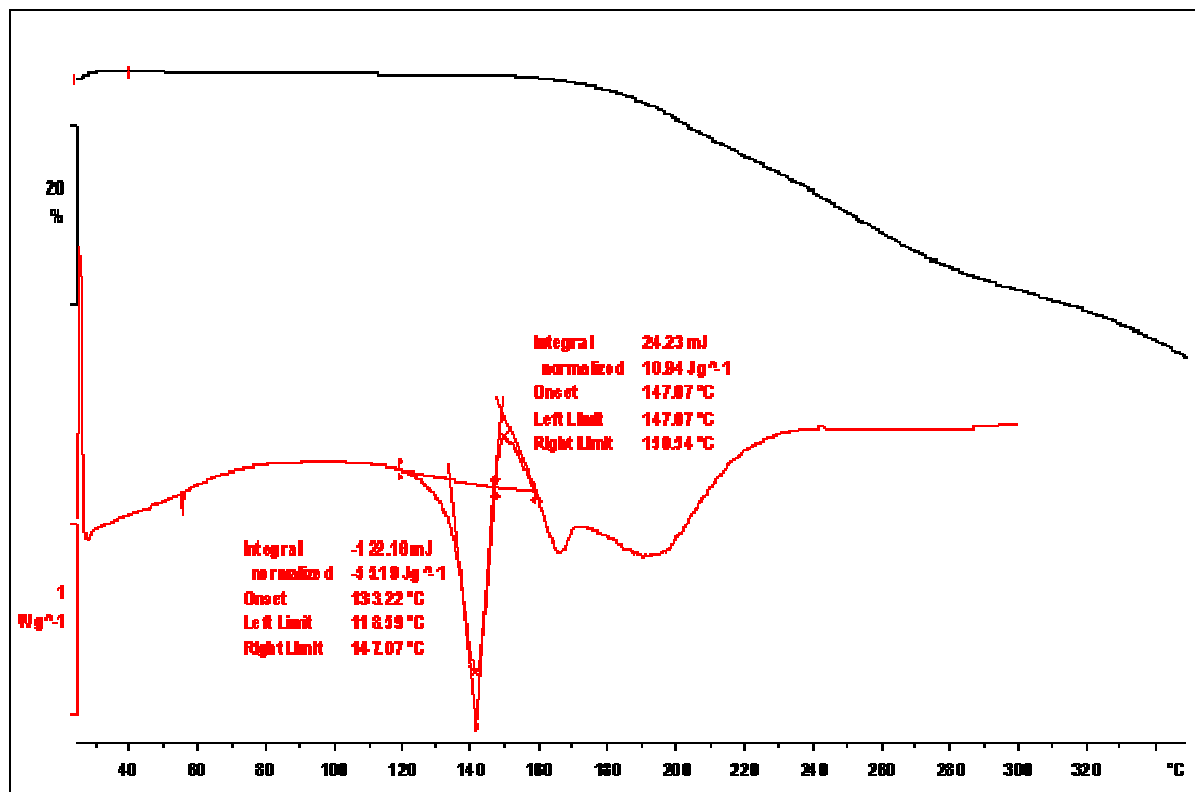
^1H -NMR spectrum is consistent with proposed structure. The stoichiometry has been confirmed by integration of the signals for the counter ion.

Figure 32 XRPD and Stability at 40°C and 75%RH of mono-maleate (Form 2)



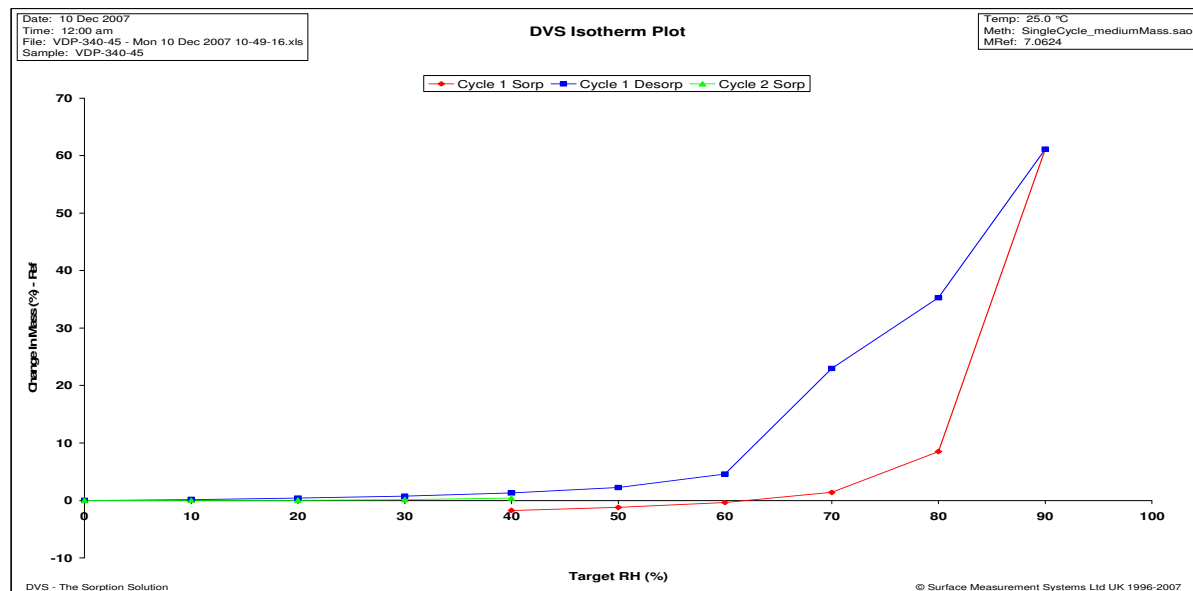
Form 2 is stable in the humidity chamber after a week. Form 2 was also obtained by storing Form 1 at 40°C and 75%RH for a week.

Figure 33 TGA/DSC of mono-maleate (Form 2)



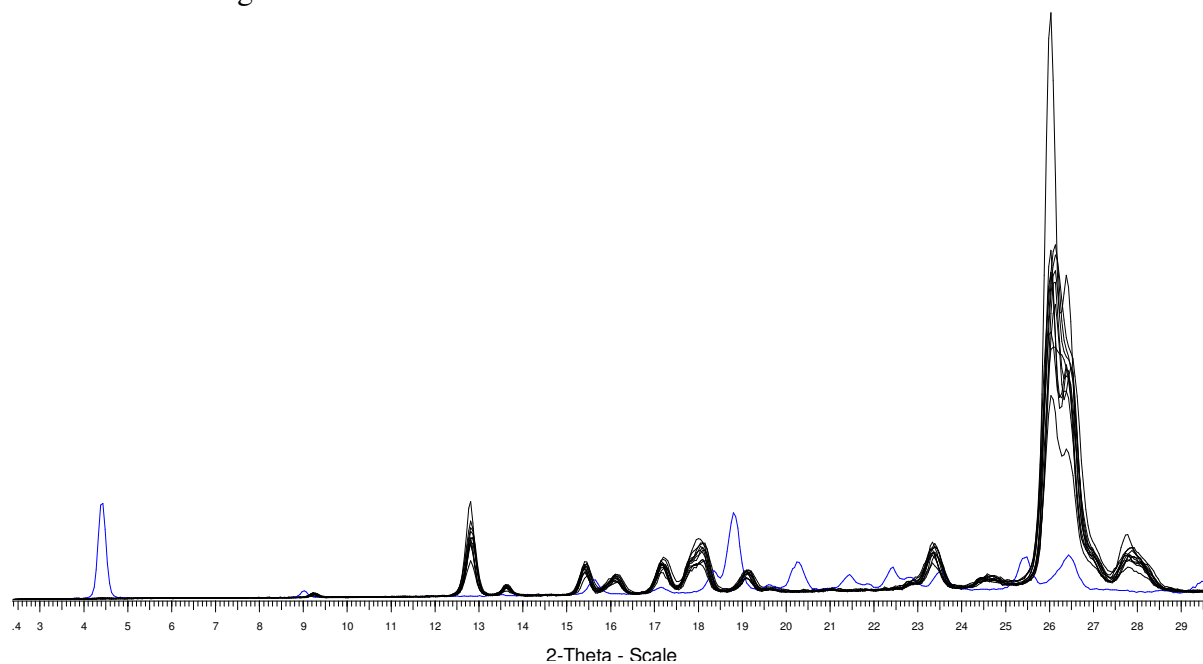
No weight loss is observed for the mono-maleate (Form 2) until decomposition occurs at ~170°C. The sample melts at 133°C and a recrystallisation follows before degradation starts (possible Form 3).

Figure 34 DVS experiment for mono-maleate (Form 2)



Very little uptake of water occurs below 80%RH (< 5%). Above this level, the uptake of water goes up to 60% at 90%RH. Reversible process, dehydration occurs at 60%RH. No changes in XRPD pattern.

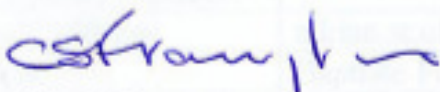
Figure 35 Maturation of mono-maleate in 15 different solvents



Maturation of the mono-maleate in 15 different solvents at 50°C/r.t. (4h cycle) for 24h, produced mostly Form 1, but a new form (Form 2, in blue) was also identified. This form also matches with the crystals formed after storage of Form 1 in the humidity chamber for a week.

Pharmorphix™

Polymorphic behaviour of TC-6499-221 for Targacept, Inc

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Proposal Reference

Q1083_001ASCB160108

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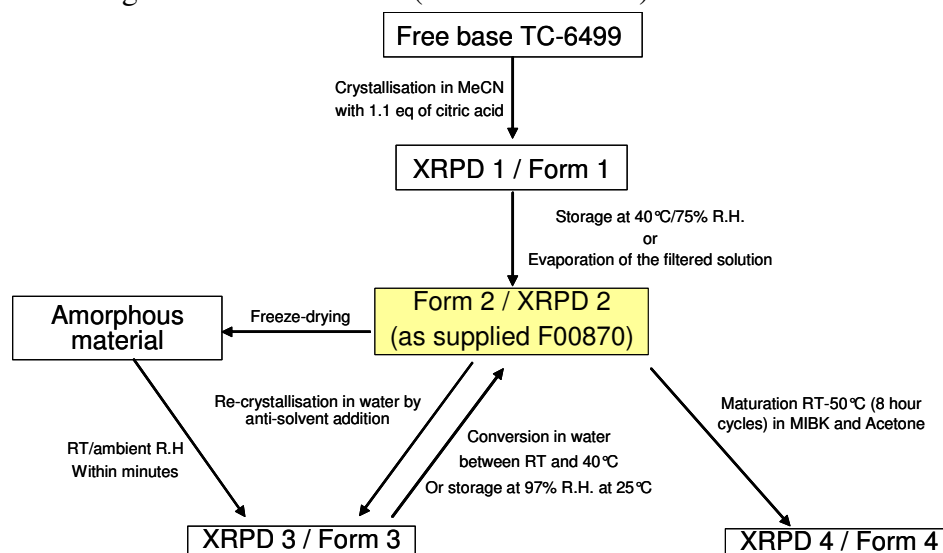
Executive Summary

Four crystalline forms of TC-6499-221 (mono-citrate) have been identified in the course of Project 1083.

- Form 1 (Crop 1/XRPD 1) was the first solid phase highlighted in the course of the salt selection study (P880; report R880_002AC060907), TGA-DSC showed that Form 1 is an anhydrous polymorphic form which melts and degrades at temperature above 149°C. However, attempts to produce a new batch of Form 1, during this study, didn't succeed and this phase was not reproducible (disappearing polymorph).
- Form 2 (F00870 as supplied/XRPD 2) is an anhydrous polymorphic form of the mono-citrate salt which melts and degrades at temperature above 164°C. Form 2 is not sensitive to high level of humidity (97% R.H at 25°C and 75% R.H at 40°C) and not hygroscopic.
- Form 3 (XRPD 3) is a new anhydrous polymorphic form which melts and degrades at temperature above 160°C. Form 3 is stable at the solid state, under normal conditions of temperature and pressure, but converts to Form 2 at high levels of humidity (97% R.H at 25°C). Furthermore, the relative stability between Form 3 and Form 2 showed that Form 3 is a metastable polymorph between 20 and 40°C.
- An additional new XRPD pattern (XRPD 4) was also identified, after maturation of Form 2 in acetone and MIBK (RT-50°C/8 hour cycles), but is less relevant than the morphic forms described above. No other characterisation has been done on this phase as the morphic form was not reproducible.

The Figure below summarises the relationship between the different solid forms of TC-6499-221 (mono-citrate salt).

Figure 1 Form diagram of TC-6499-221 (mono-citrate salt).



In summary, Form 2 has been identified as the most suitable for development (thermally stable and not sensitive to humidity). Form 3 could also be progressed but with suitable moisture control during storage. A scale up of Form 2 was made from the free base in IPA/water (1:4) mixtures by cooling from a saturated solution at 60°C down to 25°C.

1 Introduction

Targacept has a clinical development candidate TC-6499, which has been indicated for pain. A previous salt selection study performed at Pharmorphix identified several salt forms including the mono-citrate salt as suitable candidates for development, subject to a complete polymorphism study. To date, two solid forms of the mono-citrate have been identified: Crop 1 (XRPD 1) was obtained from MeCN and Crop 2 (XRPD 2) on storage of Crop 1 at 40°C/75% R.H. These two phases have been partially characterised by XRPD, ¹H NMR and thermal analyses.

Targacept has requested a further study on the propensity of the mono-citrate salt of TC-6499 to form polymorphs.

2 Objectives

3 Materials

Table 1 Details of Supplied Samples

Name of Compound	Pharmorphix Tracking Number	Customer Batch / Lot Number	Quantity Supplied
TC-6499-221	F00870	JAM-013893	20 g
TC-6499-12	E00478	GMD-011671-2	2.50 g

4 Safety Information

An MSDS has been provided for this compound which indicates that specific hazards associated with the compound have not been evaluated, and the material should be treated as toxic and/or carcinogenic compound. For this reason, directives for safe handling are to avoid contact with eyes, skin, clothing and repeated exposure.

5 Instrument and Methodology Details

5.1. X-Ray Powder Diffraction (XRPD)

5.1.1. Bruker AXS C2 GADDS

X-Ray Powder Diffraction patterns were collected on a Bruker AXS C2 GADDS diffractometer using Cu K α radiation (40 kV, 40 mA), automated XYZ stage, laser video microscope for auto-sample positioning and a HiStar 2-dimensional area detector. X-ray optics consists of a single Göbel multilayer mirror coupled with a pinhole collimator of 0.3 mm.

The beam divergence, i.e. the effective size of the X-ray beam on the sample, was approximately 4 mm. A θ - θ continuous scan mode was employed with a sample - detector distance of 20 cm which gives an effective 2θ range of $3.2^\circ - 29.7^\circ$. Typically the sample would be exposed to the X-ray beam for 120 seconds. The software used for data collection was GADDS for WNT 4.1.16 and the data were analysed and presented using *Diffraction Plus* EVA v 9.0.0.2 or v 13.0.0.2.

Ambient conditions

Samples run under ambient conditions were prepared as flat plate specimens using powder as received without grinding. Approximately 1-2 mg of the sample was lightly pressed on a glass slide to obtain a flat surface.

5.1.2. Bruker AXS D8 Advance

X-Ray Powder Diffraction patterns were collected on a Bruker D8 diffractometer using Cu K α radiation (40kV, 40mA), θ - 2θ goniometer, and divergence of V4 and receiving slits, a Mo monochromator and a Lynxeye detector. The instrument is performance checked using a certified Corundum standard (NIST 1976). The software used for data collection was *Diffraction Plus* XRD Commander v2.5.0 and the data were analysed and presented using *Diffraction Plus* EVA v 11,0.0.2 or v 13.0.0.2.

Samples were run under ambient conditions as flat plate specimens using powder as received. Approximately 20 mg of the sample was gently packed into a cavity cut into polished, zero-background (510) silicon wafer. The sample was rotated in its own plane during analysis. The details of the data collection are:

- Angular range: 2 to $42^\circ 2\theta$
- Step size: $0.05^\circ 2\theta$

Collection time: 0.5 s.step^{-1}

5.2. ^1H NMR

NMR spectra were collected on a Bruker 400MHz instrument equipped with an auto-sampler and controlled by a DRX400 console. Automated experiments were acquired using *ICON-NMR* v4.0.4 (build 1) running with *Topspin* v 1.3 (patch level 8) using the standard Bruker loaded experiments. For non-routine spectroscopy, data were acquired through the use of *Topspin* alone.

Samples were prepared in D_2O . Off-line analysis was carried out using *ACD SpecManager* v 9.09 (build 7703).

5.3. FT-IR by ATR

Data were collected on a Perkin-Elmer Spectrum One fitted with a Universal ATR sampling accessory. The data were collected and analysed using Spectrum v5.0.1 software.

5.4. Differential Scanning Calorimetry (DSC)

5.4.1. Mettler DSC 823e

DSC data were collected on a Mettler DSC 823e equipped with a 34 position auto-sampler. The instrument was calibrated for energy and temperature using certified indium. Typically 0.5-1 mg of each sample, in a pin-holed aluminium pan, was heated at 10 °C.min⁻¹ from 25 °C to 300 °C. A nitrogen purge at 50 ml.min⁻¹ was maintained over the sample. The instrument control and data analysis software was STARe v9.10.

5.5. Thermo-Gravimetric Analysis (TGA)

5.5.1. Mettler TGA/SDTA 851e

TGA data were collected on a Mettler TGA/SDTA 851e equipped with a 34 position auto-sampler. The instrument was temperature calibrated using certified indium. Typically 5-15 mg of each sample was loaded onto a pre-weighed aluminium crucible and was heated at 10 °C.min⁻¹ from ambient temperature to 300 °C. A nitrogen purge at 50 ml.min⁻¹ was maintained over the sample.

The instrument control and data analysis software was STARe v9.10.

5.6. Polarised Light Microscopy (PLM)

Samples were studied on a Leica LM/DM polarised light microscope with a digital video camera for image capture. A small amount of each sample was placed on a glass slide, mounted in immersion oil and covered with a glass slip, the individual particles being separated as well as possible. The sample was viewed with appropriate magnification and partially polarised light, coupled to a λ false-colour filter.

5.7. Gravimetric Vapour Sorption (GVS)

5.7.1. SMS DVS Intrinsic

Sorption isotherms were obtained using a SMS DVS Intrinsic moisture sorption analyser, controlled by SMS Analysis Suite software. The sample temperature was maintained at 25 °C by the instrument controls. The humidity was controlled by mixing streams of dry and wet nitrogen, with a total flow rate of 200 ml.min⁻¹. The relative humidity was measured by a calibrated Rotronic probe (dynamic range of 1.0-100 %RH), located near the sample. The weight change, (mass relaxation) of the sample as a function of %RH was constantly monitored by the microbalance (accuracy ± 0.005 mg).

Typically 5-20 mg of sample was placed in a tared mesh stainless steel basket under ambient conditions. The sample was loaded and unloaded at 40 %RH and 25 °C (typical room conditions). A moisture sorption isotherm was performed as outlined below (2 scans giving 1 complete cycle). The standard isotherm was performed at 25 °C at 10 %RH intervals over a 0.5-90 %RH range.

Table 2 HPLC Method Parameters for Solubility Measurements

Parameters	Values
Adsorption - Scan 1	40 - 90
Desorption / Adsorption - Scan 2	85 - Dry, Dry - 40
Intervals (%RH)	10
Number of Scans	2
Flow rate (ml.min ⁻¹)	200
Temperature (°C)	25
Stability (°C.min ⁻¹)	0.2
Sorption Time (hours)	6 hour time out

The sample was recovered after completion of the isotherm and re-analysed by XRPD.

5.8. Water Determination by Karl Fischer (KF)

The water content of each sample was measured on a Mettler Toledo DL39 Coulometer using Hydranal Coulomat AG reagent and an argon purge. Weighed solid samples were introduced into the vessel on a platinum TGA pan which was connected to a subseal to avoid water ingress. Approx 10 mg of sample was used per titration and duplicate determinations were made.

5.9. Thermodynamic Aqueous Solubility by HPLC

Aqueous solubility was determined by suspending sufficient compound in water to give a maximum final concentration of $\geq 30 \text{ mg.ml}^{-1}$ of the parent free-form of the compound. The suspension was equilibrated at 25 °C for 24 hours then the pH was measured. The suspension was then filtered through a glass fibre C filter into a 96 well plate. The filtrate was then diluted by a factor of 101. Quantitation was by HPLC with reference to a standard solution of approximately 0.1 mg.ml^{-1} in DMSO. Different volumes of the standard, diluted and undiluted sample solutions were injected. The solubility was calculated using the peak areas determined by integration of the peak found at the same retention time as the principal peak in the standard injection.

If there was sufficient solid in the filter plate, the XRPD was collected.

Analysis was performed on an Agilent HP1100 series system equipped with a diode array detector and using ChemStation software vB.02.01-SR1.

Table 3 HPLC Method Parameters for Solubility Measurements

Type of method:	Reverse phase with gradient elution		
Column:	Phenomenex Luna, C18 (2) 5µm 50 x 4.6 mm		
Column Temperature (°C):	25		
Standard Injections (µl):	1, 2, 3, 5, 7, 10		
Test Injections (µl):	1, 2, 3, 10, 20, 50		
Detection: Wavelength, Bandwidth (nm) :	260,80		
Flow Rate (ml.min ⁻¹):	2		
Phase A:	0.1% TFA in water		
Phase B:	0.085% TFA in acetonitrile		
Timetable:	Time (min)	% Phase A	% Phase B
	0.0	95	5
	1.0	80	20
	2.3	5	95
	3.3	5	95
	3.5	95	5
	4.4	95	5

5.10. Chemical Purity Determination by HPLC

Purity analysis was performed on an Agilent HP1100 series system equipped with a diode array detector and using ChemStation software vB.02.01-SR1.

Table 4 HPLC Method Parameters for Chemical Purity Determinations

Sample Preparation:	0.5mg/ml in acetonitrile : water 1:1 v/v		
Column:	Phenomenex Luna C18 (2), 150x4.6mm, 5µm		
Column Temperature (°C):	25		
Injection (µl):	5		
Detection: Wavelength, Bandwidth(nm):	255, 90		
Flow Rate (ml.min ⁻¹):	1		
Phase A:	0.1%TFA in water		
Phase B:	0.085% TFA in acetonitrile		
Timetable:	Time (min)	% Phase A	% Phase B
	0	95	5
	25	5	95
	25.2	95	5
	30	95	5

5.11. Ion Chromatography (IC)

Data were collected on a Metrohm 761 Compact IC (for cations) and a Metrohm 861 Advanced Compact IC (for anions) using IC Net software v2.3. Samples were prepared as 1000 ppm stocks in DMSO. Samples were diluted to 100 ppm with DMSO prior to testing. Quantification was achieved by comparison with standard solutions of known concentration of the ion being analysed.

Table 5 HPLC Method Parameters for Anion Chromatography

Type of method	Anion exchange
Column:	Metrosep A Supp 5 – 250 (4.0 x 250 mm)
Column Temperature (°C):	Ambient
Injection (µl):	20
Detection:	Conductivity detector
Flow Rate (ml.min ⁻¹):	0.7
Eluent:	3.2 mM sodium carbonate, 1.0 mM sodium hydrogen carbonate in water

Table 6 HPLC Method Parameters for Cation Chromatography

Type of method	Cation exchange
Column:	Metrosep C 2 – 250 (4.0 x 250 mm)
Column Temperature (°C):	Ambient
Injection (µl):	20
Detection:	Conductivity detector
Flow Rate (ml.min ⁻¹):	1.0
Eluent:	4.0 mM Tartaric acid, 0.75 mM Dipicolinic acid in water

6 Polymorphism Study

6.1. Introduction

An investigation into the propensity for TC-6499-221 to form polymorphs and solvates was performed using a variety of procedures, outlined below.

- Input crystalline (F00870) or amorphous material for use of the following experiments (in up to 24 solvents):
 - Maturation cycle from RT to 50°C.
 - Cooling from saturated solutions from RT to 4°C then to -20°C.
 - Solvent evaporation from saturated solutions at RT.
- Crystallisation by anti-solvent addition to saturated solutions
- DSC heat cool cycles.

The supplied batch, F00870, used for this study was fully characterised (see section 7.2). The XRPD pattern showed a good match with the pattern of the 2nd crop (XRPD 2) obtained in project 880, except for one additional peak observed at ~12°(2θ). In order to maintain consistency with the data collected during the salt selection study (report R880_002AC060907), batch F00870 is called Form 2. Diffractograms of Crop 1; Crop 2 and F00870 can be seen in Data Section 1.

Any polymorphs found were identified by XRPD and scaled-up for characterisation.

6.2. Attempt to produce amorphous material by freeze drying TC-6499-221

A solution of TC-6499-221 (F00870) in water was freeze-dried in order to produce amorphous material. The residue was analysed by XRPD to confirm the lack of crystallinity. After 2 hours in ambient conditions, the sample was re-analysed by XRPD and a new pattern was observed (XRPD 3). The evolution of the XRPD patterns can be seen in Data Section 2.

The characterisation of this new phase (batch code: BF-405-22), presented in section 7.3, highlights the existence of a new polymorphic form of TC-6499-221 called Form 3.

The amorphous state of TC-6499-221 is unstable in normal conditions (temperature and pressure) and crystallises in few minutes to a solid consistent with Form 3.

Form 3 was scaled up to be used as input material for maturation, cooling and evaporation screen (see section 6.3.2). An aqueous solution of 2 grams of TC-6499-221 was freeze-dried to obtain a second batch of Form 3, sample reference BF-405-44.

6.3. Slurry experiments/cooling and evaporation of saturated solutions

Twenty four solvents were selected for the polymorphism study, including water plus a diverse array of class 2 and class 3 solvents: see Table 6.

Table 7 TC-6499-221 Selected solvents for the polymorphism study

No.	Solvent	Solvent class	No.	Solvent	Solvent class
1	DCM	2	13	Acetone	3
2	Chlorobenzene	2	14	Ethanol	3
3	Toluene	2	15	Methanol	2
4	Anisole	3	16	2-Butanol	3
5	Heptane	3	17	1-Butanol	3
6	1,4-Dioxane	2	18	1-Propanol	3
7	Ter-Butylmethyl ether	3	19	2-Propanol	3
8	n-Butyl acetate	3	20	2-Methoxyethanol	2
9	Isopropyl acetate	3	21	Acetonitrile	2
10	Ethyl acetate	2	22	Tetrahydrofuran	2
11	Methylisobutyl Ketone	3	23	Water	N/A
12	Methylethyl Ketone	3	24	Nitromethane	2

Maturation experiments and experiments using cooling or solvent evaporation (at ambient) from saturated solutions were carried out from:

- Form 2 (F00870) in dry solvents.
- Form 3 (BF-405-44) in solvents containing 5 % v/v of water.

6.3.1. Maturation/cooling and evaporation screen from crystalline material of Form 2 in dry solvents.

The selected solvents (see Table 6) were used to prepare suspensions of Form 2 (F00870): 0.5 to 1 mL of each solvent was added to 20-30 mg of Form 2. On shaking at ambient, only the sample containing water dissolved. After 12 hours of stirring to reach equilibrium, known volumes of saturated solutions were withdrawn from the slurries prior to cooling to 4°C. If no precipitate was obtained the samples were cooled further to -21°C, prior to evaporation at ambient.

All suspensions were shaken with alternating 4-hour cycles between ambient temperature and 50°C. After 1 day, a part of each suspension was withdrawn and filtered at room temperature in order to check the nature of the solid by XRPD analysis. Suspensions were matured for a total of 5 days. The results are presented in Table 7.

Table 8 Results of maturation, cooling and evaporation experiments of Form 2 (F00870)

	Solvent	Maturation (RT/50 °C, 4 hours cycles)			cooling from saturated solution RT to -20 °C		slow evaporation of saturated solutions at RT
		volume (mL)	solid after 12 h	Solid after 5 days	withdraw volume (mL)	Solid after 24 h	
1	DCM	1	Form 2	Form 2	0.2	No solid	insufficient material for XRPD
2	Chlorobenzene	1	Form 2	Form 2	0.2		
3	Toluene	0.5	Form 2	Form 2	0.3		
4	Anisole	0.5	Form 2	Form 2	0.5		
5	Heptane	1	Form 2	Form 2	0.5		
6	1,4-Dioxane	0.5	Form 2	Form 2	0.5		
7	TBME	1	Form 2	Form 2	0.5		
8	n-butyl acetate	1	Form 2	Form 2	0.5		
9	Isopropyl acetate	1	Form 2	Form 2	0.5		
10	Ethyl acetate	1	Form 2	Form 2	0.5		
11	MIBK	1	Form 2	Form 4 + Form 2	0.5		
12	MEK	1	Form 2	Form 2	0.5		
13	Acetone	1	Form 2	Form 4 + Form 2	0.5		
14	Ethanol	1	Form 2	Form 2	0.5		
15	Methanol	1	Form 2	Form 2	0.5		
16	2-Butanol	1	Form 2	Form 2	0.5		
17	1-Butanol	1	Form 2	Form 2	0.5		
18	1-Propanol	1	Form 2	Form 2	0.5		
19	2-Propanol	1	Form 2	Form 2	0.5		
20	2-Methoxyethanol	1	Form 2	Form 2	0.5		
21	Acetonitrile	1	Form 2	Form 2	0.5		
22	Tetrahydrofuran	1	Form 2	Form 2	0.5		
23	Water	1	No solid	No solid	0.5	Form 2	
24	Nitromethane	1	Form 2	Form 2	0.5	No solid	insufficient material for XRPD

A new solid phase (Form 4/XRPD 4) was detected in the slurries in acetone and MIBK after 5 days of maturation cycles. The saturated solutions (withdrawn from the suspensions at RT) that were cooled prior to be evaporated did not give sufficient material for analyses due to the low solubility of Form 2 in these solvents. The exception was the saturated water solution which gave Form 2 solid after evaporation. XRPD analyses recorded after 1 and 5 days can be seen in Data Section 3.

6.3.2. Maturation/cooling and evaporation screen from crystalline material of Form 3 in wet solvents.

Another series of experiments was performed with Form 3 (BF-405-44) in wet solvents: 20-30 mg of Form 3 were suspended in 1 mL of solvent; 5% of water (%vol) was added to each sample in order to increase the solubility of the Form 3 material. On shaking at ambient, a number of the samples dissolved in the water to give 2 liquid immiscible phases. After 12 hours, known volumes of saturated solutions from the slurries were withdrawn prior to cooling to -20°C. If no precipitate was obtained by cooling these samples were evaporated at ambient conditions.

The slurry samples were shaken with alternating 4-hour cycles between ambient temperature and 50°C for 3 days with an intermediate XRPD analysis after 12 hours. The results are presented in Table 8.

Table 9 Results of maturation, cooling and evaporation experiments of Form 3 (BF-405-44)

			Maturation (RT/50 °C, 4 hours cycles)		cooling from saturated solution RT to -20 °C		slow evaporation of saturated solutions at RT
	Solvent	Addition of 50 µL of water	solid after 12 h	Solid after 3 days	withdraw volume (mL)	Solid after 24 h	
1	DCM	2 liquid immiscible phases	no solid				
2	Chlorobenzene	2 liquid immiscible phases	no solid				
3	Toluene	2 liquid immiscible phases	no solid				
4	Anisole	2 liquid immiscible phases	no solid				
5	Heptane	2 liquid immiscible phases	no solid				
6	1,4-Dioxane	suspension	Form3	Form3	0.5	no solid	insufficient material for XRPD
7	TBME	2 liquid immiscible phases	no solid				
8	n-Butyl acetate	2 liquid immiscible phases	no solid				
9	Isopropyl acetate	2 liquid immiscible phases	no solid				
10	Ethyl acetate	suspension	Form 2	Form 2	0.5	no solid	insufficient material for XRPD
11	MIBK	suspension	Form 2	Form 2	0.5	no solid	
12	MEK	suspension	Form 2	Form 2	0.5	no solid	
13	Acetone	suspension	Form 3+ Form 2	Form 3+ Form 2	0.5	no solid	
14	Ethanol	suspension	Form 3+ Form 2	Form 3+ Form 2	0.5	no solid	
15	Methanol	suspension	Form 2	Form 2	0.5	no solid	
16	2-Butanol	suspension	Form 3+ Form 2	Form 3+ Form 2	0.5	no solid	
17	1-Butanol	suspension	Form 3+ Form 2	Form 2	0.5	no solid	
18	1-Propanol	suspension	Form 3+ Form 2	Form 2	0.5	no solid	
19	2-Propanol	suspension	Form 3+ Form 2	Form 2	0.5	no solid	
20	2-Methoxyethanol	suspension	Form 2	Form 2	0.5	no solid	
21	Acetonitrile	suspension	Form3	Form 3+ Form 2	0.5	no solid	
22	Tetrahydrofuran	suspension	Form 2	Form 2	0.5	no solid	
24	Nitromethane	2 liquid immiscible phases	no solid				

After 3 days of maturation, suspensions of Form 3 converted to Form 2 or to mixtures of Form 2 and 3, except for 1,4-dioxane (no change in XRPD pattern). However, no solid was obtained after full evaporation of the saturated solutions due to the poor solubility of Form 3 in these solvents.

XRPD analyses recorded after 1 and 5 days can be seen in Data Section 4.

6.3.3. Crystallisation experiments by anti-solvent addition at room temperature

Two saturated solutions of Form 2 (F00870) were prepared at room temperature in DMSO and water using the following method: suspensions of Form 2 were prepared in water and DMSO and stirred for 12 hours (at least) to reach equilibrium. Then, the suspensions were filtered to isolate the liquid phase. Each water and DMSO saturated solution was heated at 50°C for 1 hour in order to ensure the dissolution of residual seeds.

Each solution was divided into 2 vials; 0.5 mL of the solution per vial. Twelve solvents were selected to be used as anti-solvents according to their miscibility with water and/or DMSO.

At room temperature, 1 mL of anti-solvent was added to each saturated solution. The solid phases precipitated were filtered under vacuum and analysed by XRPD. The results are presented in Table 9.

Table 10 Anti-solvent addition crystallization experiments at RT.

	Solvent	1 mL added in a saturated water solution	solid	1 mL added in a saturated DMSO solution	solid
1	Methanol	no solid formed	no crystallisation	Not miscible in DMSO	
2	Ethanol	crystallisation after few seconds	Form 3	Crystallisation after few seconds	Form 3
3	IPA	Precipitation	Form 3	Demixion and crystallisation after few seconds	Form 2
4	1,4-dioxane	Demixion and crystallisation after few seconds	Form 3	Demixion	Form 2
5	Acetonitrile	Demixion and crystallisation after few seconds	Form 3	Demixion and crystallisation after few seconds	Form 2
6	Acetone	Demixion and crystallisation after few seconds	Form 3	Demixion	Form 2
7	MEK	Not miscible in water		Demixion and crystallisation after few seconds	Form 2
8	THF	Demixion and crystallisation after few seconds	Form 3	Demixion and crystallisation after few seconds	Form 2
9	Ethyl acetate	Not miscible in water		Demixion and crystallisation after few seconds	Form 2
10	DCM			Demixion and crystallisation after few seconds	Form 2
11	TBME			Demixion	Form 2
12	Toluene			Demixion and crystallisation after few seconds	Form 2

No solid precipitation was obtained in methanol. All the experiments carried out in water lead to precipitation of Form 3. Apart from the solid from ethanol, all precipitates from DMSO solution were consistent with Form 2. The XRPD patterns recorded can be seen in Data Section 5.

6.4. DSC heat cool cycle of Form 2

A DSC heat/cool cycle experiment was carried on Form 2 (F00870). The cycle was performed by cooling the sample from 25 to -80°C at 2°C/min and then heating up to 170°C at 10°C/min. The thermogram can be seen in Data Section 6. No solid-solid transition phenomenon was detected and the product melted at about 165°C.

6.5. Summary of the polymorphism study

During this polymorphism study two new solid phases of TC-6499-221 had been highlighted:

- Form 3 obtained by freeze drying an aqueous solution of TC-6499-221 or by anti-solvent addition (from a range of solvents) to a saturated solution in water at ambient temperature.
- Form 4 obtained by maturation of Form 2 in acetone and MIBK.

7 Characterisation of polymorphs

7.1. Characterisation of Form 1 (Crop 1)

A partial characterisation of Form 1 (Crop 1/XRPD 1) had been carried out in the course of the salt selection study (Project 880; see report R880_002AC060907). Table 10 is a summary of the characterisation of this form. The experimental data can be seen in Data Section 7.

Crystallisation experiments were carried out from the free base (freshly prepared) in MeCN, IPAc and EtOH, with the addition of 1 equivalent of citric acid. The aim was to obtain a new batch of Form 1 for complete characterisation and for a stability study. However these experiments did not succeed in crystallising Form 1.

The following experimental procedure was used:

The fresh free base (30 mg, 1.71 mmol, freshly prepared from the hemi-galactarate salt (E00478) following the procedure described in section 9.2.1) was suspended in MeCN, IPAc or EtOH. The solutions were then treated with 1.1 eq. of citric acid (32.6 mg), warmed up to 50°C (for homogenisation) and cooled slowly to room temperature. After precipitation, the solids were immediately analysed by XRPD analysis: all the patterns obtained showed profiles of Form 2. Form 1 was not reproducible.

Table 11 Characterisation of Form 1

Experiment	Comment
¹ H NMR (400 MHz, D ₂ O)	¹ H NMR spectrum was consistent with the structure. No impurities were identified by NMR. Stoichiometry was confirmed by integration of the signal of the counter ion and was consistent with a mono-citrate stoichiometry.
HPLC Purity	99.94% (using Pharmorphix generic method)
XRPD Stability	The material was crystalline. The pattern transformed completely to Form 2 after the sample was stored at 40°C /75% R.H. for one week. In the solid state, and after 6 months of storage at 4°C (in a fridge), a batch of Form 1 was re-analysed and the pattern showed a partial conversion to Form 2 and 3.
TGA/DSC	No weight loss was observed until the decomposition occurs at ~170°C. Form 1 melts at 149°C.
GVS at 25°C	No hydration was observed. Less than 1.5% of water was taken up below 70% R.H. then another 2.5% w/w was taken up 90% R.H. No major changes were observed in the XRPD after GVS experiment.
Thermodynamic solubility in water	> 10 mg/mL (pH of unfiltered solution:3.82)

7.2. Characterisation of Form 2 as supplied (F00870)

The table below is a summary of the batch characterisation of F00870 (Form 2). The experimental data can be found in Data Section 7.

Table 12 Characterisation of TC-6499-221 Form 2

Experiment	Comment
¹ H NMR (400 MHz, D ₂ O)	¹ H NMR spectrum was consistent with the proposed structure. No impurities were identified by NMR. Stoichiometry was confirmed by integration of the signal of the counter ion and was consistent with a mono-citrate stoichiometry.
HPLC Purity	99.78% (using Pharmorphix generic method)
XRPD Stability	The material supplied was crystalline. No changes were observed in the humidity chamber after the sample was stored at 40°C /75% R.H. and 25°C / 97% R.H. for one week.
Optical Microscopy	Thin plate shapes. Birefringence was clearly observed with polarised light.
TGA/DSC	No weight loss was observed up to the decomposition. The material melted with decomposition at 164.2°C (onset).
GVS at 25°C	No hydration was observed. The mass change was less than 0.45% w/w between 0 and 90% R.H., the material is not hygroscopic. No changes observed in the XRPD after GVS analysis.
Thermodynamic solubility in water	> 30 mg/mL (pH of unfiltered solution: 3.77)

7.3. Characterisation of Form 3

Table 12 is a summary of the characterisation of Form 3. The experimental data can be found in Data Section 7. This batch of Form 3 was produced by freeze-drying (see section 6.2) and has sample reference BF-405-22.

Table 13 Characterisation of Form 3

Experiment	Comment
¹ H NMR (400 MHz, D ₂ O)	¹ H NMR spectrum was consistent with the structure. No impurities were identified and the stoichiometry was confirmed by integration of the signal of the counter ion and was consistent with a mono-citrate.
HPLC Purity	99.67% (using Pharmorphix generic method)
XRPD Stability	XRPD pattern of Form 3 showed good crystallinity. No change was observed after the sample was stored at 40°C /75% R.H for one week. However, the pattern partially evolved to Form 2 after one week of storage at 25°C /97% R.H.
Optical Microscopy	Plate shapes. Birefringence was clearly observed with polarised light.
TGA/DSC	No weight loss was observed up to the decomposition. The material melted with decomposition at 164.6°C (onset).
GVS at 25°C	No hydration phenomenon was observed. The mass change was less than 0.40 % w/w between 0 and 90% R.H. No changes observed in the XRPD after GVS analysis.
Thermodynamic solubility in water	> 30 mg/mL (pH of unfiltered solution: 3.77)

7.4. Characterisation of Form 4

Form 4 was only detected by XRPD analysis (XRPD 4) as a mixture with Form 2. Two additional peaks were observed on the XRPD pattern (at 3 and 30° (2θ)) after maturation experiment. The diffractogram is presented in Data Section 3 (Figure 6).

7.5. Summary of characterisation results

According to the data collected from the characterisation studies:

- Form 1 is the first polymorphic form highlighted during the salt selection study (Project 880) but seems to be metastable and was not reproducible.
- Form 2 (batch provided, F00870) is a polymorphic form stable at ambient conditions of pressure and temperature and not sensitive to humidity (not hygroscopic).
- Form 3 is a polymorphic form stable, at the solid state, at ambient conditions (temperature and pressure) but is sensitive to high levels of humidity and converts to Form 2 at 25°C /97% R.H.

- Form 4 was only detected by XRPD analysis from solids isolated after maturation cycles of Form 2 in acetone and MIBK. No further characterisation has been done and this phase was not reproducible. Form 4 could be a solvate (metastable or efflorescent in normal conditions).

8 Stability Study of Form 2 and 3

Two relevant polymorphic forms have been characterised during this project: Form 2 as the batch supplied (F00870) and Form 3, which was obtained after freeze drying an aqueous solution of the mono-citrate salt (BF-405-44).

Two cross seeding experiments were carried out at 20 and 40°C to investigate the relative stabilities of Form 2 and Form 3 at these temperatures. Water was used as a medium to speed up the transition toward the most stable polymorph.

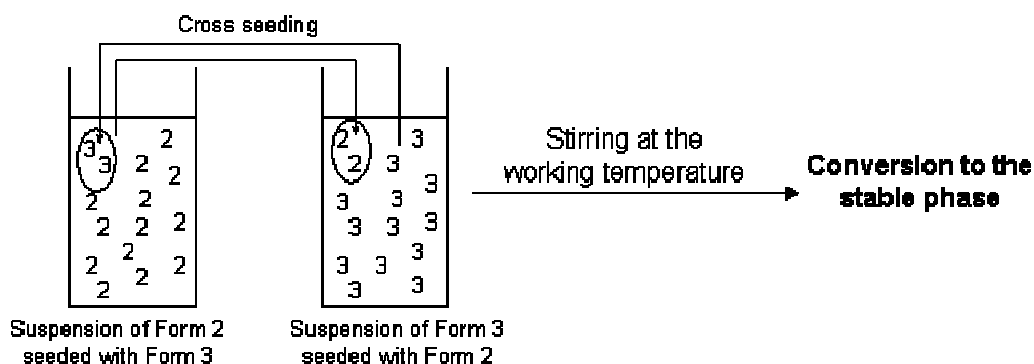
No results were obtained above 40°C. Degradation of the product was visually observed by slurrying Form 2 in water at 60°C. After 24 hours, the saturated liquor took a dark red color.

8.1. Method

Two suspensions of Form 2 and 3 were prepared in pure water (0.5 mL) and sealed in 2 mL vials to prevent solvent loss.

After 12 hours of stirring (to reach the thermodynamic equilibrium) the suspensions of Form 2 and 3 were respectively seeded with Form 3 and 2. After 3 days, all solid phases were filtrated under vacuum through a glass filter and XRPD analysis was performed on the dried solids.

Figure 2 Cross seeding experiment leading to the stable polymorph.



8.2. Results and conclusion of cross seeding experiments

After 3 days, the solid phases recovered from the cross seeding experiments carried out at 20 and 40°C were analysed by XRPD analysis. Only patterns of Form 2 were obtained. The diffractograms are represented in Data Section 8.

Therefore Form 2 is the most stable form between 20 and 40°C.

9 Scale up of Form 2 in IPA/H₂O

The aim was to produce a method for the reliable production of the desired stable polymorphic form (Form 2). Two crystallisation experiments were carried out on a gram scale:

- Recrystallisation from the supplied batch (F00870)
- Crystallisation from the free base TC-6499 with seeding with Form 2.

9.1. Method for the recrystallisation from the supplied batch (F00870)

- In a 100 mL round bottom flask, a suspension of 2.05 g of Form 2 (F00870) was prepared in 20 mL of IPA. The suspension was heated at 60°C with magnetic stirring.
- At 60°C, the addition of 4.5 mL of pure water was necessary to dissolve the entire solid.
- Then, the homogeneous system was cooled from 60 to 25°C at 1°C/min.
- After crystallisation, the solid (batch BF-405-69) was filtered under vacuum and dried in a vacuum oven at 25°C for 12 hours.

During the cooling, the temperature and the turbidity profiles were recorded by probes placed in the reaction vessel (see thermal and turbidity curves in Data Section 9, Figure 27).

9.2. Method for the crystallisation of Form 2 from the free base

9.2.1. Free base preparation

The free base was extracted from the mono-citrate salt. A batch of free base was prepared by treatment of the starting material with sodium hydroxide as required.

F00870 (3 g) was suspended in dichloromethane (30 mL) and treated with an excess of aqueous sodium hydroxide (1 M, 20 mL). The mixture was shaken at room temperature for a few minutes and the two phases were separated. The aqueous phase was washed with DCM (2 x 10 mL) and the combined organic extracts were washed with water (10 mL), dried with magnesium sulphate and concentrated. The residue (colourless oil) was analysed by ¹H NMR to check the structure.

9.2.2. Crystallisation method from the free base

- In a 100 mL round bottom flask, 0.966 mg (5.51 mmol) of fresh free base was diluted with 20 mL of IPA. The suspension was heated at 60°C with magnetic stirring.
- At 60°C, 4.5 mL of aqueous solution of 1.23 M citric acid (5.52 mmol) was added to the system: a homogeneous solution was obtained.
- The system was directly cooled to 50°C and the supersaturated solution was seeded with Form 2 obtained in the first scale up experiment (BF-405-69).
- After few minutes, the system was cooled from 50°C to 25°C at 1°C/min.
- After crystallisation, the solid (batch BF-405-71b) was filtered under vacuum and dried in a vacuum oven at 25°C for 12 hours.

During the cooling, the temperature and the turbidity profiles were recorded by probes placed in the reaction vessel (see thermal and turbidity curves in Data Section 9, Figure 28).

9.3. Results

Table 13 is a summary of the yields and crop characterisations obtained from the scale up experiments.

Table 14 Results of the scale up experiments

	BF-405-69	BF-405-71b
Theoretical mass of salt (g)	2.05	2.02
Experimental mass obtained (g)	1.522	1.411
Yield	76%	70%
¹ H NMR	Consistent with the structure and with the stoichiometry of a mono-citrate salt.	
XRPD	Form 2/XRPD 2. Significant increase of crystallinity (intensities and resolution) compared to the supplied batch F00870	
Chemical purity (% area)	99.83	99.93

The data collected during characterisation of the scale up experiments can be seen in Data Section 9.

9.4. Summary of the scale up experiments

The crops of Form 2 made on scale-up had a better crystallinity than the provided batch, F00870. The chemical purity was also increased to 99.93% (generic Pharmorphix method) for the crop crystallised from the free base.

The yield can also be improved by adjusting the IPA/H₂O ratio.

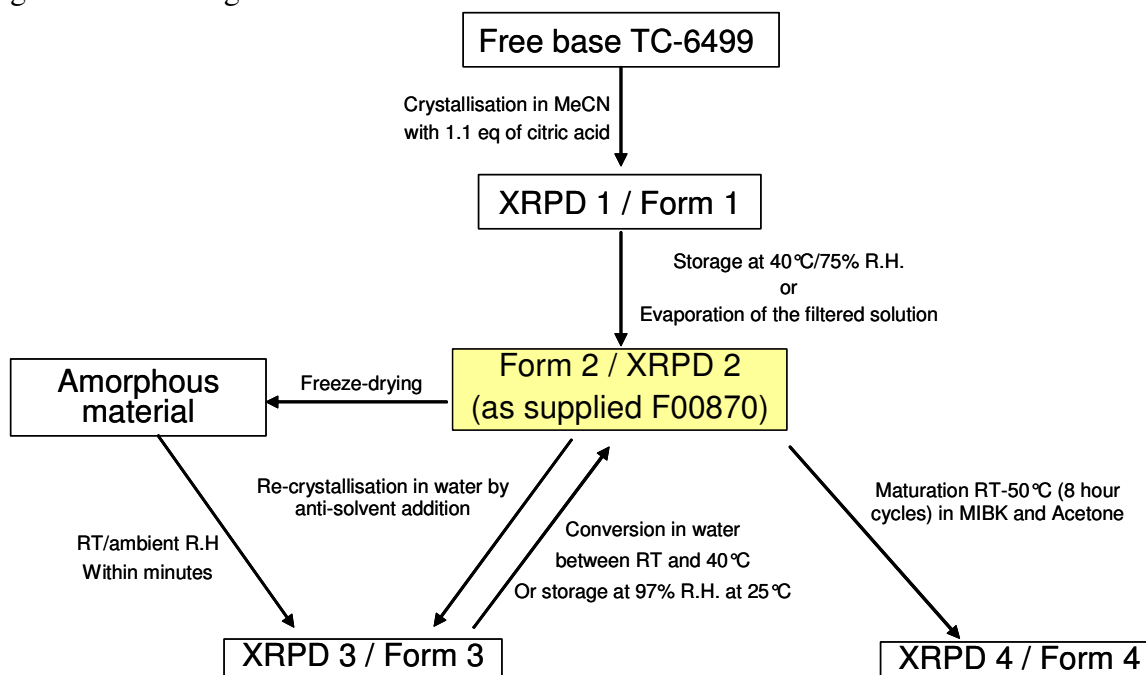
10 Conclusions

Four crystalline forms of TC-6499-221 have been identified in the course of Project 1083.

- Form 1 (Crop 1/XRPD 1) was the first solid phase highlighted in the course of the salt selection study (P880; report R880_002AC060907). Partial characterisation was carried out using techniques such as XRPD and TGA-DSC. The thermal techniques showed that Form 1 is an anhydrous polymorphic form which melts and degrades at temperature above 149°C. However, attempts to produce a new batch of Form 1, during this study, didn't succeed and this phase was not reproducible (disappearing polymorph).
- Form 2 (F00870 as supplied/XRPD 2) is an anhydrous polymorphic form of the mono-citrate salt which melts and degrades at temperature above 164°C. Form 2 is not sensitive to high level of humidity (97% R.H at 25°C and 75% R.H at 40°C) and is not hygroscopic.
- Form 3 (XRPD 3) is a new anhydrous polymorphic form which melts and degrades at temperature above 160°C. Form 3 is stable at the solid state, under normal conditions of temperature and pressure, but converts to Form 2 at high levels of humidity (97% R.H at 25°C). Furthermore, the relative stability between Form 3 and Form 2 showed that Form 3 is a metastable polymorph between 20 and 40°C.
- An additional new XRPD pattern (XRPD 4) was also identified, after maturation of Form 2 in acetone and MIBK (RT-50°C/8 hour cycles), but is less relevant than the morphic forms described above. No other characterisation has been done on this phase which was not reproducible.

The figure below summarises the relationship between the different solid forms of the TC-6499 mono-citrate salt.

Figure 3 Form diagram of TC-6499 mono-citrate salt.



In summary, Form 2 has been identified as the most suitable form for development (thermally stable and not sensitive to humidity). Form 3 could also be progressed for development but with suitable moisture control during the storage. A scale up of Form 2 was also made from the free base in IPA/H₂O mixtures.

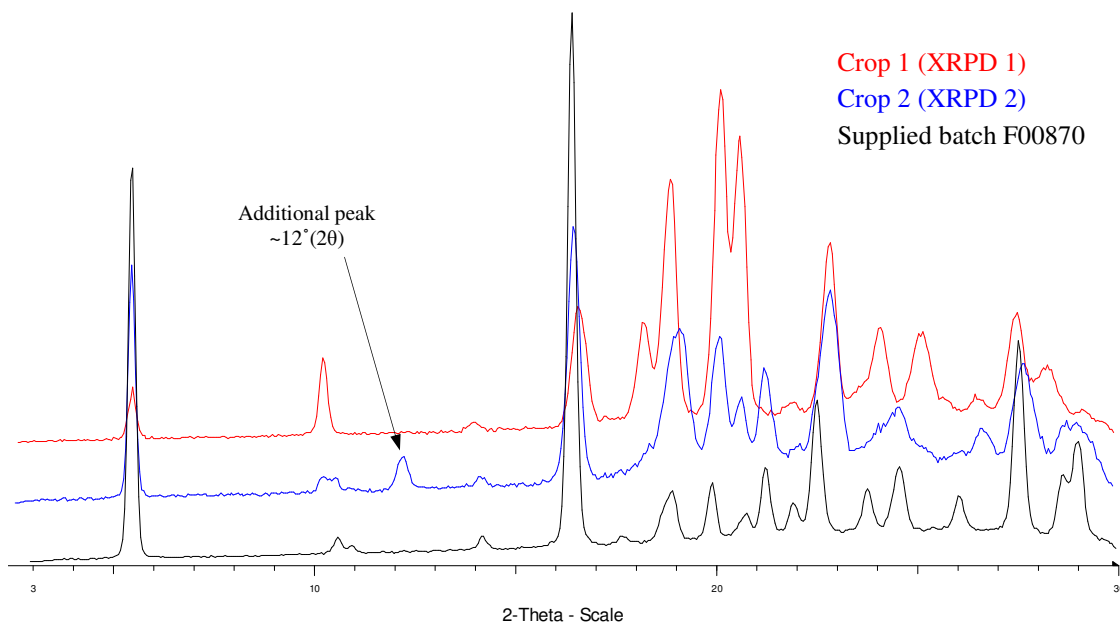
11 Abbreviations

Table 15 List of Abbreviations

DCM	dichloromethane, methylene chloride
DMF	dimethylformamide
D ₂ O	deuterium oxide
EtOAc	ethyl acetate
EtOH	ethanol, ethyl alcohol
H ₂ O	water - distilled or HPLC grade
IPA	iso-propyl alcohol, propan-2-ol
i-PrOAc	iso-propyl acetate
MeCN	methyl cyanide, acetonitrile
MEK	methyl ethyl ketone, butanone
MeOH	methanol, methyl alcohol
MIBK	methyl isobutyl ketone, 2,2-dimethyl butan-3-one
NMP	N--methyl-2- pyrrolidone
RT	room temperature
t-BME / TBME	t-butylmethyl ether
THF	tetrahydrofuran

Data Section 1

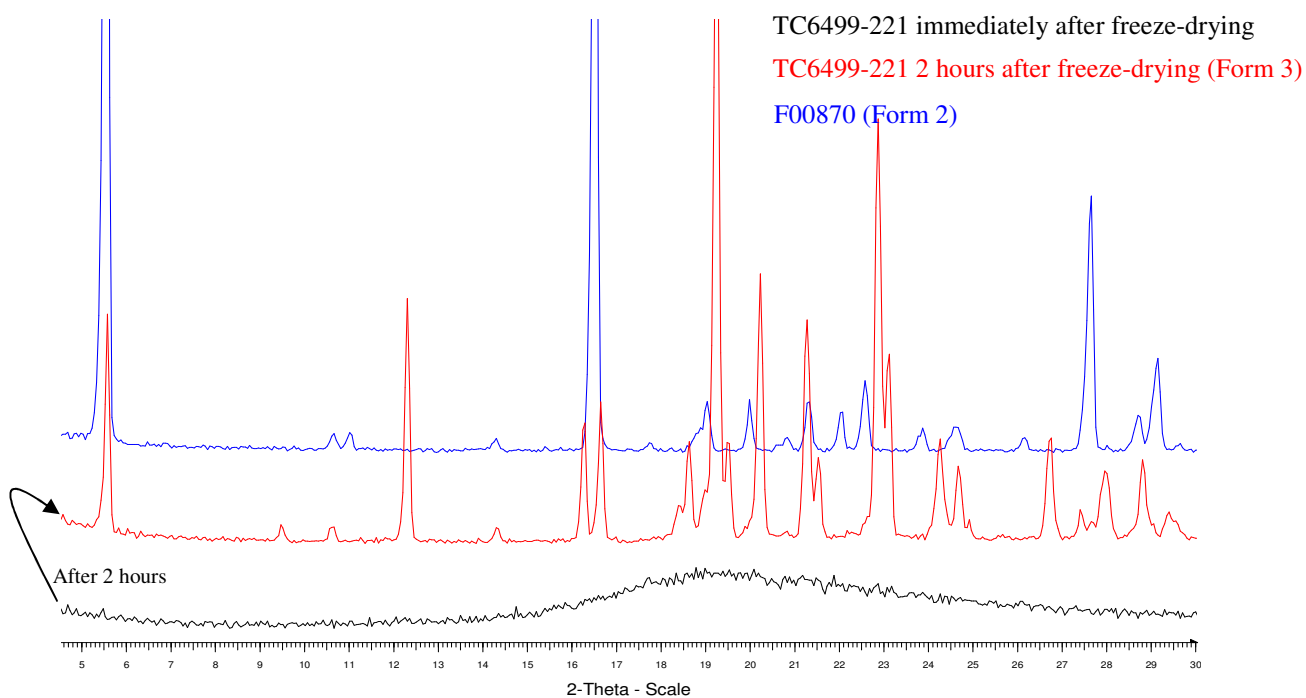
Figure 4 XRPD patterns of Crop 1, 2 and F00870.



The XRPD pattern of the supplied batch mainly corresponds to Crop 2 obtained in the course of the salt selection study (Project 880; see report R880_002AC060907).

Data Section 2

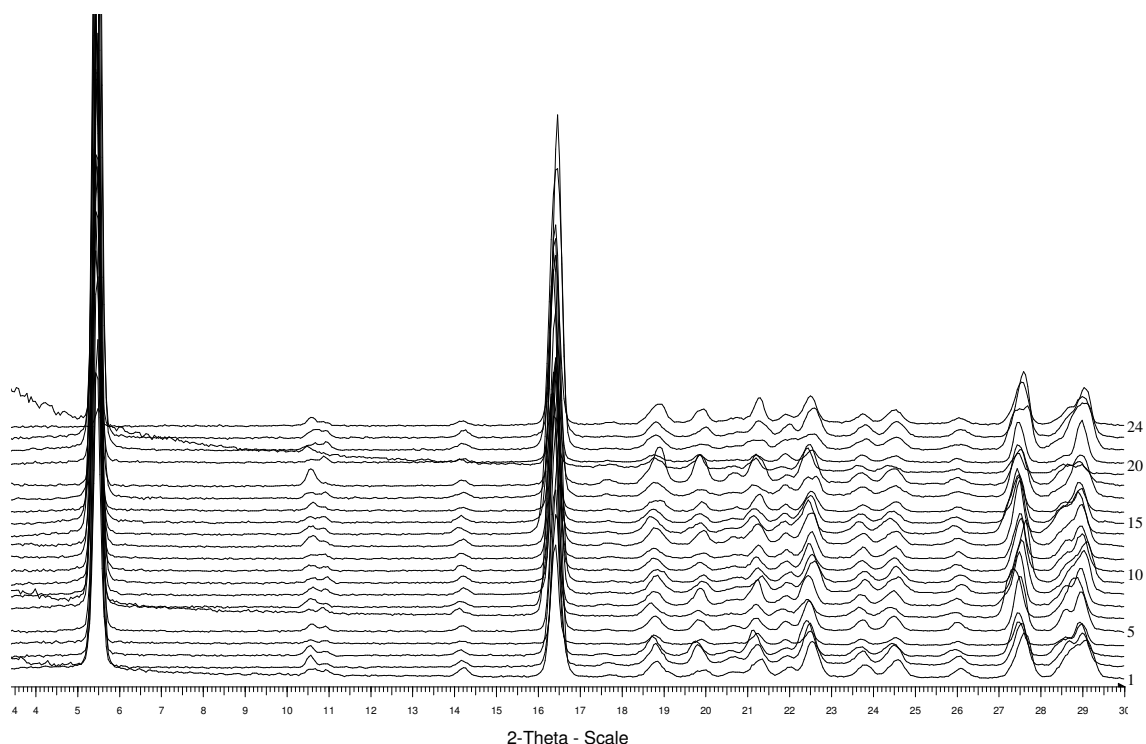
Figure 5 XRPD patterns of TC6499-221 post freeze-drying experiment.



After 2 hours, the amorphous state of TC6499-221 crystallised to a new Form 3.

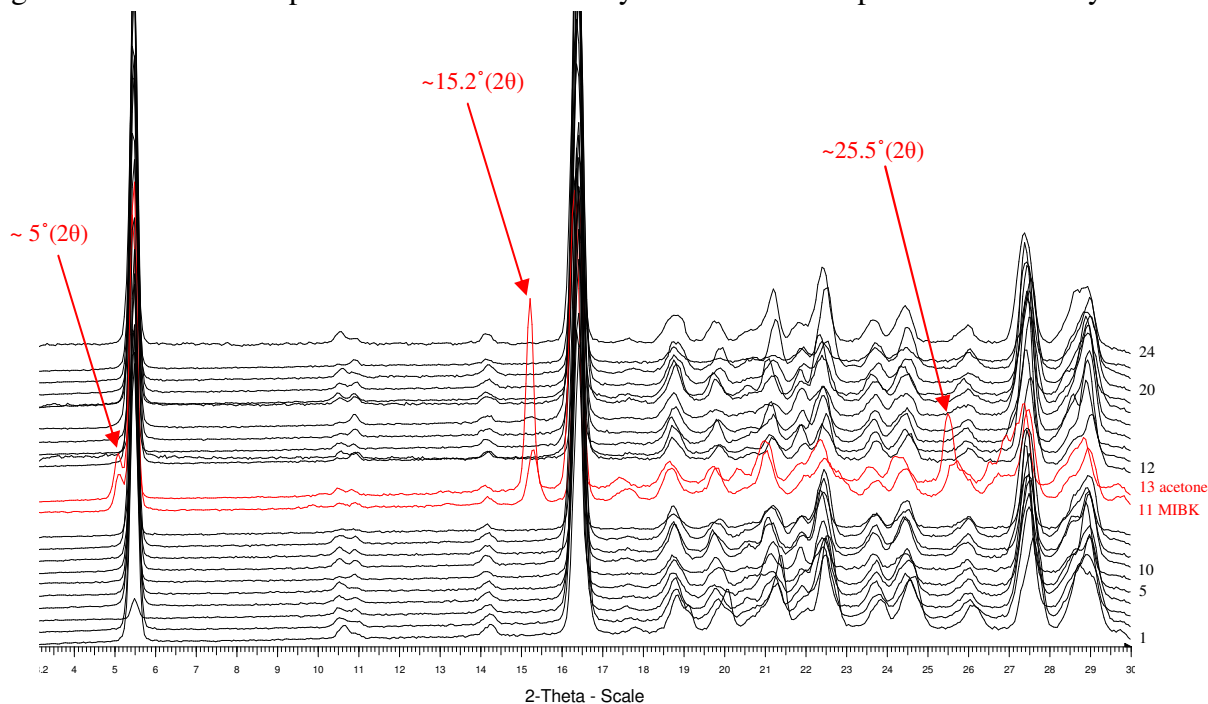
Data Section 3

Figure 6 Maturation experiments of Form 2 in dry solvents: XRPD patterns after 1 day.



No change was observed on the XRPD patterns after 1 day of maturation cycles (RT-50°C /4 hours cycles)

Figure 7 Maturation experiments of Form 2 in dry solvents: XRPD patterns after 5 days.



After 5 days of maturation cycles, mixtures of Form 2 and Form 4 were obtained in acetone and MIBK. Three characteristic additional peaks were observed at ~5; 15.2 and 25.5° (2θ).

Data Section 4

Figure 8 Maturation experiments of Form 3 in wet solvents: XRPD patterns after 12 hours.

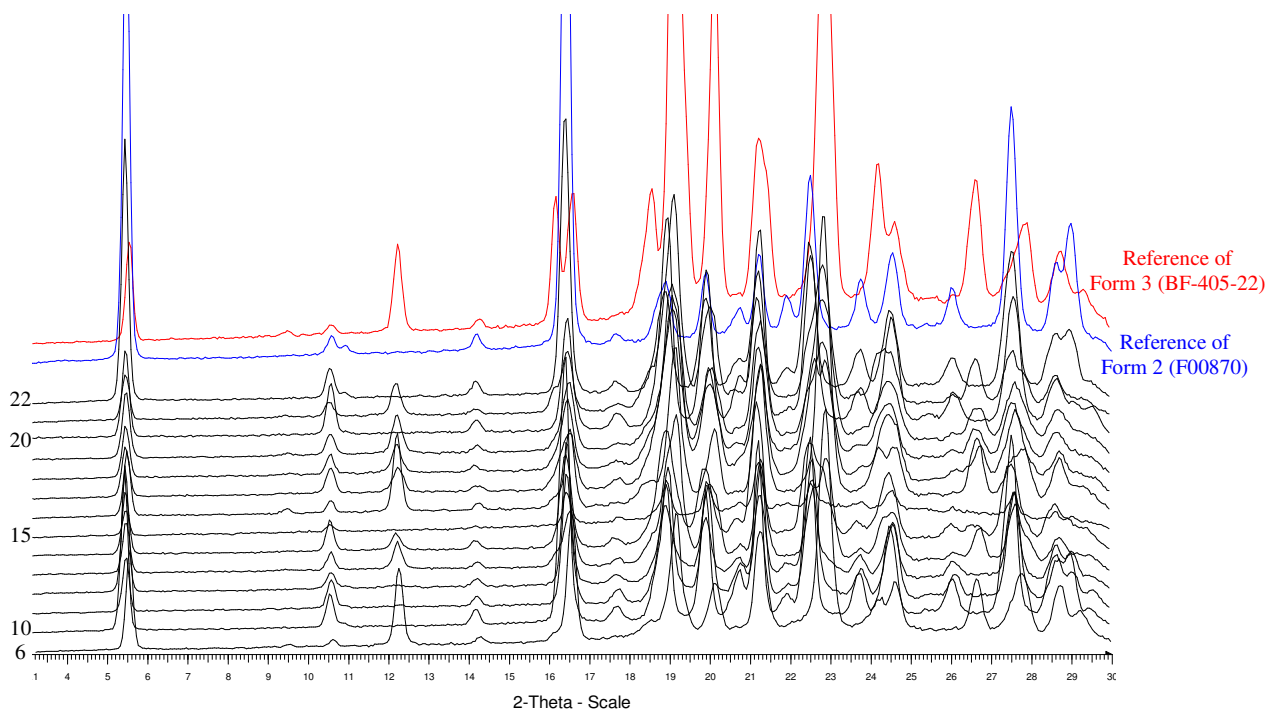
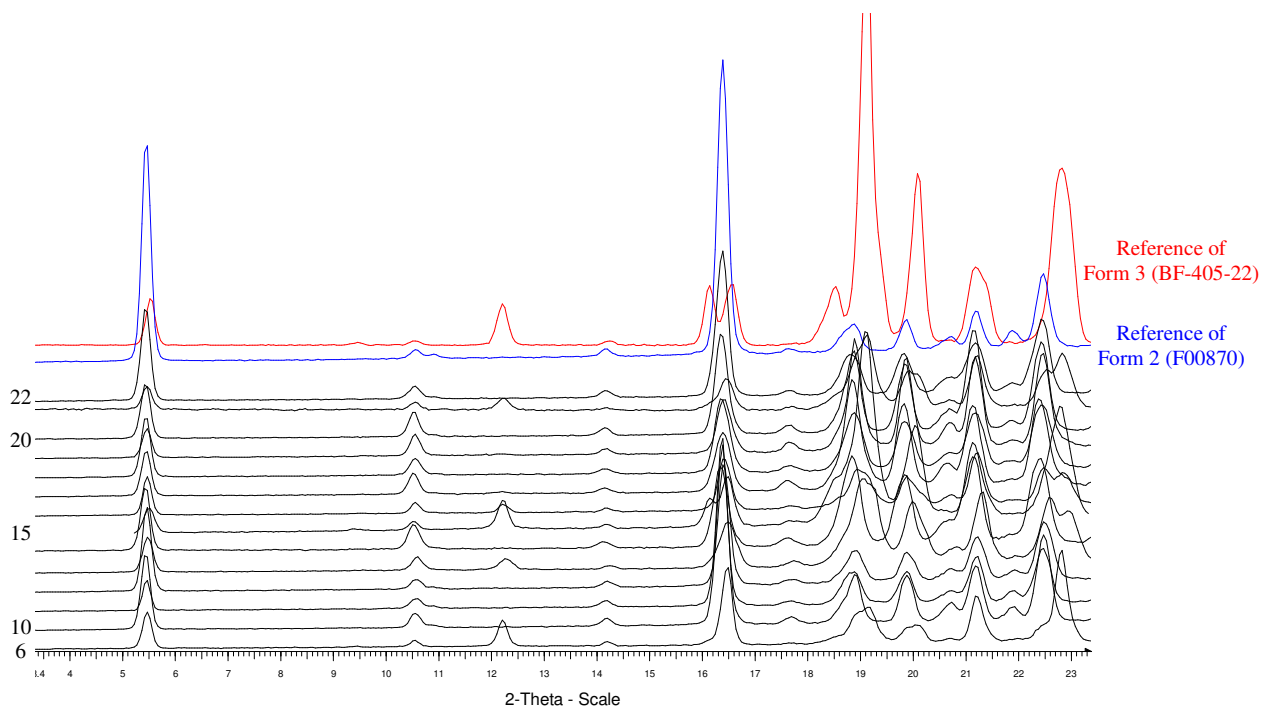


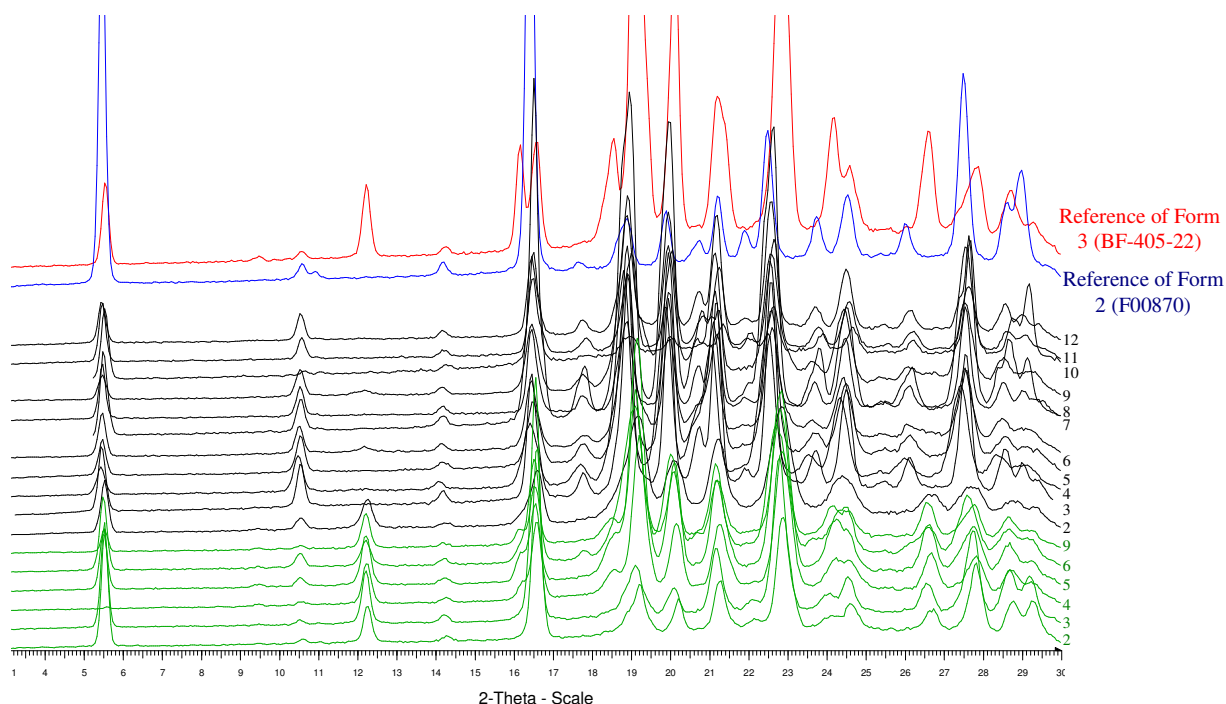
Figure 9 Maturation experiments of Form 3 in wet solvents: XRPD patterns after 3 days.



Except in 1,4-dioxane where no change was observed on the XRPD pattern, all suspensions of Form 3 lead to partial or complete transformations of Form 2.

Data Section 5

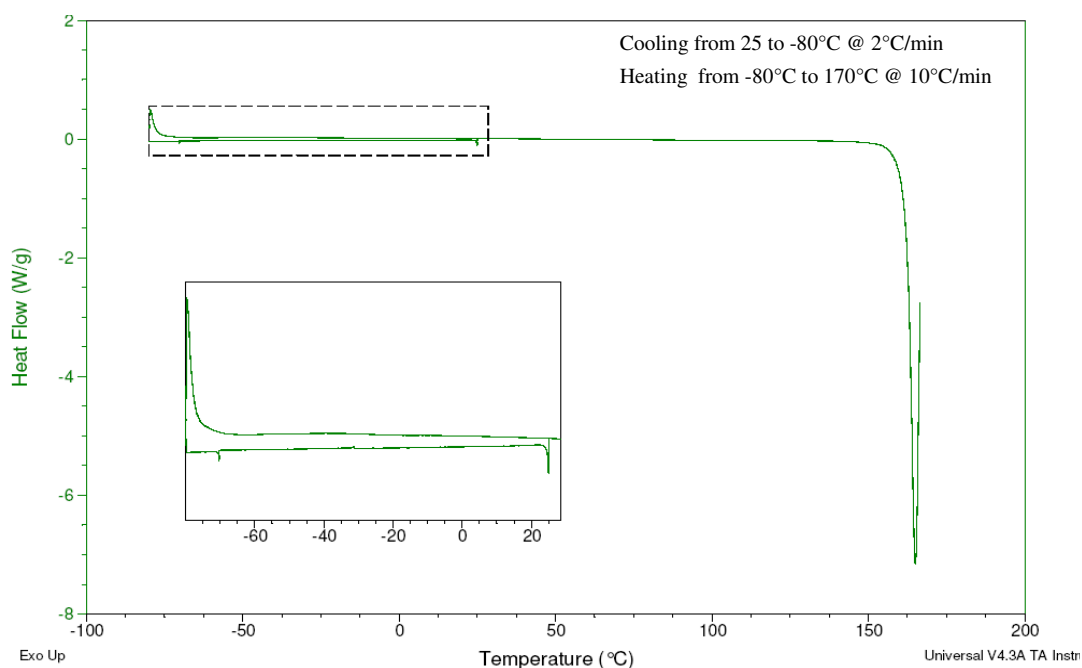
Table 16 Anti-solvent addition crystallisation experiments: XRPD patterns.



Green traces: solid obtained by anti-solvent addition in saturated solution of water. Black traces: solid obtained by anti-solvent addition in saturated solution of DMSO. All XRPD patterns collected on solids obtained in water corresponded to Form 3, and all from DMSO were Form 2 (except where ethanol was used as anti-solvent, which gave Form 3).

Data Section 6

Figure 10 DSC heat/cool cycle thermogram of Form 2



Data Section 7: Characterisation of polymorphs

Figure 11 XRPD pattern references of Forms 1, 2, 3, and mixture of Form 2 and 4.

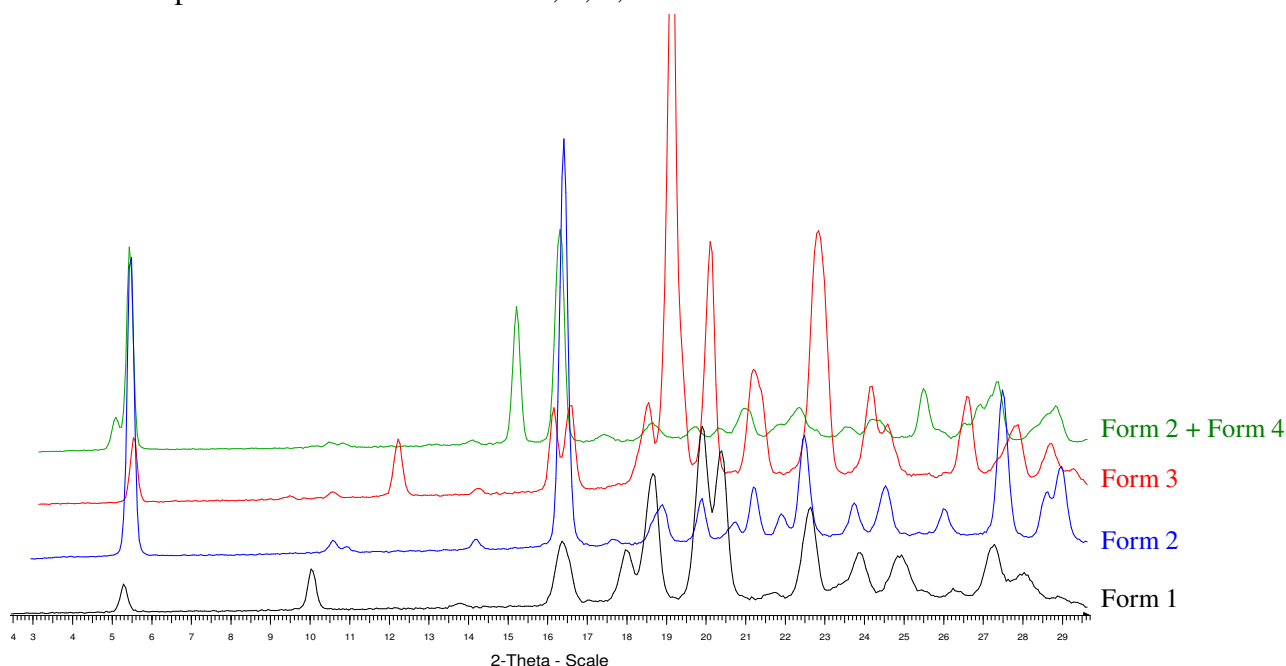
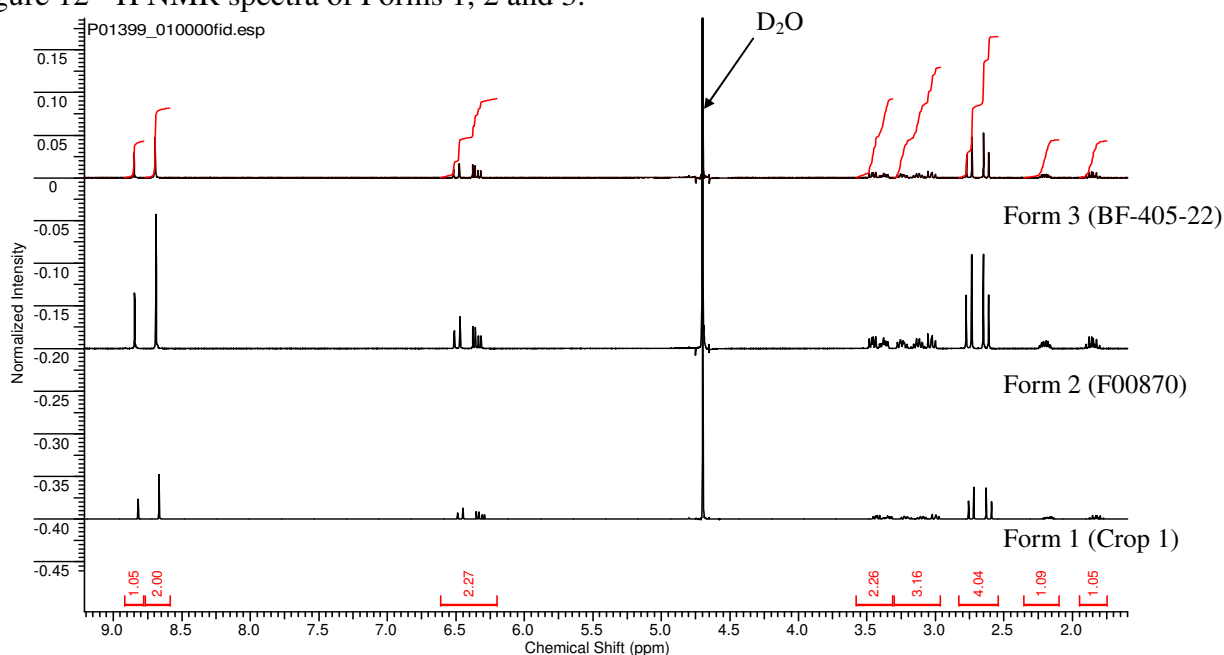
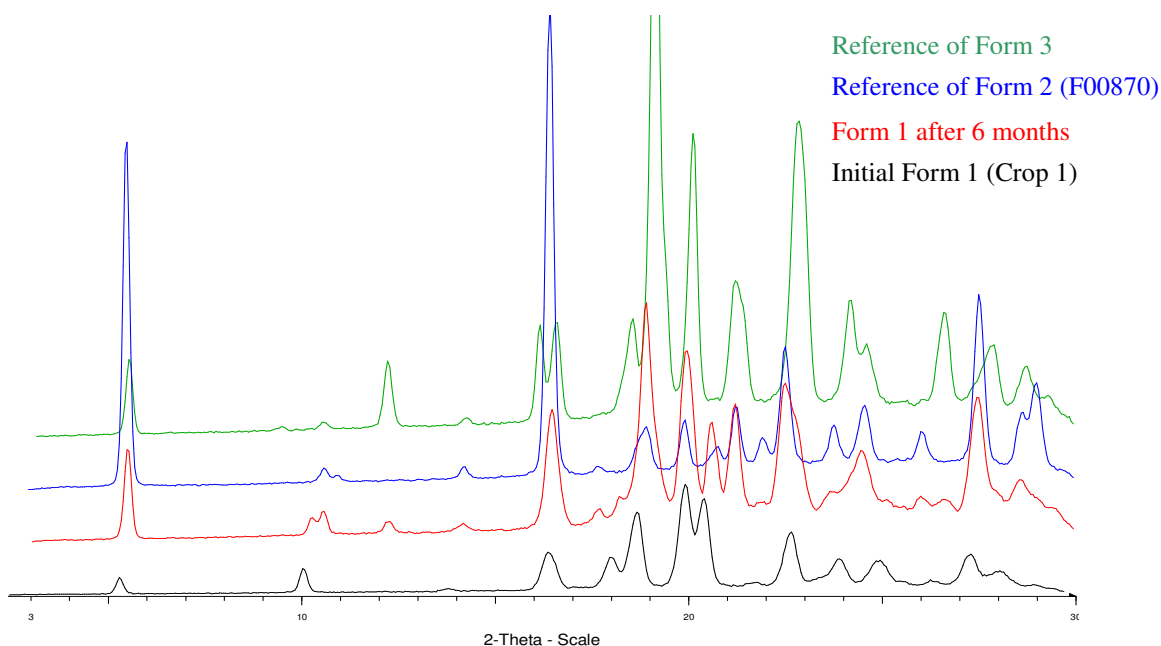


Figure 12 ^1H NMR spectra of Forms 1, 2 and 3.



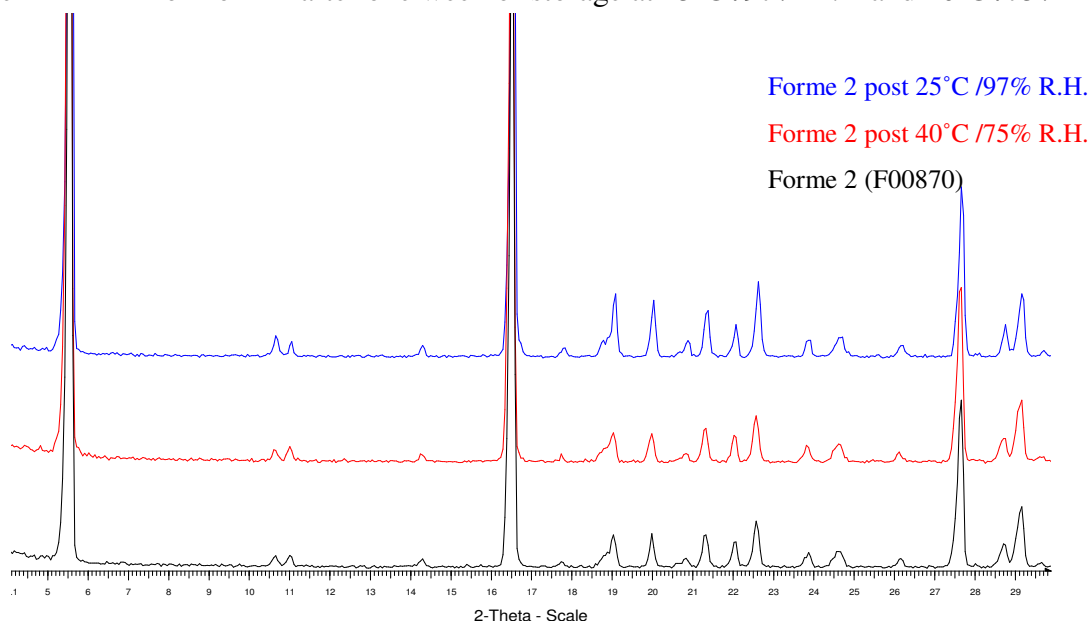
^1H NMR spectra of the 3 polymorphs are consistent with a mono-citrate structure. No impurities can be seen by NMR. The stoichiometries were confirmed by integration of the signals of the counter ion.

Figure 13 XRPD of Form 1 after 6 months of storage at 4°C.



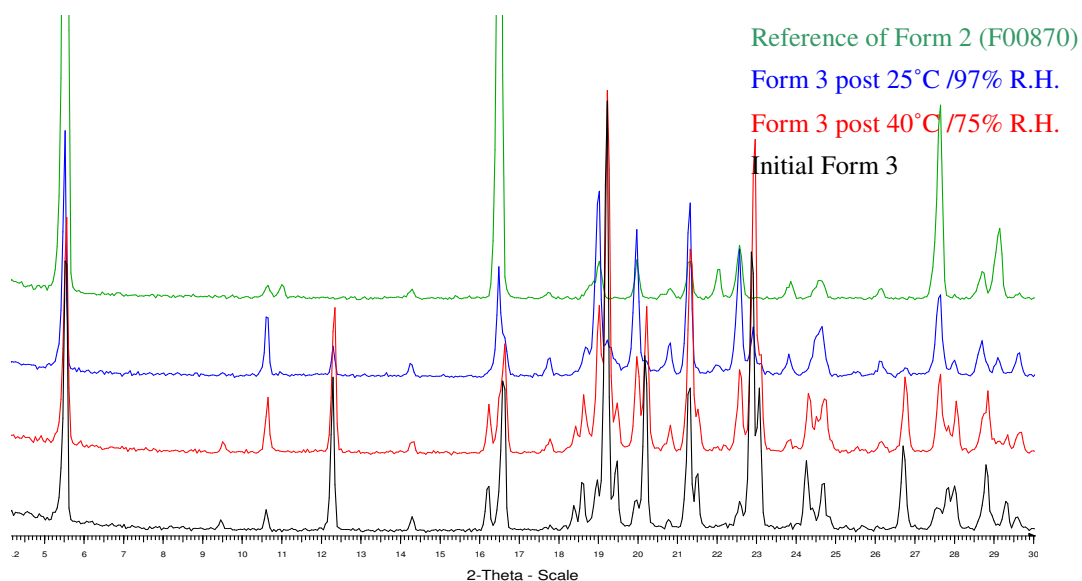
After 6 months of storage at 4°C, the batch of Form 1 (Crop 1) obtained during the salt selection study (Project 880 ; see report R880_002AC060907) seems to have transformed to a mixture of Form 2 and 3. This observation confirms the metastable character of this phase.

Figure 14 XRPD of Form 2 after one week of storage at 25°C /97% R.H and 40°C /75% R.H.



No change was observed in the XRPD patterns of Form 2 after storage at 40 °C /75 % R.H. or 25°C /97%R.H. for 1 week. Therefore Form 2 is not sensitive to humidity.

Figure 15 XRPD of Form 3 after one week of storage at 25°C /97% R.H. and 40°C /75% R.H.



No change was observed in the pattern of Form 3 after one week of storage at 40°C /75% R.H. However, after one week at 25°C /97% R.H, the XRPD pattern had changed to one that was consistent with Form 2. Therefore, Form 3 is metastable at these specific conditions.

Figure 16 TGA-DSC of Form 1 (Crop 1).

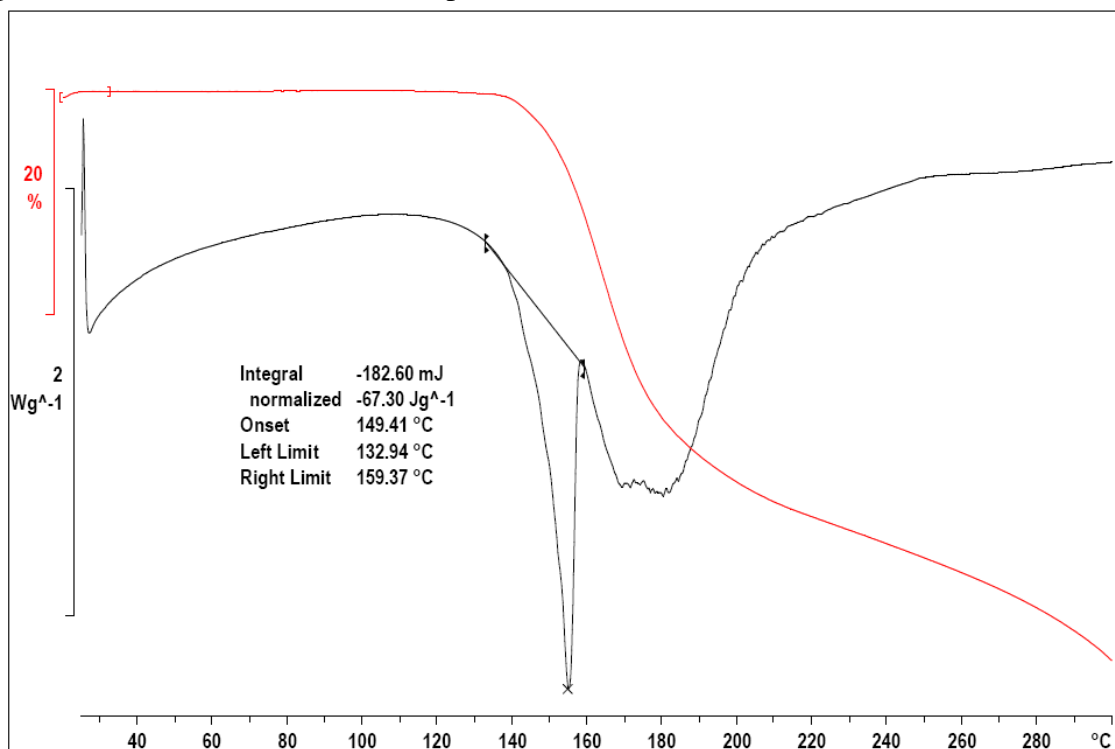


Figure 17 TGA-DSC of Form 2 (F00870).

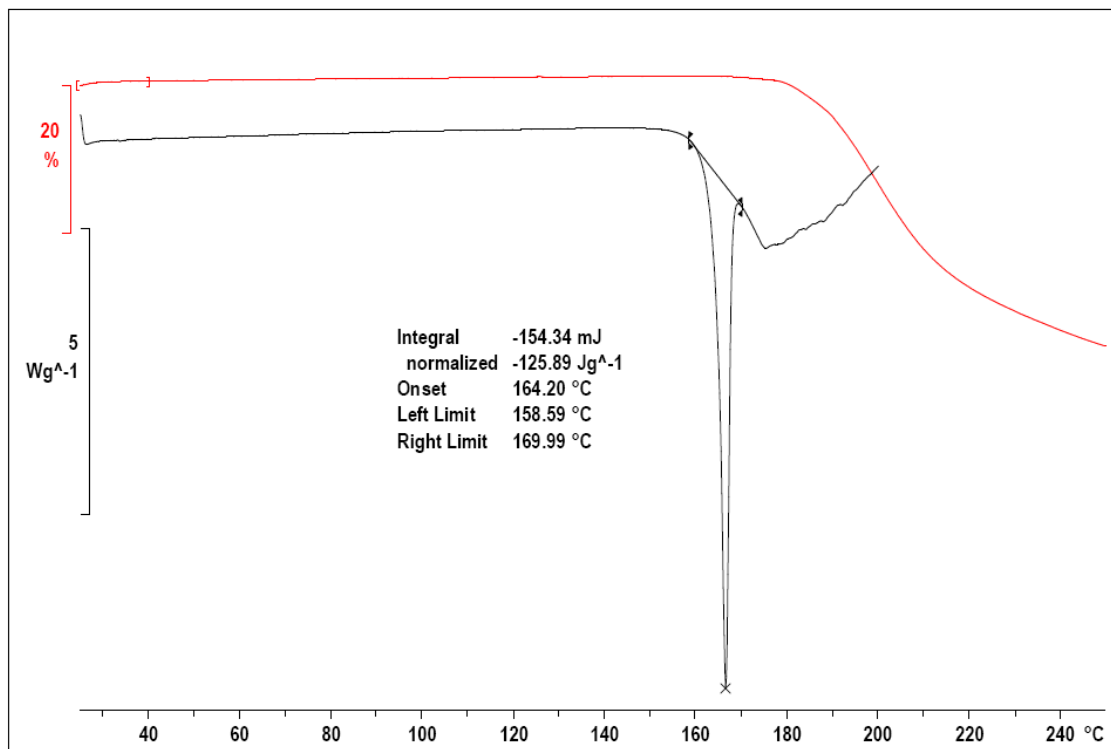


Figure 18 TGA-DSC of Form 3.

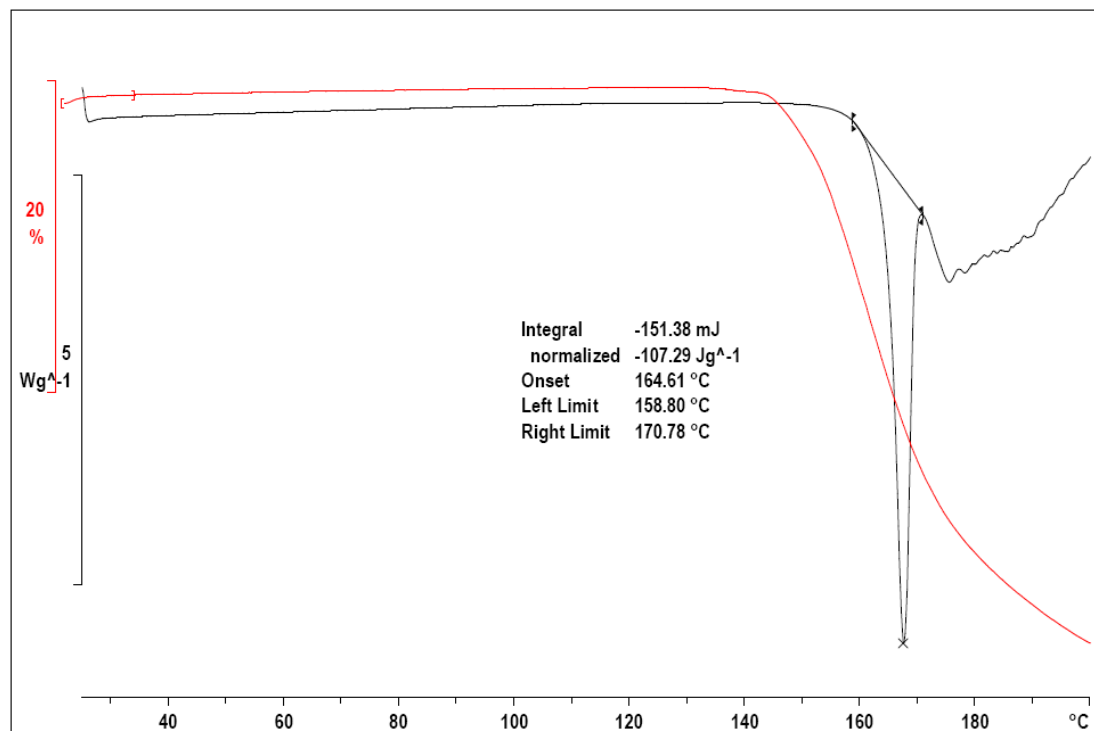
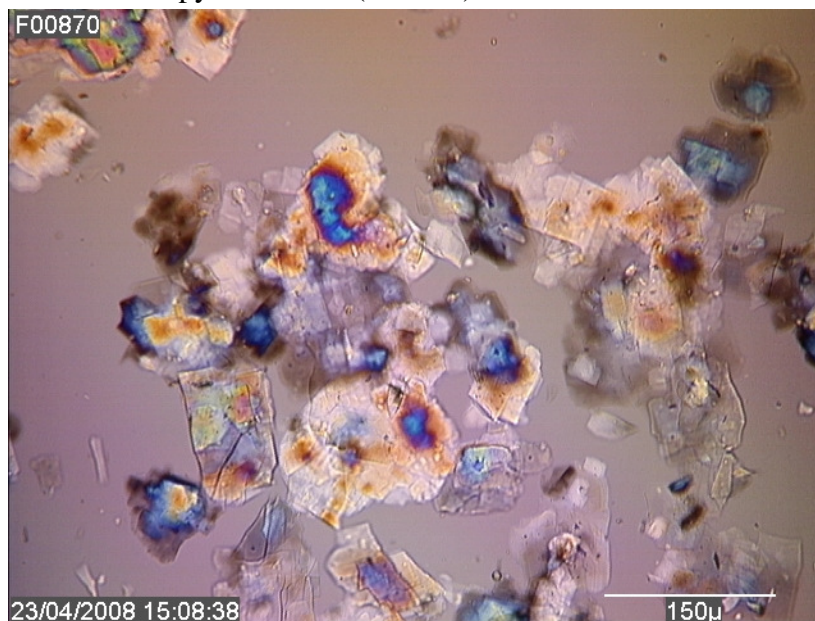
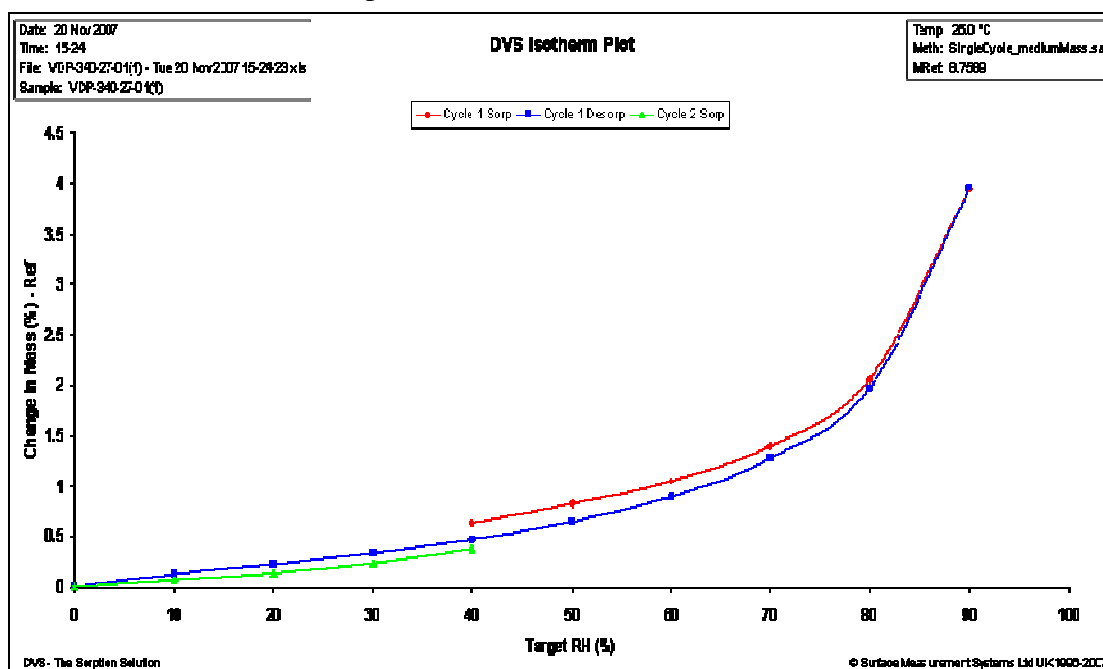


Figure 19 Optical Microscopy of Form 2 (F00870).



The material provided was initially crystalline showing clear birefringence. Form 2 is made up of particles with a thin plate shape morphology..

Figure 20 GVS of Form 1 (Crop 1) at 25°C.



Less than 1.5% of water was taken up between 0 and 70% R.H, then a further 2.5% between 70 and 90% R.H. No major changes are observed on the XRPD pattern.

Figure 21 XRPD of Form 1 post GVS at 25°C .

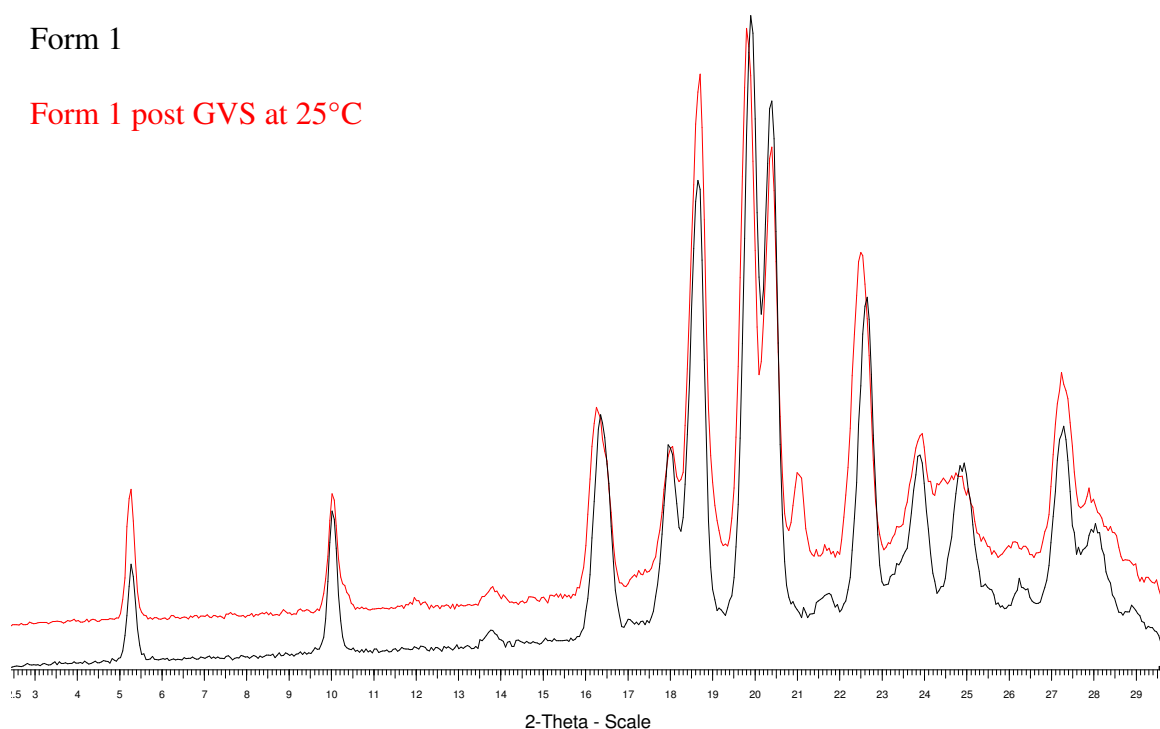
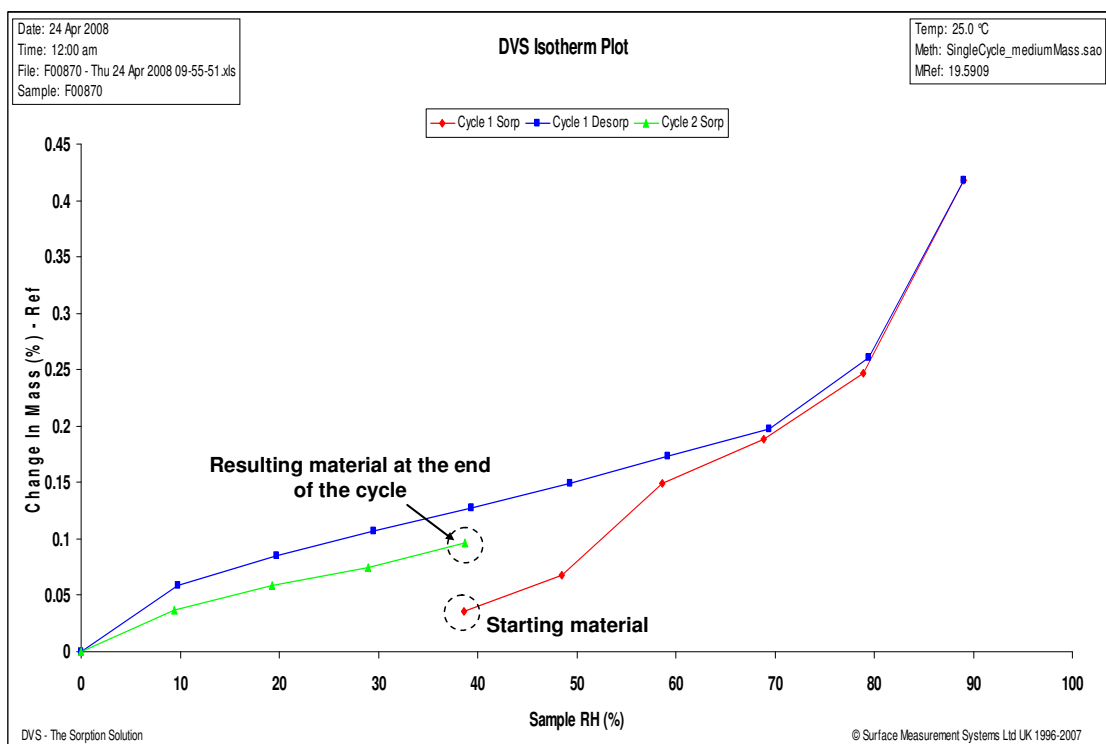


Figure 22 GVS of Form 2 (F00870) at 25°C.



No hydration was observed. Form 2 is not hygroscopic. No change was observed in the XRPD after analysis.

Figure 23 XRPD of F00870 post GVS at 25°C.

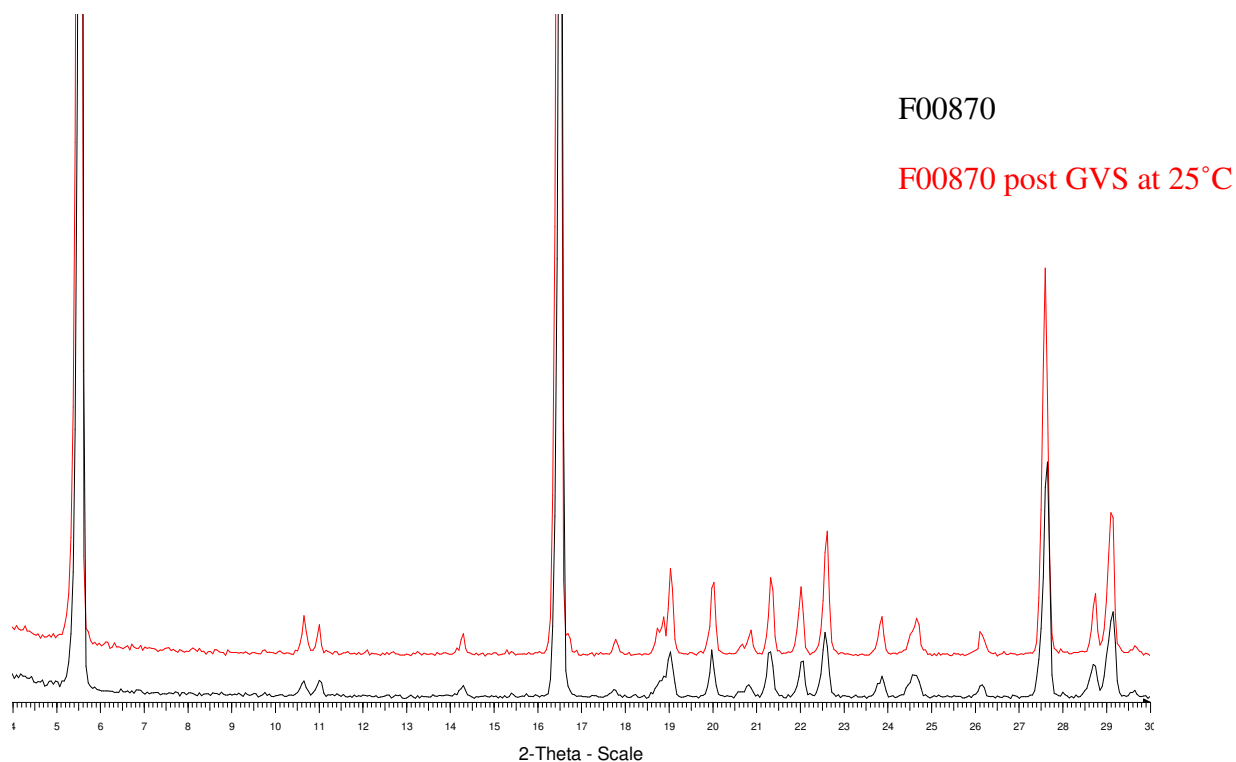
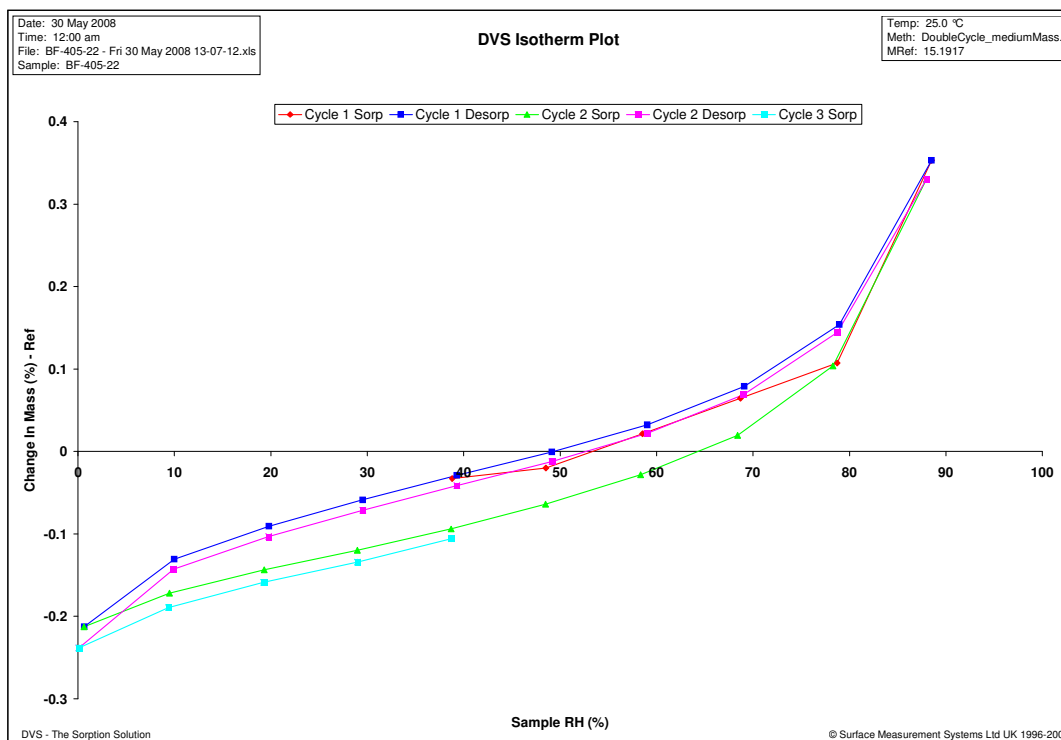


Figure 24 GVS analysis of Form 3 (two cycles) at 25°C.



No hydration observed. Form 3 is not hygroscopic. No change was observed in the XRPD after analysis.

Figure 25 XRPD of Form 3 post GVS at 25°C .

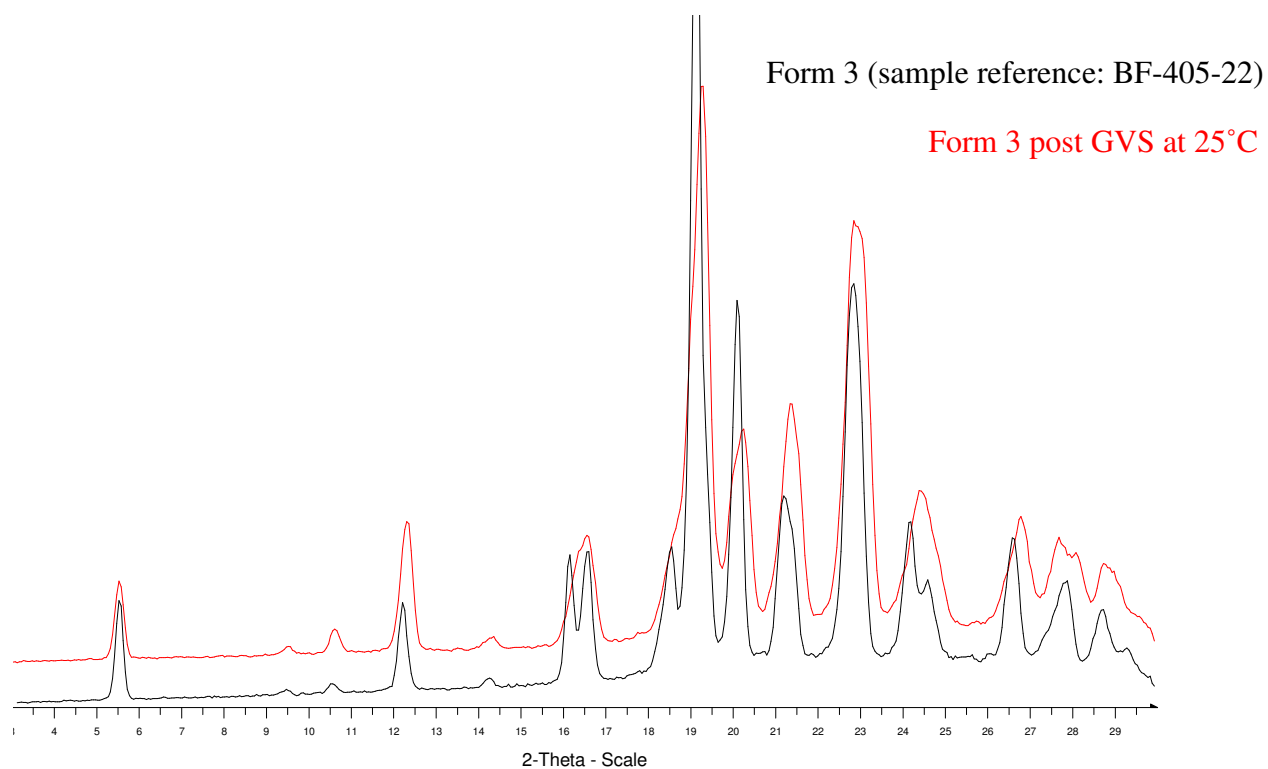
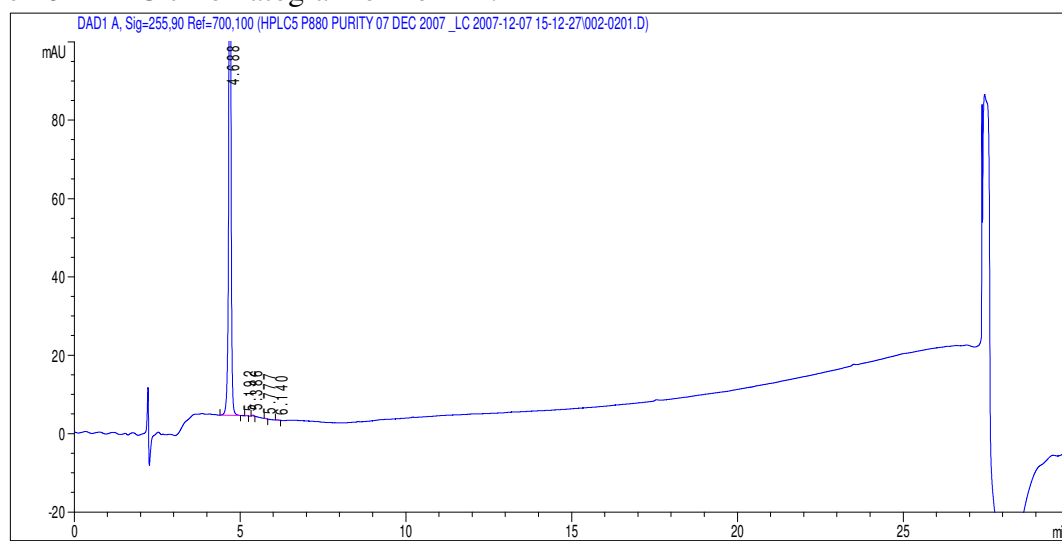
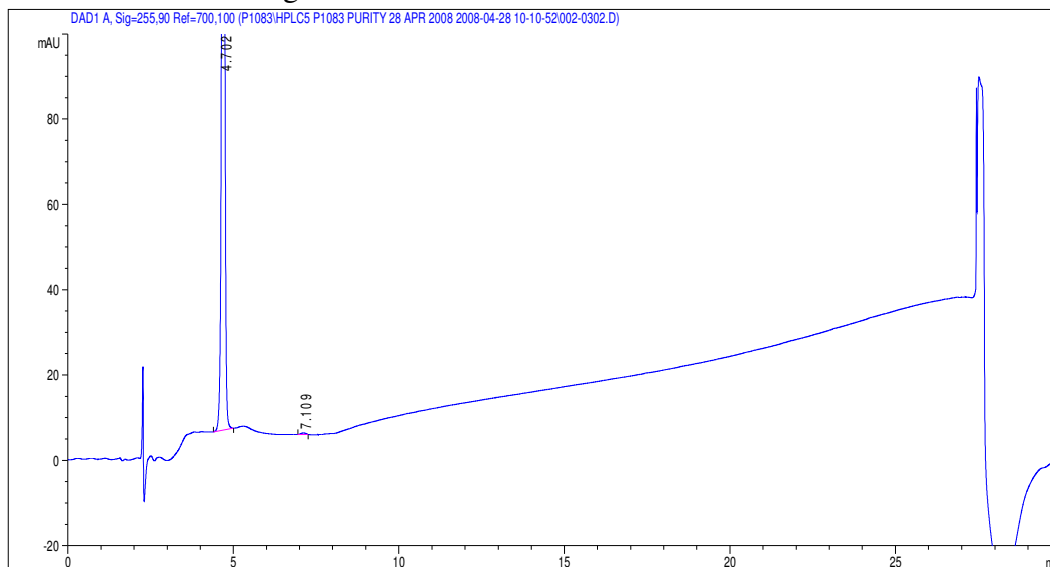


Figure 26 HPLC chromatogram of Form 1.



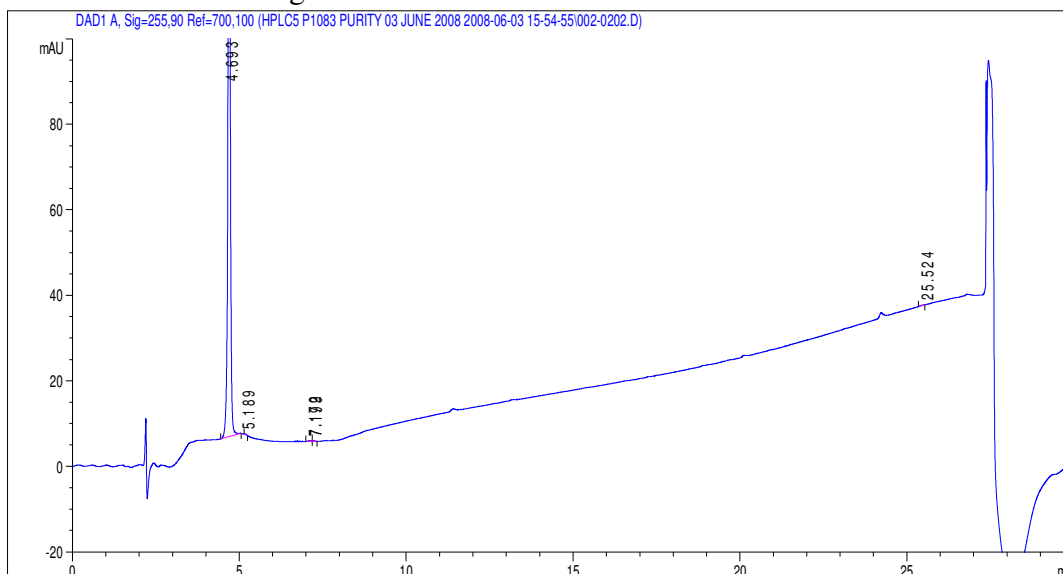
The sample purity of Form 1 was measured at 99.82% using the Pharmorphix generic method.

Figure 27 HPLC chromatogram of Form 2.



The sample purity of Form 2 was measured at 99.78% using the Pharmorphix generic method.

Figure 28 HPLC chromatogram of Form 3.



The sample purity of Form 3 was measured at 99.67% using the Pharmorphix generic method.

Table 17 HPLC purity values.

RRT	Area %		
	Form 1	Form 2	Form 3
1	99.82	99.78	99.67
1.15	0.1	-	-
1.51	-	0.22	-
1.53	-	-	0.15

Only purities above 0.10% are reported.

Data Section 8: Cross seeding experiments

Figure 29 Cross seeding experiments carried out at 25°C.

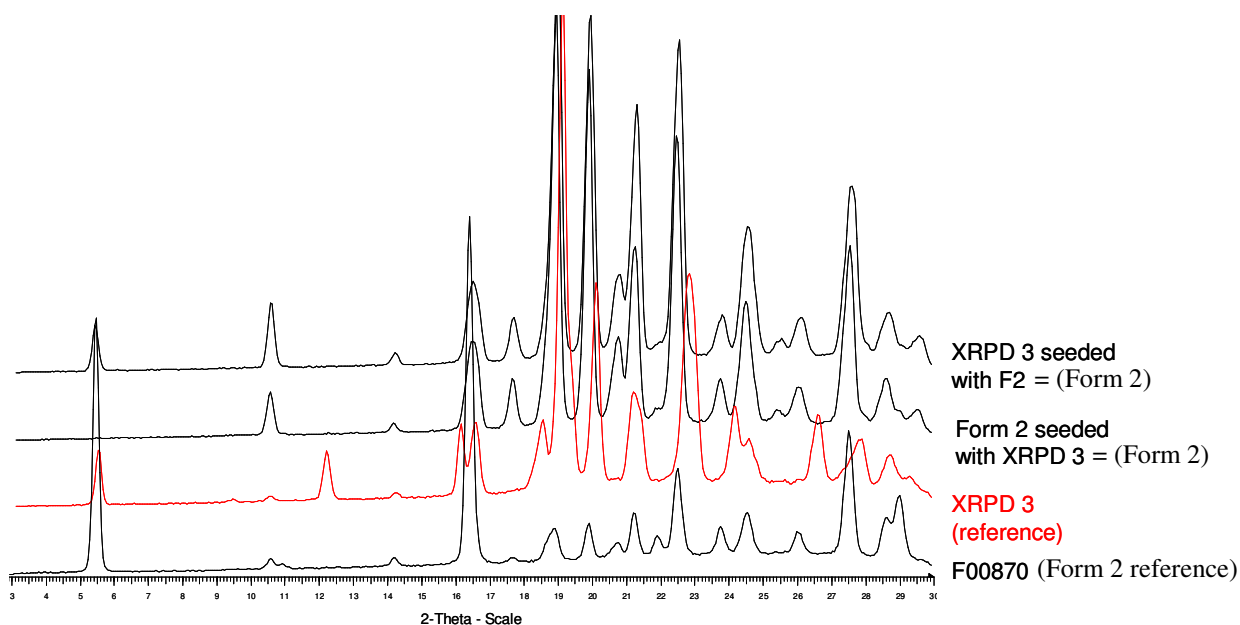
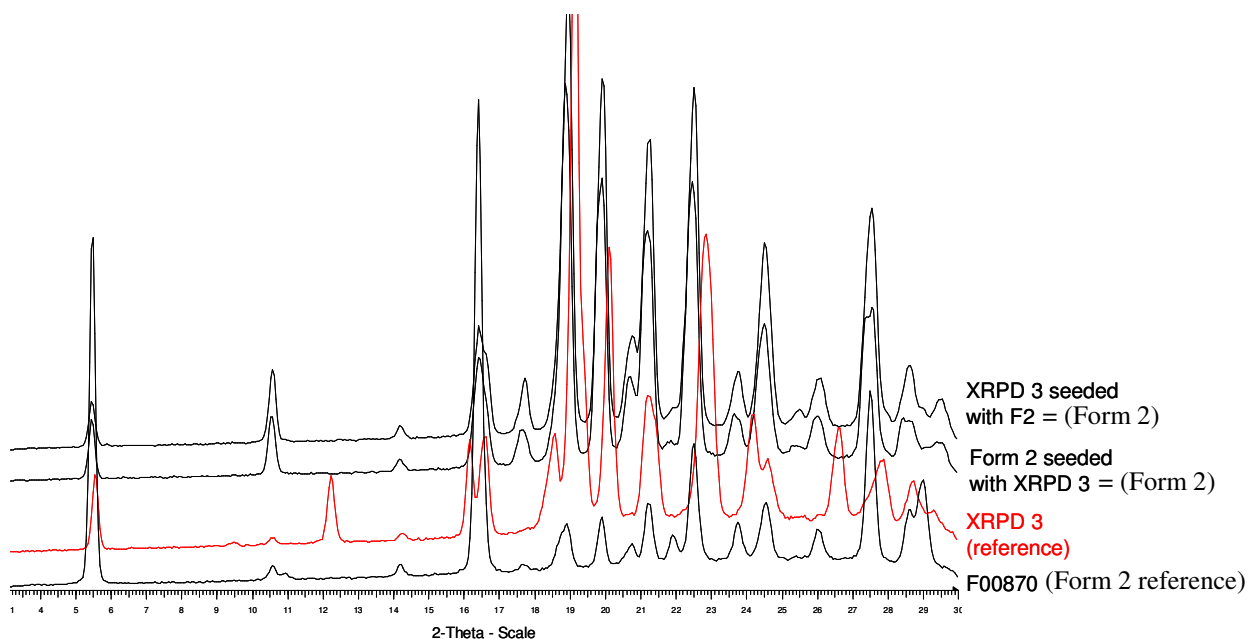


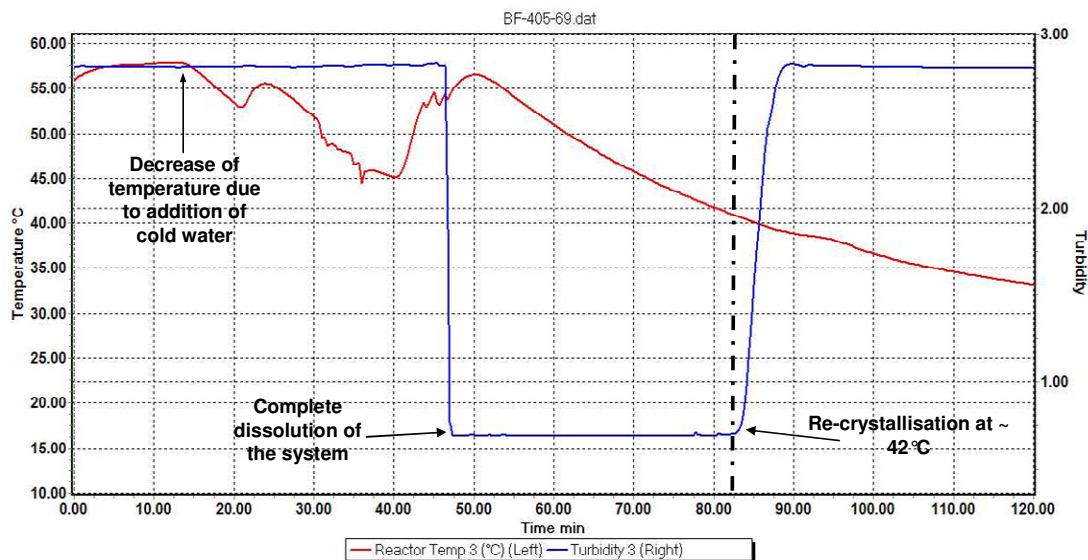
Figure 30 Cross seeding experiments carried out at 40°C.



After 4 days at 20°C and 5 days at 40°C, diffractograms recorded from the dry filtered solids showed profiles consistent with Form 2. Form 3 had completely converted to Form 2.

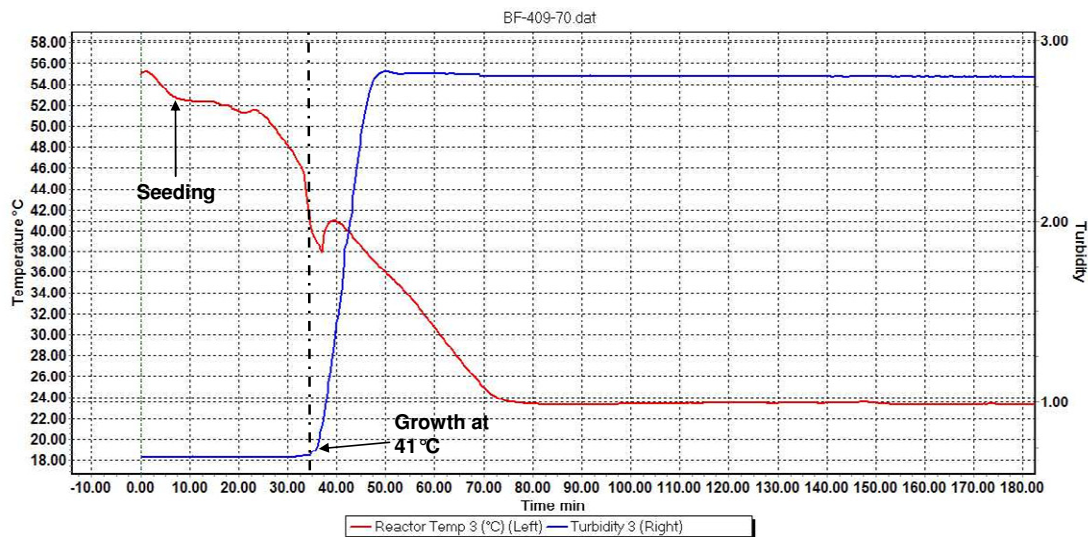
Data Section 9: Scale up of Form 2

Figure 31 Thermal and turbidity curves recorded during the recrystallisation of Form 2 (BF-405-69).



The crystallisation of Form 2 was detected at about 42°C corresponding to a large increase in turbidity.

Figure 32 Thermal and turbidity curves of Form 2 (BF-405-71b).



The system was seeded at 50°C and the crystalline growth was detected at about 41°C.

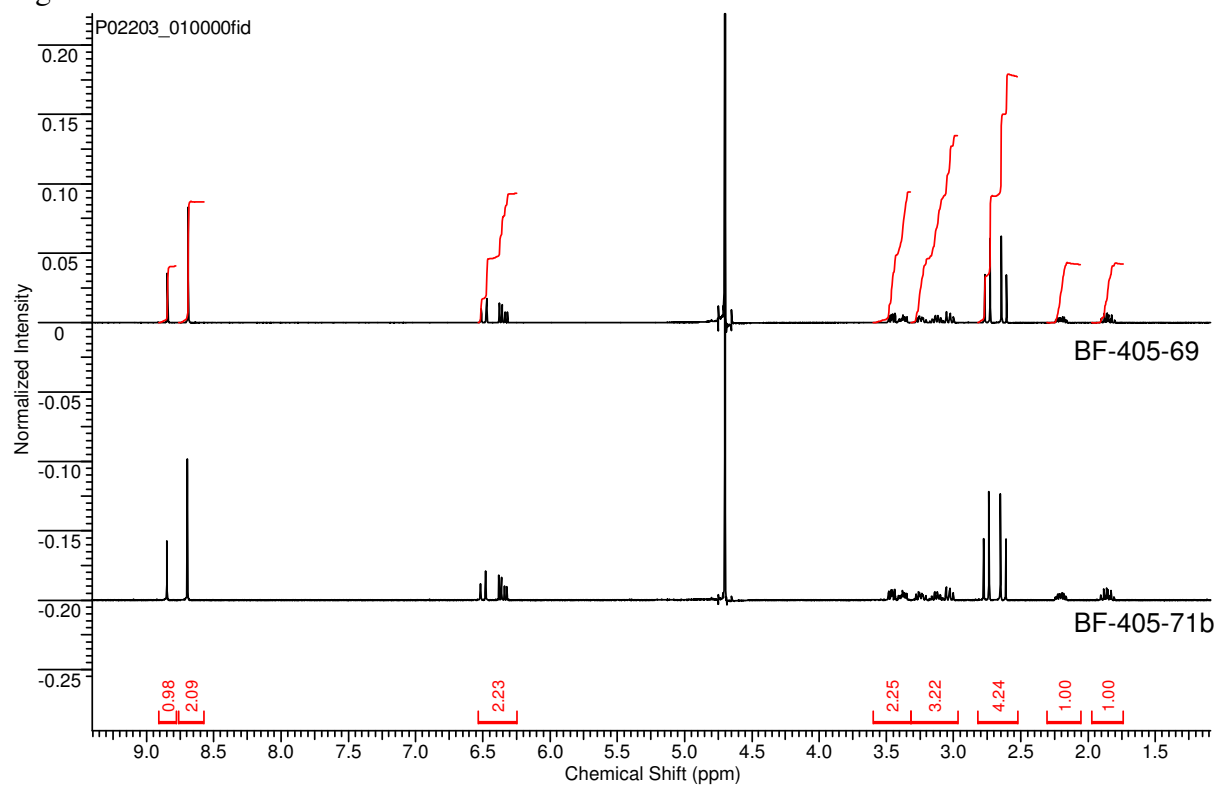
Figure 33 ^1H NMR of BF-405-69 and BF-405-71b.

Figure 34 XRPD patterns of BF-405-69 and BF-405-71b.

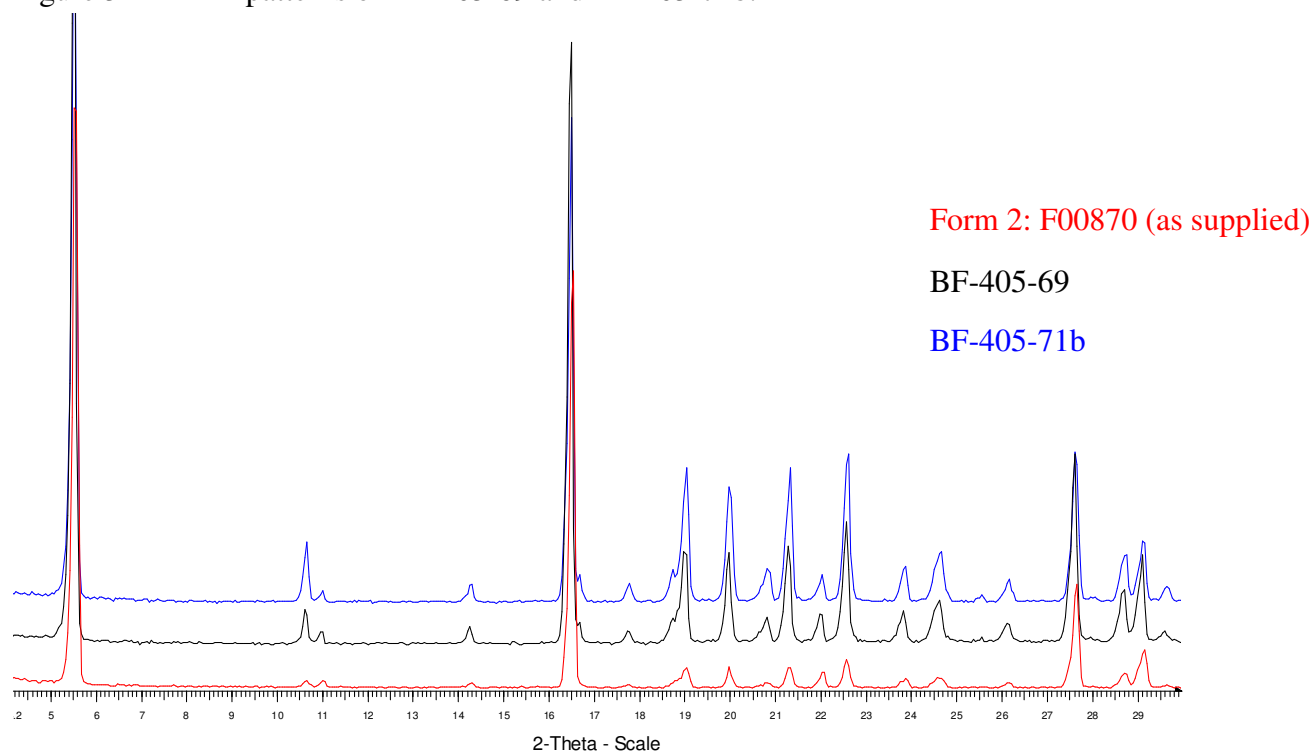
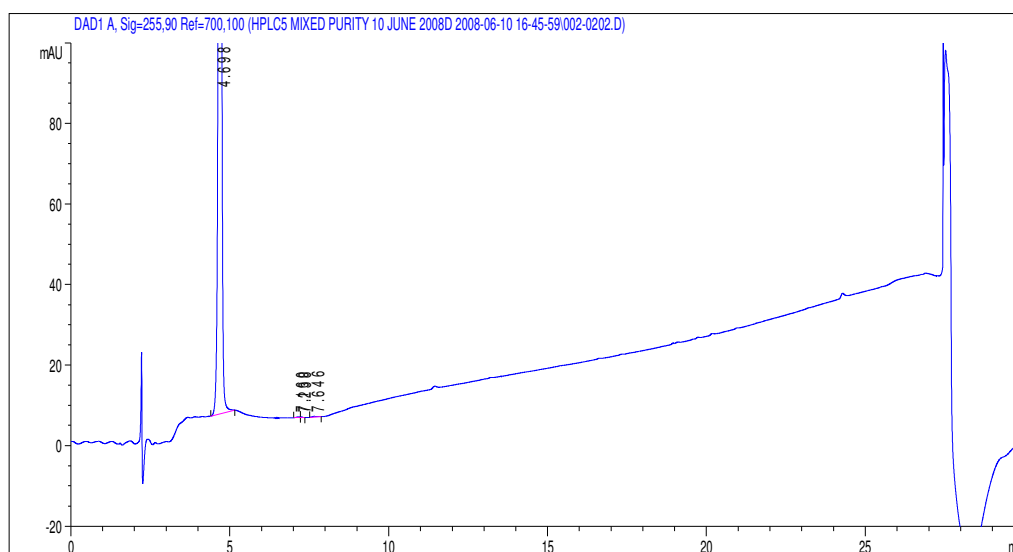
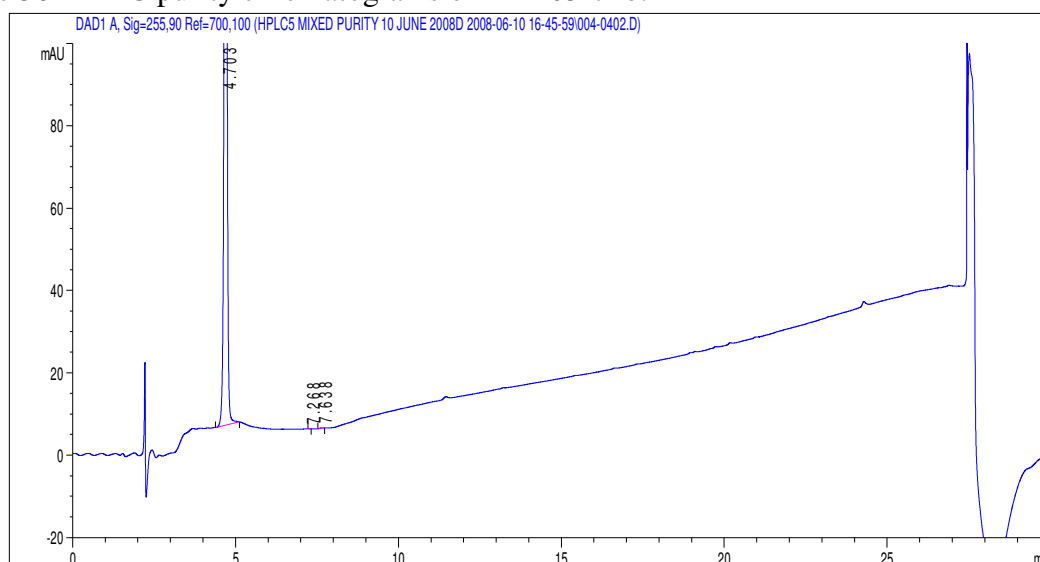


Figure 35 HPLC purity chromatograms of BF-405-69.



The purity of the crop BF-405-69 was measured at 99.83% area.

Figure 36 HPLC purity chromatograms of BF-405-71b.



The purity of the crop BF-405-71b was measured at 99.93% area.

Figura 14 XRPD de la Forma 2 después de una semana de almacenamiento a 25°C/97% de humedad relativa y a 40°C/75% de humedad relativa

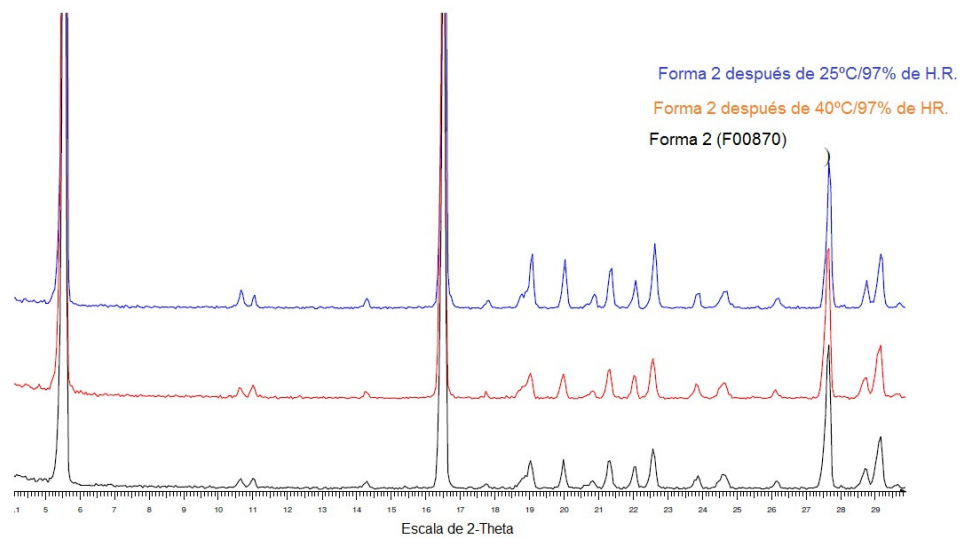


Tabla 9. Resumen de detección de monosal

Ácido	IPAc				MeCN			
	Observaciones Iniciales	Después de Evaporación de Solvente	THF de Maduración	Cámara de Humedad	Observaciones Iniciales	Después de Evaporación de Solvente	IPA de Maduración	Cámara de Humedad
Ácido clorhídrico	<i>Se investigará por separado</i>				<i>Se investigará por separado</i>			
Ácido sulfúrico	Sólido pegajoso	La muestra se licúa	Sólido pegajoso amarillo/se licúa		Sólido pegajoso	La muestra se licúa	Parcialmente cristalino - XRPD 1	
Ácido metano sulfónico	Sólido	Cristalino - XRPD 1	N/A	La muestra se licúa	Sólido	La muestra se licúa	N/A	
Ácido maleico	Sólido	Cristalino - XRPD 1	N/A	La muestra se licúa	Sólido	Cristalino - XRPD 1	N/A	La muestra se licúa
Ácido fosfórico	Sólido pegajoso	La muestra se licúa	Sólido pegajoso amarillo		Sólido pegajoso	Aceite	Aceite	
Ácido 1-hidroxi-2-naftoico	Ningún sólido producido	Aceite oscuro	Aceite oscuro		Ningún sólido producido	Aceite oscuro	Vidrio/ aceite oscuro	
Ácido cetoglutárico	Sólido pegajoso	Sólido pegajoso	Espuma pegajosa		Ningún sólido producido	Aceite oscuro	Vidrio	
Ácido malónico	Aceite	Aceite amarillo	Aceite amarillo		Ningún sólido producido	Aceite amarillo	Aceite	
Ácido L-tartárico	Sólido pegajoso	La muestra se licúa	Sólido pegajoso amarillo		Sólido pegajoso	La muestra se licúa	Sólido pegajoso	
Ácido fumárico	Sólido pegajoso	Cristalino - XRPD 1	N/A	La muestra se licúa	Sólido pegajoso	Sólido pegajoso	N/A	

Ácido cítrico	Sólido pegajoso	La muestra se licúa	Cristalino - XRPD 1	XRPD 1	Sólido pegajoso	Vidrio	Cristalino - XRPD 1	XRPD1
Ácido L-málico	Aceite	Aceite amarillo	Aceite oscuro		Ningún sólido producido	Aceite amarillo	Vidrio / aceite	
Ácido hipúrico	Ningún sólido producido	Aceite oscuro	Aceite oscuro		Ningún sólido producido	Aceite oscuro	Aceite oscuro	
Ácido L-láctico	Aceite	Aceite oscuro	Aceite oscuro		Ningún sólido producido	Aceite oscuro	Aceite	
Ácido benzoico	Ningún sólido producido	Aceite oscuro	Aceite oscuro		Ningún sólido producido	Aceite oscuro	Aceite	
Ácido succínico	Ningún sólido producido	Aceite oscuro	Aceite oscuro		Ningún sólido producido	Aceite oscuro	Aceite	
Ácido adípico	Aceite	Aceite oscuro	Aceite oscuro		Aceite	Aceite oscuro	Vidrio / aceite	
Ácido acético	Ningún sólido producido	Sólido pegajoso oscuro	Aceite oscuro		Ningún sólido producido	Aceite oscuro	Aceite oscuro	
Ácido nicotínico	Aceite	Aceite oscuro	Sólido pegajoso oscuro		Ningún sólido producido	Aceite oscuro	Vidrio / aceite	
Ácido propiónico	Ningún sólido producido	Aceite oscuro	Aceite oscuro		Ningún sólido producido	Aceite oscuro	Aceite oscuro	
Ácido orótico	Sólido	Principalmente amorfo - XRPD1	N/A	XRPD1	Sólido	Cristalino - XRPD 1	N/A	XRPD 1
Ácido 4-hidroxibenzoico	Sólido	XRPD 1	N/A	La muestra se licúa	Sólido	XRPD 1	N/A	La muestra se licúa
Ácido di-p-toluil-D-tartárico	Sólido	XRPD 1	N/A	XRPD 1	Sólido	XRPD 1	N/A	XRPD 1

Figura 16 - Curva de DSC de la forma A de AVE3183A (lote P-31448.030-1), referencia: ropa1303b

Muestra: RPR263183B

Tamaño: 4,7020 mg

Método: 263183B

Comentario: ROPA1303b/ACD4/1-2

DSC

Archivo: C:\...AVE3183A\ACD_DSC2010\ropa1303b.dat

Operador: ropa

Fecha de ejecución: 28 de agosto de 2002 13:53

Instrumento: 2010 DSC V4.4E

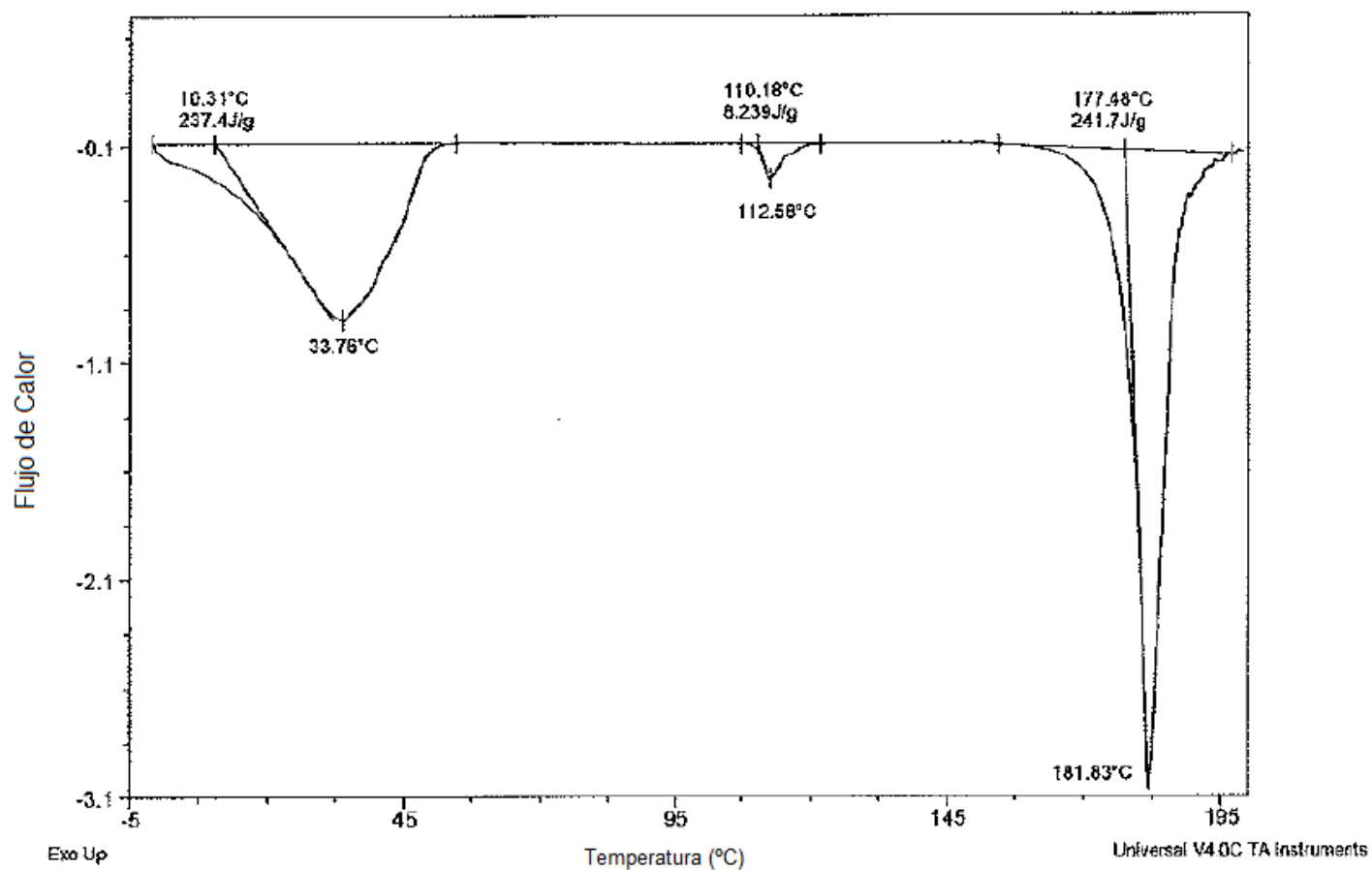


Figura 39 - Formas sólidas de AVE3183A identificadas hasta el momento en las condiciones experimentales que permiten el paso de una forma a otra

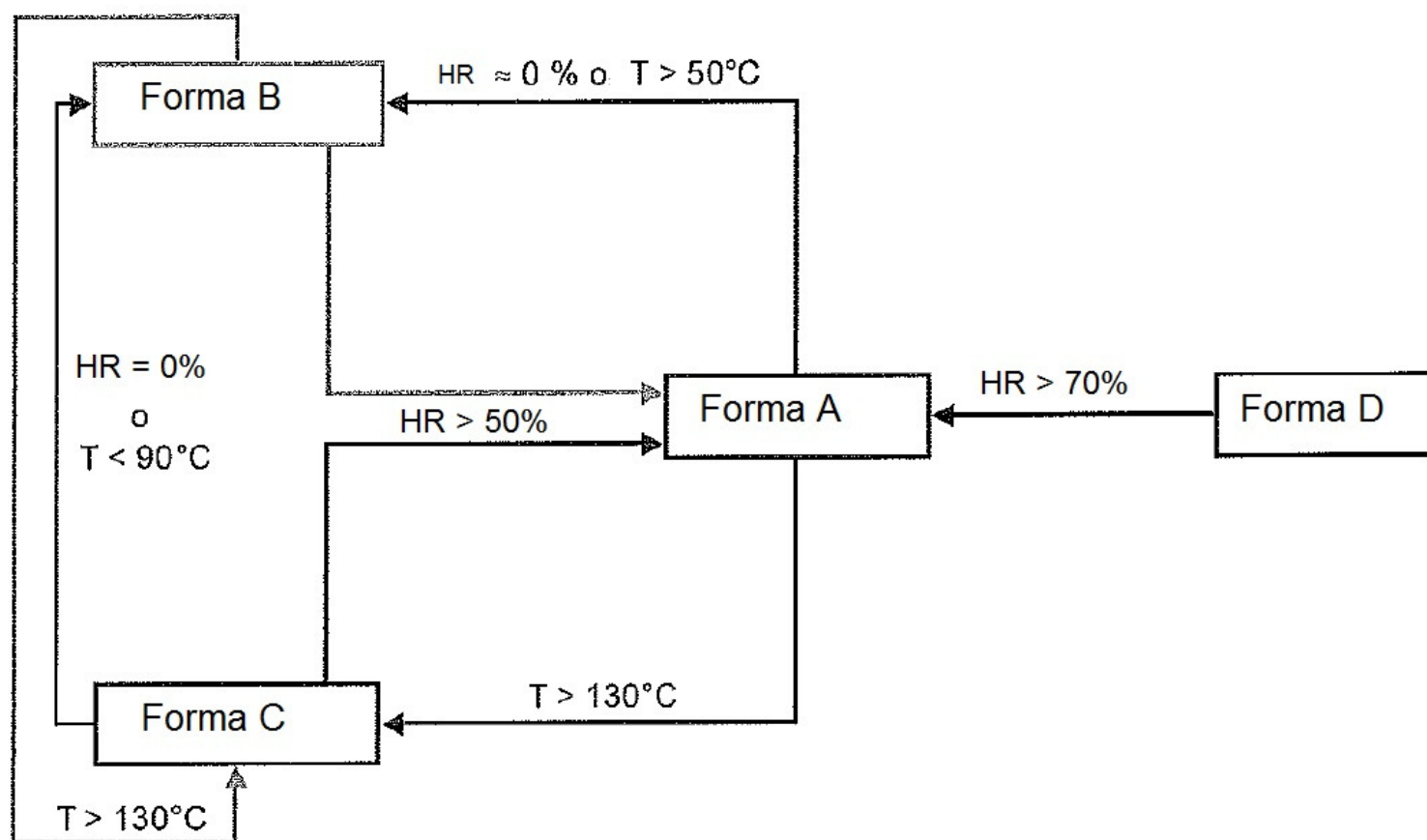


Tabla 10. Resumen de detección de hemisal

Ácido	IPAc				MeCN			
	Observaciones Iniciales	Después de Evaporación de Solvente	THF de Maduración	Cámara de Humedad	Observaciones Iniciales	Después de Evaporación de Solvente	IPA de Maduración	Cámara de Humedad
Ácido sulfúrico	Aceite	Aceite amarillo	Aceite amarillo		Aceite	Aceite	Aceite	
Ácido maleico	Aceite	Sólido pegajoso oscuro	Sólido pegajoso oscuro		Aceite	Aceite	Aceite	
Ácido fosfórico	Aceite	Sólido pegajoso oscuro	Sólido pegajoso amarillo		Aceite	Sólido pegajoso oscuro	Sólido pegajoso oscuro	
Ácido cetoglutárico	Aceite	Aceite	Aceite		Aceite	Aceite	Aceite	
Ácido malónico	Aceite	Aceite oscuro	Aceite oscuro		Aceite	Aceite amarillo	Aceite oscuro	
Ácido L-tartárico	Sólido	Sólido / Se licúa	Aceite oscuro		Sólido	Sólido / Se licúa	Sólido / Se licúa	La muestra se licúa
Ácido fumárico	Aceite	Vidrio / aceite oscuro	Sólido / Se licúa	La muestra se licúa	Aceite	Sólido / Se licúa	Principalmente amorfo	La muestra se licúa
Ácido cítrico	Aceite	Espuma pegajosa oscura	XRPD 1	La muestra se licúa parcialmente	Aceite	Sólido / Se licúa	Aceite	
Ácido L-málico	Aceite	Aceite oscuro	Aceite oscuro		Aceite	aceite	Aceite	
Ácido succínico	Aceite	Aceite oscuro	Aceite oscuro		Aceite	Aceite	Aceite	

Ácido adípico	Aceite	Aceite oscuro	Aceite oscuro		Aceite	Aceite	Aceite
Ácido di-p- toluil-D- tartárico	Sólido	XRPD 1	XRPD 1	XRPD 1	Sólido	XRPD 1	XRPD 1

Figura 17 - TGA-DSC de la Forma 2 (F00870)

