CA 2940902 A1 2015/09/24

(21) **2 940 902**

(12) **DEMANDE DE BREVET CANADIEN**
CANADIAN PATENT APPLICATION

(13) **A1**

(86) **Date de dépôt PCT/PCT Filing Date:** 2015/03/17

(87) **Date publication PCT/PCT Publication Date:** 2015/09/24

(85) **Entrée phase nationale/National Entry:** 2016/08/26

(86) **N° demande PCT/PCT Application No.:** EP 2015/055531

(87) **N° publication PCT/PCT Publication No.:** 2015/140153

(30) **Priorités/Priorities:** 2014/03/18 (US61/954,715); 2014/07/03 (US62/020,570)

(51) **Cl.Int./Int.Cl.** ***C07H 17/08*** (2006.01), ***A61K 31/7048*** (2006.01), ***A61P 31/04*** (2006.01), *C30B 29/54* (2006.01)

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(54) **Titre : NOUVEAUX POLYMORPHES ET NOUVEAUX ETATS SOLIDES DE LA TIACUMICINE B**
(54) **Title: NEW POLYMORPHS AND NEW SOLID STATES OF TIACUMICIN B**

(57) **Abrégé/Abstract:**

The present invention relates to new polymorphs consiting in crystallines solvates of Tiacumicin B, the solvates being propanol, isopropanol, acetic acid, isopropyl acetate, chlorobenzene and methyl-ethyl-ketone. The present invention also relates to a new amorphous form of Tiacumicin B.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
24 September 2015 (24.09.2015)

WIPO | PCT

(10) International Publication Number
WO 2015/140153 A1

(51) International Patent Classification:
C07H 17/08 (2006.01) *A61P 31/04* (2006.01)

(21) International Application Number:
PCT/EP2015/055531

(22) International Filing Date:
17 March 2015 (17.03.2015)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/954,715 18 March 2014 (18.03.2014) US
62/020,570 3 July 2014 (03.07.2014) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*

WO 2015/140153 A1

(54) **Title**: NEW POLYMORPHS AND NEW SOLID STATES OF TIACUMICIN B

(57) **Abstract**: The present invention relates to new polymorphs consiting in crystallines solvates of Tiacumicin B, the solvates being propanol, isopropanol, acetic acid, isopropyl acetate, chlorobenzene and methyl-ethyl-ketone. The present invention also relates to a new amorphous form of Tiacumicin B.

New polymorphs and new solid states of Tiacumicin B

The present invention relates to new polymorphs and new solid states of Tiacumicin B. Tiacumicin B can be produced as disclosed in US4918174 or WO2004014295. There are several publications mentioning various polymorphs and crystals related to tiacumicins e.g. US8722863, US7863249, US8518899, CN103275153, J. Chem. Soc. Perkin Trans, by Arnone and Nasini, 1987, page 1353-1359.

The following background is based on Florence et al, Physiochemical Principles of Pharmacy, 5th edition, August 2011:
The physical properties of the solid state seen in crystals and powders of both drugs and pharmaceutical excipients are of interest because they can affect both the production of dosage forms and the performance of the finished product.

Crystalline solids can exist in several subphases, such as polymorphs, solvates, hydrates, and cocrystals. Polymorphs are different crystalline forms (at different free energy states) of the same compound. On the other hand, solvates, hydrates and co-crystals are similar in that they comprise a stoichiometric or significant amount of an additional compound. E.g., a drug together with an organic solvent (to form a solvate) or water (to form a hydrate), or another crystalline solid (to form co-crystals). Both types of compounds participate in the short-range and long-range orders of the crystal and therefore these subphases are regarded as single crystalline forms consisting of two types of molecules.

The nature of the crystalline form of a drug substance may affect its stability in the solid state, its solution properties and its absorption.

The solid state is important for a variety of reasons: morphology, particle size, polymorphism, solvation or hydration can affect filtration, flow, tableting, dissolution and bioavailability. The crystals of a given substance may vary in size, the relative development of the given faces and the number and kind of the faces (or forms) present; that is, they may have different crystal habits. The habit describes the overall shape of the crystal in rather general terms and includes, for example, acicular (needle-like), prismatic, pyramidal, tabular, equant, columnar and lamellar types.

A more fundamental difference in properties may be found when the compounds crystallise as different polymorphs. When polymorphism occurs, the molecules arrange themselves in two or more different ways in the crystal; either they may be packed differently in the crystal lattice or there may be differences in the orientation or conformation of the molecules at the lattice sites. These variations cause differences in the X-ray diffraction patterns of the polymorphs and this technique is one of the main methods of detecting the existence of polymorphs. The polymorphs have different physical and chemical properties; for example, they may have different melting points and solubilities and they also usually exist in different habits.

Polymorphism is common with pharmaceutical compounds, but predictability of the phenomenon is difficult. Its pharmaceutical importance depends very much on the stability and solubility of the forms concerned. It is difficult, therefore, to generalise, except to say that where polymorphs of insoluble compounds occur there are likely to be biopharmaceutical implications.

The term “polymorph” as used herein is meant to embrace crystalline solid states of a pure compound including solvates, co-crystals and crystals.

However, polymorphs also have different crystal lattices and consequently their energy contents may be sufficiently different to influence their stability and biopharmaceutical behaviour.

The most important consequence of polymorphism is the possible difference in the bioavailability of different polymorphic forms of a drug; particularly when the drug is poorly soluble. The rate of absorption of such a drug is often dependent upon its rate of dissolution. The most stable polymorph has the lowest solubility and slowest dissolution rate and consequently often a lower bioavailability than the metastable polymorph. It has been proposed that when the free energy differences between the polymorphs are small there may be no significant differences in their biopharmaceutical behaviour as measured by the blood levels they achieve.

When some compounds crystallise they may entrap solvent in the crystal. Crystals that contain a solvent as part of the crystal lattice are called crystal solvates, or crystal hydrates when water is the solvent of crystallisation. Crystals that contain no water of crystallisation are termed anhydrates. Crystal solvates exhibit a wide range of behaviour depending on the interaction between the solvent and the crystal structure. With some solvates the solvent plays a key role in holding the crystal together; for example, it may be part of a hydrogen-bonded network within the crystal structure. These solvates are very stable and are difficult to desolvate. When these crystals lose their solvent they may collapse and re-crystallise in a new crystal form. We can think of these as polymorphic solvates. In other solvates, the solvent is not part of the crystal bonding and merely occupies voids in the crystal. These solvates lose their solvent more readily and desolvation does not destroy the crystal lattice.

Certain impurities can inhibit the growth pattern and favour the growth of metastable polymorphs. Impurities in a crystallization process have been found to have vast effects on the morphology of the resulting crystal, with both beneficial and detrimental effects possible for the resultant product.
Impurities occur because materials are never 100% pure. An impurity compound can often be incorporated at a regular site in the crystal structure.

Brief description of the figures

Figure 1A: Graphical XRPD of the Tiacumicin B starting material

Figure 1B: Graphical XRPD of the amorphous Tiacumicin B material

Figure 2A: Graphical XRPD of the chlorobenzene crystal solvate of Tiacumicin B

Figure 2B: XRPD of the chlorobenzene crystal solvate of Tiacumicin B in table format showing the relative intensity of the peaks, highest peak ≡ 100. The peaks are designated w for weak intensity, m for medium intensity, s for strong intensity and vs for very strong intensity.

Figure 3A: XRPD of the n-propanol crystal solvate of Tiacumicin B

Figure 3B: XRPD of the n-propanol crystal solvate of Tiacumicin B in table format showing the relative intensity of the peaks, highest peak ≡ 100. The peaks are designated w for weak intensity, m for medium intensity, s for strong intensity and vs for very strong intensity.

Figure 4A: Graphical XRPD of the isopropanol crystal solvate of Tiacumicin B

Figure 4B: XRPD of the isopropanol crystal solvate of Tiacumicin B in table format showing the relative intensity of the peaks, highest peak ≡ 100. The peaks are designated w for weak intensity, m for medium intensity, s for strong intensity and vs for very strong intensity.

Figure 5A: Graphical XRPD of the methyl-ethyl-ketone crystal solvate of Tiacumicin B.

Figure 5B: XRPD of the methyl-ethyl-ketone crystal solvate of Tiacumicin B in table format showing the relative intensity of the peaks, highest peak ≡ 100. The peaks are designated w for weak intensity, m for medium intensity, s for strong intensity and vs for very strong intensity.

Figure 6A: Graphical XRPD of the acetic acid crystal solvate of Tiacumicin B

Figure 6B: XRPD of the acetic acid crystal solvate of Tiacumicin B in table format showing the relative intensity of the peaks, highest peak ≡ 100. The peaks are designated w for weak intensity, m for medium intensity, s for strong intensity and vs for very strong intensity.

Figure 7A: XRPD of high purity amorphous Tiacumicin material stored at 0 days (top), 1 day (middle) and 7 days (bottom).

Figure 7B: XRPD of high purity amorphous Tiacumicin material stored at 0 months (bottom), 1 month (middle) and 2 months (top).

Figure 7C: Glass transition temperature of high purity amorphous Tiacumicin material by using Fox Equation.

Figure 8A: XRPD of the Tiacumicin solvate form α during storage.

Figure 8B: XRPD of the Tiacumicin solvate form α with peaks assigned with 2θ values.

Figure 8C: XRPD in table format of Tiacumicin solvate form α.

Figure 9A: XRPD of the Tiacumicin solvate form β during storage.

Figure 9B: XRPD of the Tiacumicin solvate form β with peaks assigned with 2θ values.

Figure 9C: XRPD in table format of Tiacumicin solvate form β.

Figure 10: Chromatogram of high purity Tiacumicin material.
A 4.6x150 mm Agilent Zorbax Eclipse XDB-C8 3.5 μm column was used and the detector wavelength was 230 nm. A flow rate of 1.0 mL/min was used. Injection volume was 10 μL and a total runtime of 21 min. The gradient program was 0 min: 60%A, 40%B. 3 min; 50% A, 50% B, 14 min 39% A, 61% B, 14.5 60% A, 40% B until 21 min.
Mobile Phase A: 2.0 mL of trifluoroacetic acid was added to 2 L of Millli-Q water.
Mobile Phase B: 1.0 mL of trifluoroacetic acid was added to 2 L of acetonitrile.
App 0.2 mg of high purity Tiacumicin material was scaled into a HPLC vial and diluted with citric buffer at pH 4.0+-0.1 that has been mixed with Acetonirile at a ratio of 2:3.

Figure 11: Comparison of XRPDs of three polymorphs according to the present invention.

Summary of the invention:

The present invention concerns new polymorphs or solid states of Tiacumicin B. The new polymorphs and new solid states are alternatives to the existing polymorphs.

In one aspect, the present polymorph is a crystal solvate of Tiacumicin B selected from the propanol crystal solvate, the acetic acid crystal solvate, the chlorobenzene crystal solvate, the methyl-ethyl-ketone crystal solvate, the isopropyl-acetate solvate or the isopropanol crystal solvate.

In one aspect, the present polymorph is a crystal solvate of Tiacumicin B selected from the propanol crystal solvate, the acetic acid crystal solvate, the chlorobenzene crystal solvate, the methyl-ethyl-ketone crystal solvate, the isopropyl-acetate solvate or the isopropanol crystal solvate characterized by a XRPD displaying a peak at diffraction angle 2θ of 19.9 ± 0.1

In one aspect, the present polymorph is a crystal solvate of Tiacumicin B selected from the propanol crystal solvate, the acetic acid crystal solvate, the methyl-ethyl-ketone crystal solvate, the isopropyl-acetate solvate or the isopropanol crystal solvate characterized by a XRPD displaying a peak at diffraction angle 2θ of 3.3 and 19.9 ± 0.1

In one aspect, the present polymorph is a crystal solvate of Tiacumicin B selected from the propanol crystal solvate, the acetic acid crystal solvate or the methyl-ethyl-ketone crystal solvate characterized by a XRPD displaying a peak at diffraction angle 2θ of 3.3 and 19.9 ± 0.1

In one aspect, the n-propanol crystal solvate of Tiacumicin B is characterized by a XRPD displaying peaks at diffraction angles 2θ of 3.3, 7.5, 7.7, 18.8 and 19.9

In one preferred aspect, the acetic acid crystal solvate of Tiacumicin B is characterized by a XRPD displaying peaks at diffraction angles 2θ of 6.7, 7.6, 18.7 and 19.9

In one aspect, the chlorobenzene crystal solvate of Tiacumicin B is characterized by a XRPD displaying peaks at diffraction angles 2θ of 6.7, 18.8 and 19.9

In one preferred aspect, the methyl-ethyl-ketone crystal solvate of Tiacumicin B is characterized by a XRPD displaying peaks at diffraction angles 2θ of 3.3, 7.5, 15.7 and 18.6

In one preferred aspect, the isopropanol crystal solvate of Tiacumicin B is characterized by a XRPD displaying peaks at diffraction angles 2θ of 6.5, 9.9, 18.6 and 19.8

In one aspect of the present invention, the new solid state is a high purity amorphous Tiacumicin B material.

In one aspect of the present invention, the new solid state is a high purity amorphous Tiacumicin B material with a Tg of ca 113 °C as measured by DSC.

In one aspect of the present invention, the new solid state is a high purity amorphous Tiacumicin B material comprising less than 5% w/w water.

In one aspect of the present invention, the new solid state is a high purity amorphous Tiacumicin B material comprising less than 2% w/w water.

In one aspect of the present invention, the new solid state is a high purity amorphous Tiacumicin B material comprising less than 0.5 % w/w water.

In one aspect of the present invention, the new solid state is a high purity amorphous Tiacumicin B material suitable for storage, comprising less than 2% w/w water and has a Tg of ca 113°C as measured by DSC.

In one aspect of the present invention, the new solid state is a high purity amorphous Tiacumicin B material comprising more than 98% Tiacumicin B as measured by HPLC, which is suitable for storage and comprises less than 2% w/w water and has a Tg of ca 113°C as measured by DSC.

In one aspect of the present invention, a new polymorph of Tiacumicin B is provided which is suitable for storage and displays XRPD with peaks at diffraction angles 2θ of 16.6 and 19.9 ± 0.1

In one aspect of the present invention, the new polymorph of Tiacumicin B is an acetic acid solvate which is suitable for storage and displays XRPD with peaks at diffraction angles 2θ of 7.1, 16.6, 18.7 and 19.9 ± 0.1

In one aspect of the present invention, the new polymorph of Tiacumicin B is an acetic acid solvate which is suitable for storage and displays XRPD with peaks at diffraction angles 2θ of 7.1, 16.6, 18.7 and 19.9 ± 0.1 substantially as shown in Figure 8B. This form is called Tiacumicin solvate Form α.

In one aspect of the present invention, the new polymorph of Tiacumicin B is an isopropyl-acetate solvate which is suitable for storage and displays XRPD with peaks at diffraction angles 2θ of 7.3, 15.7, 16.6, 18.8, 19.9 and 20.2 ± 0.1

In one aspect of the present invention, the new polymorph of Tiacumicin B is an isopropyl-acetate solvate which is suitable for storage and displays XRPD with peaks at diffraction angles 2θ of of 7.3, 15.7, 16.6, 18.8, 19.9 and 20.2 ± 0.1 substantially as shown in Figure 9B. This form is called Tiacumicin solvate Form β.

In one aspect of the present invention, a new polymorph of Tiacumicin B is provided which is suitable for storage and displays XRPD with peaks at diffraction angles 2θ of 16.6, 18.8 and 19.9 ± 0.1

In one aspect, the present polymorph is any crystal solvate of Tiacumicin B characterized by a XRPD displaying a peak at diffraction angle 2θ of 3.3, 9.9 and 18.6 ± 0.1 or substantially as shown in Figure 11.

The various aspects and more of the present invention, including various embodiments, will be described in further detail, with reference to the detailed description, examples and appended drawings.

Detailed description of the invention

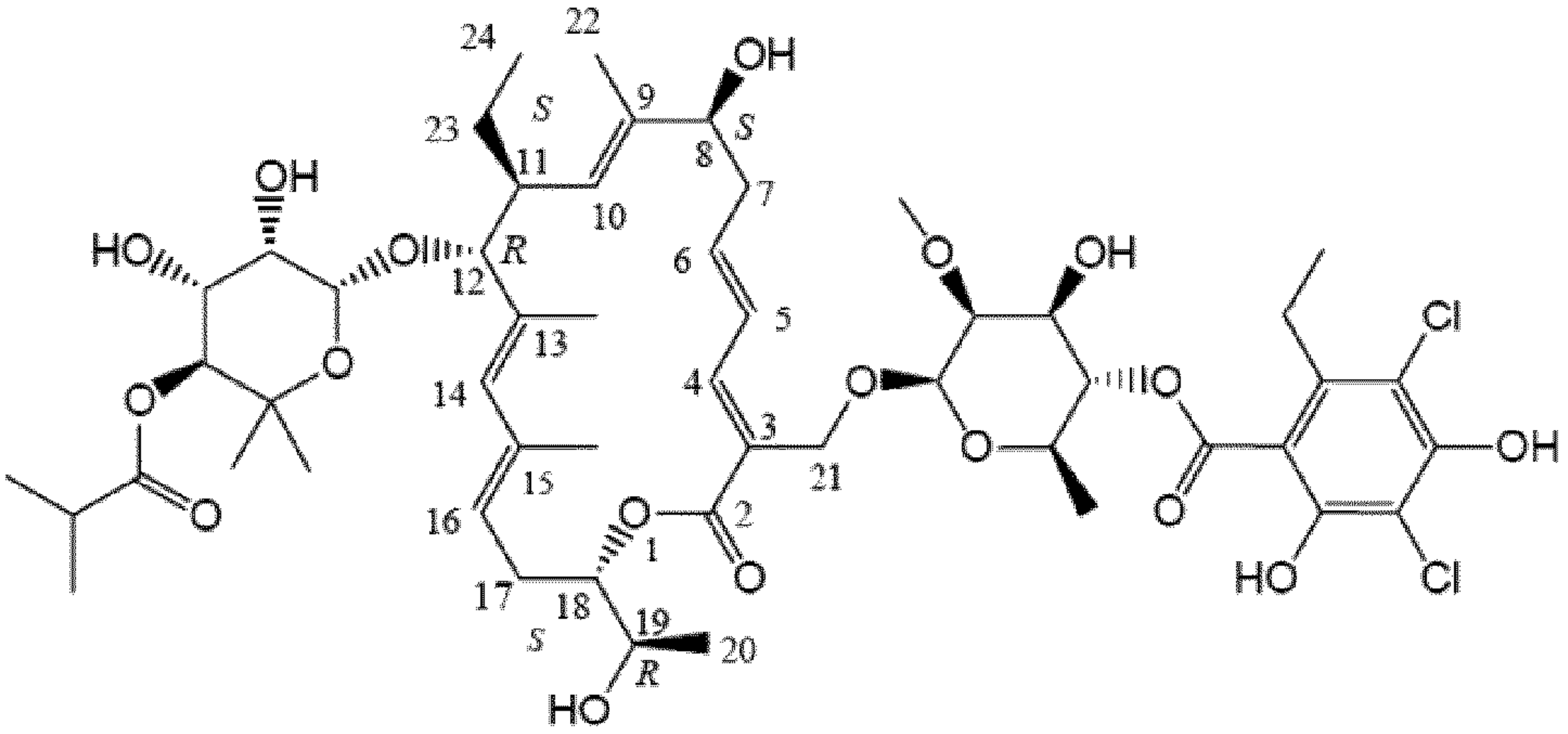
According to one embodiment, Tiacumicin B can be produced by fermentation of Dactylosporangium aurantiacum subspecies hamdenensis NRRL 18085 or Actinoplanes deccanensis ATCC 21983.

The term "polymorph" as used herein is meant to embrace crystalline solid states of a pure compound including solvates, co-crystals and crystals.

A "pure compound" as used herein is meant to cover compounds with a HPLC purity of at least 95%, or more preferred at least 97% and even more preferred at least 99%.

A "crystal solvate" as used herein is meant to cover polymorphs comprising a significant amount of a solvent relative to the pure compound. By "significant amount of a solvent relative to the pure compound" we mean a molar ratio of solvent : compound from 1:2 or more.

Tiacumicin B is meant to cover the compound represented by the following structure:



The term "XRPD" is meant to embrace any method using X-rays for obtaining a diffraction pattern of a solid state material. E.g. a method employing copper Kα radiation wavelength 1.54 Å. The methods used herein are described in more detail in the experimental part.

A "peak" as used herein concerning XRPD is a relatively sharp rise and fall of the signal between two 2θ values separated by a maximum of 2 degrees. Most peaks in the XRPDs herein have a relatively sharp rise and fall of the signal between two 2θ values separated by 1 degree or less.

A peak at a specific 2θ value is to be understood as a peak displaying the highest intensity at this value ± 0.1 degrees. A crystal solvate displaying peaks at 2θ values x, y and z ± 0.1 means that the pertaining XRPD comprises peaks at x ± 0.1, y ±0.1 and z ± 0.1.

Amorphous Tiacumicin B can be obtained from solutions comprising dissolved Tiacumicin B by a variety of methods well known to the skilled person. E.g adding in an anti-solvent followed by solvent removal, sudden cooling of a saturated solution, freeze drying/lyophilization of the solutions, spray drying of the solutions etc.

Solutions comprising dissolved Tiacumicin B can be obtained by dissolving Tiacumicin B of any solid state or by purification of a fermentation broth from Dactylosporangium aurantiacum subspecies hamdenensis NRRL 18085 or Actinoplanes deccanensis ATCC 21983.

A "high purity amorphous Tiacumicin material" is a material comprising 97% or more of Tiacumicin B as measured by HPLC and displays a XRPD without any peaks. It can be obtained as described in Example 1.

The high purity amorphous Tiacumicin material is hygroscopic with near linear water uptake (see Figure 7C), but it’s not deliquescent. The high purity amorphous Tiacumicin material is suitable for storage as demonstrated in example 7 or even longer-term storage. Thus the high purity amorphous Tiacumicin material is relatively stable with respect to crystallization and suitable for long-term storage (e.g storage for 3 months, 6 months, 9 months etc.).

A “crystal solvate of Tiacumicin B” is any crystal solvate comprising pure Ticaumicin B.

Pure Tiacumicin B as used herein is meant to cover Tiacumicin B with a HPLC purity of at least 95%, or more preferred at least 97% and even more preferred at least 99% by detection at 230 nm.

Tiacumicin B can be produced as disclosed in the prior art but is also commercially available (e.g. Bioaustralis Fine Chemicals). The crystalline Tiacumicin B starting material is also commercially available (e.g Brightgene Bio-MedicalTechnology).

Experimental data:

XRPD methods:
Stoe Stadi P; Mythen1K Detector; Cu-Kα1 radiation; standard measurement conditions: transmission; 40 kV and 40 mA tube power; curved Ge monochromator; 0.02°2θ step size, 12 s step time, 1.5-50.5°2θ scanning range; detector mode: step scan; 1°2θ detector step; standard sample preparation: 10 to 20 mg sample was placed between two acetate foils; sample holder: Stoe transmission sample holder; the sample was rotated during the measurement. Filenames of patterns recorded on this instrument consist of a three-digit number followed by a letter.
or
Bruker D8; Copper K_a radiation, 40 kV/ 40 mA; LynxEye detector, 0.02°2θ step size, 37 s step time.

Sample preparation:
The samples were generally measured without any special treatment other than the application of slight pressure to get a flat surface. Silicon single crystal sample holder types: a) standard holder for polymorphism screening, 0.1 mm deep, less than 20 mg sample required; b) 0.5 mm deep, 12 mm cavity diameter for c. 40 mg; c) 1.0 mm deep, 12 mm cavity diameter for c. 80 mg. All samples measured on the Bruker D8 are rotated during the measurement.

Example 1: Amorphous Tiacumicin B

Crystalline Tiacumicin B starting material (see XRPD in Figure 1A) with a HPLC purity of ca 99% and a water content of less than 1% w/w was dissolved in water/THF (5:1, v:v) and lyophilized. The lyophilized product was amorphous (see Figure 1B).

Example 2: Chlorobenzene crystal solvate of Tiacumicin B

Amorphous Tiacumicin B obtained in example 1 (69 mg) was mixed with 1.5 ml n-heptane/chlorobenzene 1:2 (v:v). The suspension was shaken at r.t. for 24 hours. Finally, 50% of the solvents were evaporated at r.t. under gentle N_2 flow (no flow control). The resulting solid was filtered off. The wet material thus obtained comprised 23% w/w chlorobenzene as measured by TG-FTIR. XRPD of the chlorobenzene crystal solvate of Tiacumicin B is shown in Figure 2A and 2B.

Example 3: n-propanol crystal solvate of Tiacumicin B

Amorphous Tiacumicin B obtained in example 1 (100mg) was mixed 2 ml n-propanol to form a slurry. The suspension was stirred at r.t. for 24 hours. Finally, the solvent was evaporated at r.t. under gentle N_2 flow. The wet material obtained comprised 4% w/w n-propanol as measured by TG-FTIR.
XRPD of the n-propanol crystal solvate of Tiacumicin B is shown in Figure 3A and 3B.

Example 4: iso-propanol crystal solvate of Tiacumicin B

Amorphous Tiacumicin B obtained in example 1 (73 mg) was mixed with 1.5 ml iso-propanol to form a slurry. The suspension was shaken at r.t. for 24 hours. Finally, 50% of the solvents were evaporated at r.t. under gentle N_2 flow. The slurry was then filtered and the wet material obtained comprised 17% w/w iso-propanol as measured by TG-FTIR. The isopropanol was lost in two steps; first 11% w/w and then 6% w/w.
XRPD of the iso-propanol crystal solvate of Tiacumicin B is shown in Figure 4A and 4B.

Example 5: methyl-ethyl-ketone (MEK) crystal solvate of Tiacumicin B

Amorphous Tiacumicin B obtained in example 1 (71 mg) was mixed with 1.5 ml n-heptane/MEK 1:2 (v:v). The suspension was shaken at r.t. for 24 hours. Finally, 50% of the solvents were evaporated at r.t. under gentle N_2 flow (no flow control). The resulting solid was filtered off. The wet material obtained comprised 6% iso-propanol as measured by TG-FTIR. XRPD of the methyl-ethyl-ketone crystal solvate of Tiacumicin B is shown in Figure 5A and 5B.

Example 6: Acetic acid crystal solvate of Tiacumicin B

Crystalline Tiacumicin B starting material (100 mg) with a HPLC purity of ca 99% and a water content of less than 1% w/w was mixed with 2 ml water/acetic acid

(1:1, v:v) to form a slurry. The suspension was stirred at r.t. for 48 hours. Finally, the solvents were evaporated at r.t. under gentle N_2 flow. The obtained material comprised 6% w/w acetic acid as measured by TG-FTIR. XRPD of the acetic acid crystal solvate of Tiacumicin B is shown in Figure 6A and 6B.

Example 7: Stability of high purity amorphous Tiacumicin material
Several samples of high purity amorphous Tiacumicin B material were stored at 40°C/75% relative humidity. XRPDs obtained at start , 24 hours, 7 days and 1 month show amorphous material (se Figure 7A).

Several samples of high purity amorphous Tiacumicin B material were stored at room temperature under normal light conditions. XRPDs obtained at start, 1 month and 2 months show amorphous material (se Figure 7B). XRPDs of high purity amorphous Tiacumicin B material also show amorphous material at 3 months.

Example 8: Crystal solvate of Tiacumicin B (form α)
Crystalline Tiacumicin B starting material (220 mg) with a HPLC purity of ca 99% and a water content of less than 1% w/w was mixed with 4.5 ml water/acetic acid (1:1, v:v) to form a slurry. The suspension was stirred at r.t. for 96 hours. Finally, the resulting solid was filtered off and dried in air (5 min).
The obtained material comprised 16% w/w acetic acid as measured by TG-FTIR. XRPD of the storage suitable form α of Tiacumicin B is shown in Figure 8A. XRPD of the storage suitable form α of Tiacumicin B with peaks assigned with 2θ values is shown in Figure 8B. XRPD of the storage suitable form α of Tiacumicin B in table format of is shown in Figure 8C.

Example 9: Crystal solvate of Tiacumicin B (form β)
Crystalline Tiacumicin B starting material (100 mg) with a HPLC purity of ca 99% and a water content of less than 1% w/w was mixed with isopropyl-acetate (1:1, v:v) to form a slurry. The suspension was stirred at r.t. for 48 hours. Finally, the solvents were evaporated at r.t. under gentle N_2 flow. Crystalline Tiacumicin B starting material (220 mg) with a HPLC purity of ca 99% and a water content of less than 1% w/w was mixed with 3 ml isopropyl acetate (most solid dissolved) and 3 ml n-heptane to form a slurry. The suspension was stirred at r.t. for 96 hours. Finally, the resulting solid was filtered off and dried in air (5 min).
The obtained material comprised 9% w/w isopropylacetate as measured by TG-FTIR. XRPD of the storage suitable form β of Tiacumicin B is shown in Figure 9A. XRPD of the storage suitable form β of Tiacumicin B with peaks assigned with 2θ values is shown in Figure 9B. XRPD of the storage suitable form β of Tiacumicin B in table format of is shown in Figure 9C.

CLAIMS

1.
A crystal solvate of Tiacumicin B selected from the propanol crystal solvate, the acetic acid crystal solvate, the chlorobenzene crystal solvate, the methyl-ethyl-ketone crystal solvate, the isopropyl-acetate solvate or the isopropanol crystal solvate.

2.
A crystal solvate according to claim 1 characterized by a XRPD displaying a peak at diffraction angle 2θ of 19.9 ± 0.1

3.
A crystal solvate according to claim 1 or 2 selected from the n-propanol crystal solvate, the acetic acid crystal solvate, the methyl-ethyl-ketone crystal solvate, the isopropyl-acetate or the isopropanol crystal solvate characterized by a XRPD displaying peaks at diffraction angle 2θ of 3.3 and 19.9 ± 0.1

4.
A crystal solvate according to claim 3 selected from the propanol crystal solvate, the acetic acid crystal solvate or the methyl-ethyl-ketone crystal solvate.

5.
A crystal solvate according to claim 1 which is the propanol crystal solvate characterized by a XRPD displaying peaks at diffraction angles 2θ of 3.3, 7.5, 7.7, 18.8 and 19.9 ± 0.1

6.
A crystal solvate according to claim 1 which is the acetic acid crystal solvate characterized by a XRPD displaying peaks at diffraction angles 2θ of 6.7, 7.6, 18.7 and 19.9 ± 0.1

7.
A crystal solvate according to claim 1 which is the chlorobenzene crystal solvate characterized by a XRPD displaying peaks at diffraction angles 2θ of 6.7, 18.8 and 19.9 ± 0.1

8.
A crystal solvate according to claim 1 which is the methyl-ethyl-ketone crystal solvate characterized by a XRPD displaying peaks at diffraction angles 2θ of 3.3, 7.5, 15.7 and 18.6 ± 0.1

9.
A crystal solvate according to claim 1 which is the isopropanol crystal solvate characterized by a XRPD displaying peaks at diffraction angles 2θ of 6.5, 9.9, 18.6 and 19.8 ± 0.1

10.
A crystal solvate of Tiacumicin B suitable for storage, characterized by a XRPD displaying peaks at diffraction angles 2θ of 16.6 and 19.9 ± 0.1

11.
A crystal solvate of Tiacumicin B suitable for storage, characterized by a XRPD displaying peaks at diffraction angles 2θ of 16.6, 18.8 and 19.9 ± 0.1

12.
A high purity amorphous Tiacumicin B material.

13.
A material according to claim 12, comprising more than 98% Tiacumicin B as measured by HPLC, and comprising less than 2% w/w water.

14.
A material according to claim 12 or 13 having Tg of ca 113 °C as measured by DSC.

15.
A material according to claim 12, 13 or 14 comprising less than 5% w/w water.

16.
A material according to claim 12, 13 or 14 comprising less than 2% w/w water.

17.
A material according to claim 12, 13 or 14 comprising less than 0.5% w/w water.

18.
A high purity amorphous Tiacumicin B material according to any of claims 12-17, which is undeliquescent and suitable for storage.

19.

A crystal solvate of Tiacumicin B characterized by a XRPD displaying a peak at diffraction angle 2θ of 3.3, 9.9 and 18.6 ± 0.1 or substantially as shown in Figure 11.

XRPD of the starting material:

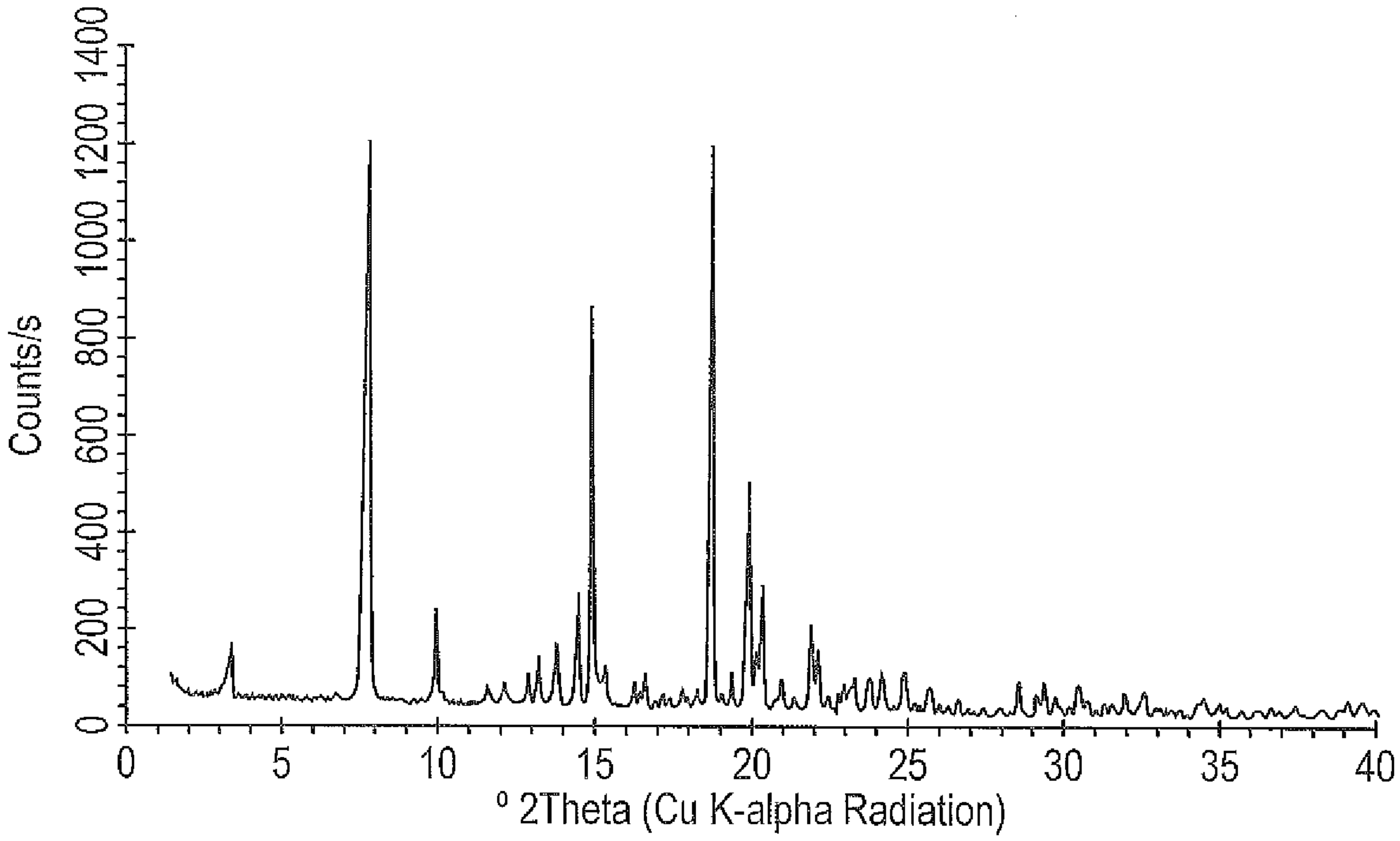


FIG. 1A

XRPD of amorphous material:

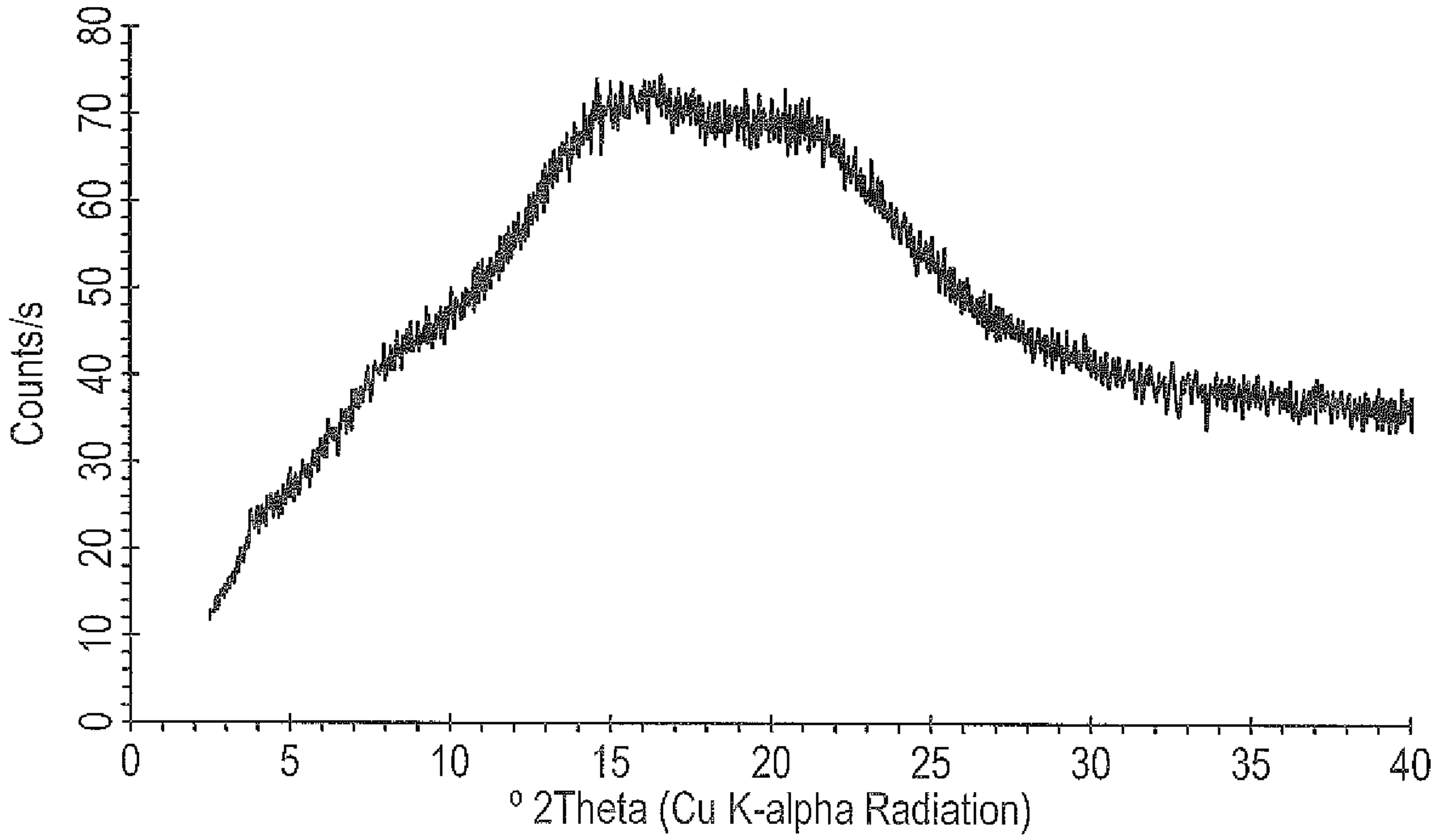


FIG. 1B

XRPD of the chlorobenzene crystal solvate:

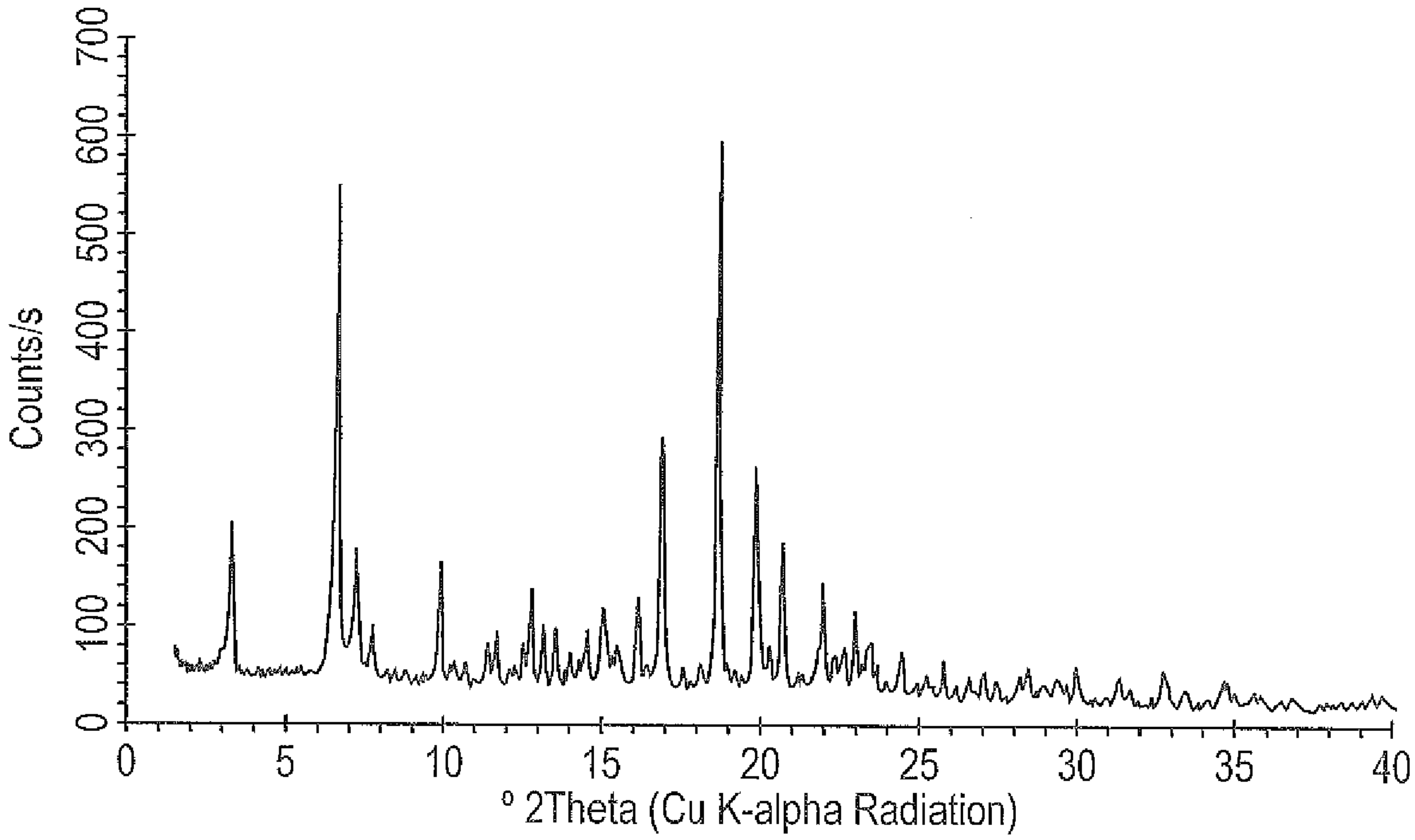


FIG. 2A

XRPD of the chlorobenzene crystal solvate in table format.

Angle [°2Θ]	d value [Å]	Intensity [Cps]	Intensity %
3.32	26.6	s	34.2
6.66	13.3	vs	91.5
7.27	12.1	m	29.9
7.78	11.4	m	17.1
9.94	8.9	m	27.9
10.75	8.2	w	10.9
11.48	7.7	w	14.0
11.73	7.5	m	18.4
12.61	7.0	w	14.1
12.85	6.9	m	23.4
13.25	6.7	m	17.3
13.65	6.5	m	16.4
14.13	6.3	w	12.5
14.65	6.0	m	16.5
15.10	5.86	m	20.3
15.58	5.68	w	13.6
16.23	5.46	m	21.9
16.97	5.22	s	49.1
17.59	5.04	w	10.1
18.20	4.87	w	10.7
18.77	4.72	vs	100.0
19.93	4.45	s	44.4
20.34	4.36	w	13.8
20.77	4.27	s	31.3
22.03	4.03	m	24.5
22.41	3.96	w	12.4
22.67	3.92	w	13.5
23.05	3.86	m	19.9
23.55	3.77	w	14.5
24.50	3.63	w	12.9
25.29	3.52	w	8.8
25.83	3.45	w	11.3
26.63	3.34	w	8.8
27.09	3.29	w	9.0
27.51	3.24	w	8.0
28.51	3.13	w	10.3
30.07	2.97	w	10.1
31.43	2.84	w	8 6
31.75	2.82	w	7.0
32.79	2.73	w	9.8
33.43	2.68	w	6.3

FIG. 2B

XRPD of the n-propanol crystal solvate:

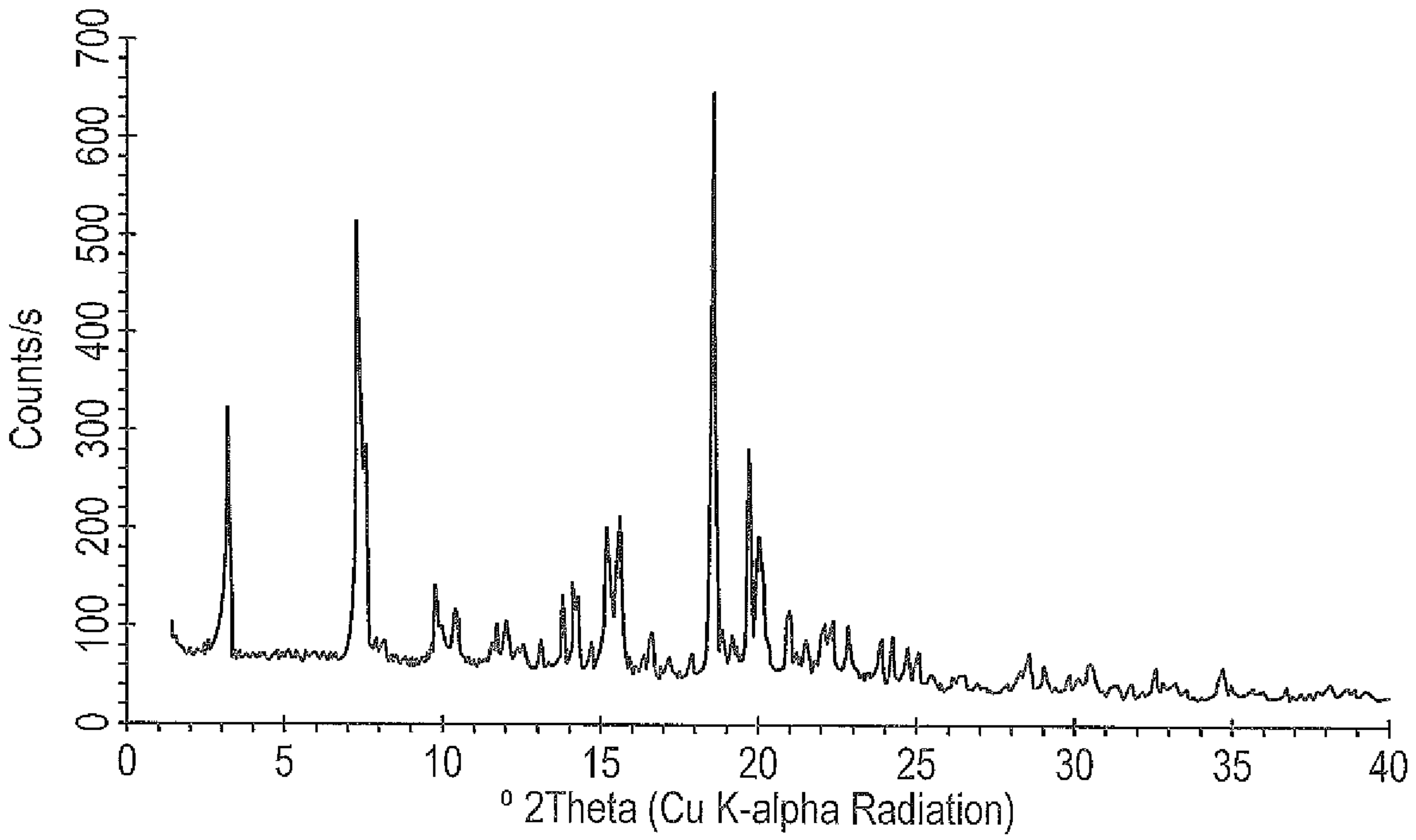


FIG. 3A

XRPD of the n-propanol crystal solvate in table format:

Angle [°2Θ]	d value [Å]	Intensity [Cps]	Intensity %
3.32	26.6	s	49.6
7.51	11.8	vs	79.2
7.70	11.5	s	43.7
9.94	8.9	m	21.3
10.54	8.4	m	17.8
10.64	8.3	m	23.1
11.93	7.4	m	15.4
12.17	7.3	m	20.0
13.26	6.7	w	12.9
13.97	6.3	m	20.3
14.30	6.2	m	26.2
14.44	6.1	m	20.1
14.87	5.95	w	12.5
15.39	5.75	s	30.7
15.79	5.61	s	32.4
16.52	5.36	w	10.6
16.80	5.27	m	15.4
17.33	5.11	w	10.2
18.76	4.73	vs	100.0
19.04	4.66	w	14.4
19.38	4.58	w	13.9
19.91	4.46	s	43.2
20.21	4.39	m	29.4
21.13	4.20	m	17.2
21.71	4.09	w	12.8
22.52	3.94	m	15.9
23.00	3.86	m	15.1
24.05	3.70	w	13.1
24.40	3.65	w	13.6
24.89	3.57	w	11.5
25.23	3.53	w	10.7
26.65	3.34	w	7.7
28.71	3.11	w	10.9
29.23	3.05	w	8.8
30.04	2.97	w	7.5
30.66	2.91	w	9.1
32.74	2.73	w	8.6
34.83	2.57	w	8.3

FIG. 3B

XRPD of the iso-propanol crystal solvate:

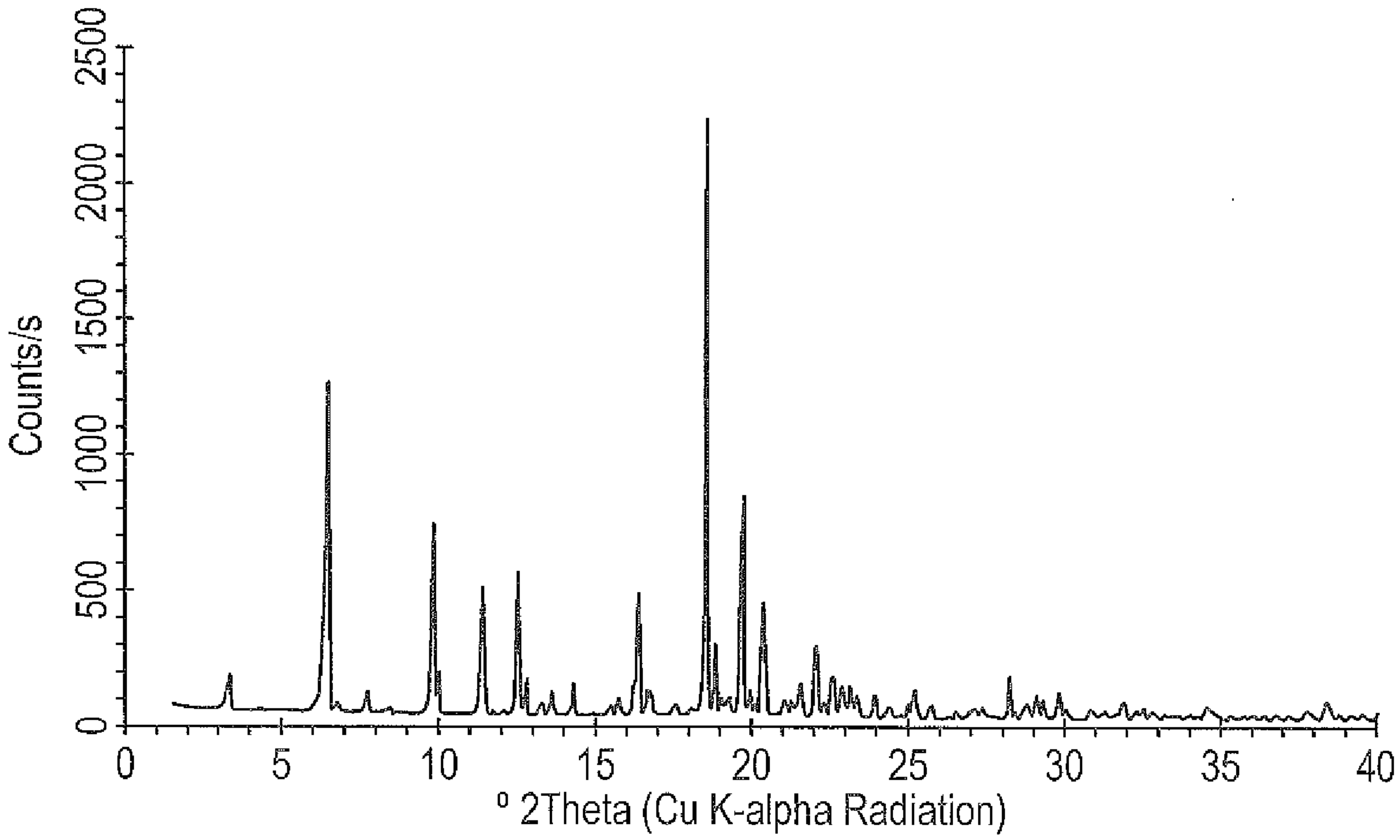


FIG. 4A

XRPD of the iso-propanol crystal solvate in table format:

Angle	d value	Intensity	Intensity %
2-Theta°	Angstrom	Cps	%
3.35	26.4	w	8.1
6.49	13.6	s	56.1
7.78	11.4	w	5.5
9.88	8.9	s	32.9
10.04	8.8	w	8.5
11.46	7.7	m	22.4
12.59	7.0	m	25.1
12.83	6.9	w	8.0
13.36	6.6	vw	3.5
13.66	6.5	w	5.5
14.39	6.1	w	6.4
15.57	5.69	vw	3.3
15.86	5.58	vw	4.9
16.45	5.39	m	21.7
16.78	5.28	w	5.8
17.66	5.02	vw	3.7
18.64	4.76	vs	100.0
18.94	4.68	w	13.2
19.38	4.58	vw	4.6
19.79	4.48	s	37.5
20.04	4.43	w	5.7
20.50	4.33	m	19.8
21.15	4.20	vw	4.2
21.67	4.10	w	6.6
22.15	4.01	w	13.0
22.68	3.92	w	8.1
22.99	3.87	w	6.3
23.28	3.82	w	6.3
23.45	3.79	vw	4.9
24.03	3.70	vw	4.9
24.54	3.62	vw	3.2
25.29	3.52	w	5.6
25.81	3.45	vw	3.3
28.31	3.15	w	7.6
28.91	3.09	vw	3.4
29.21	3.06	vw	4.7
29.41	3.03	vw	3.9
29.91	2.98	w	5.2
30.93	2.89	vw	2.6
31.98	2.80	vw	3.8
32.63	2.74	vw	2.9
34.61	2.59	vw	3.0
37.80	2.38	vw	2.3
38.40	2.34	vw	4.0

FIG. 4B

XRPD of the MEK crystal solvate:

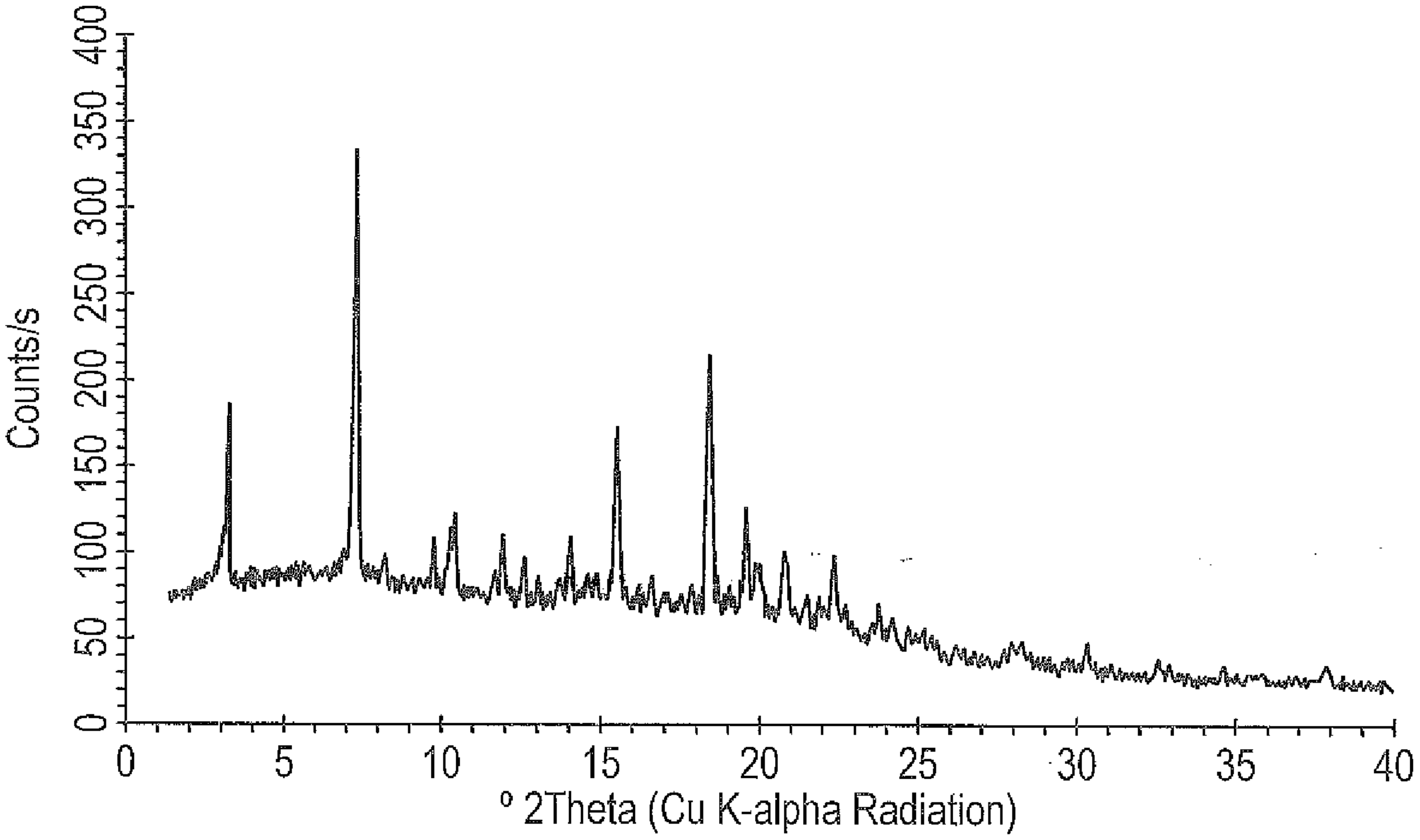


FIG. 5A

XRPD of the MEK crystal solvate in table format:

Angle [°2Θ]	d value [Å]	Intensity [Cps]	Intensity %
3.32	26.6	s	55.6
7.48	11.8	vs	100.0
8.38	10.5	m	29.2
9.94	8.9	s	32.0
10.48	8.4	s	34.0
10.62	8.3	s	38.8
11.89	7.4	m	26.7
12.13	7.3	s	32.9
12.81	6.9	m	28.6
13.23	6.7	m	25.2
14.29	6.2	s	32.5
15.72	5.63	s	51.9
16.81	5.27	m	25.5
18.06	4.91	m	24.1
18.61	4.76	s	64.2
18.88	4.70	m	25.7
19.75	4.49	s	37.7
20.07	4.42	m	29.0
21.00	4.23	m	29.9
21.71	4.09	m	21.9
22.12	4.01	m	22.0
22.55	3.94	m	29.3
22.92	3.88	m	20.7
23.99	3.71	m	20.8
24.34	3.65	m	18.6
30.50	2.93	w	13.8
34.80	2.58	w	10.5

FIG. 5B

XRPD of the acetic acid crystal solvate:

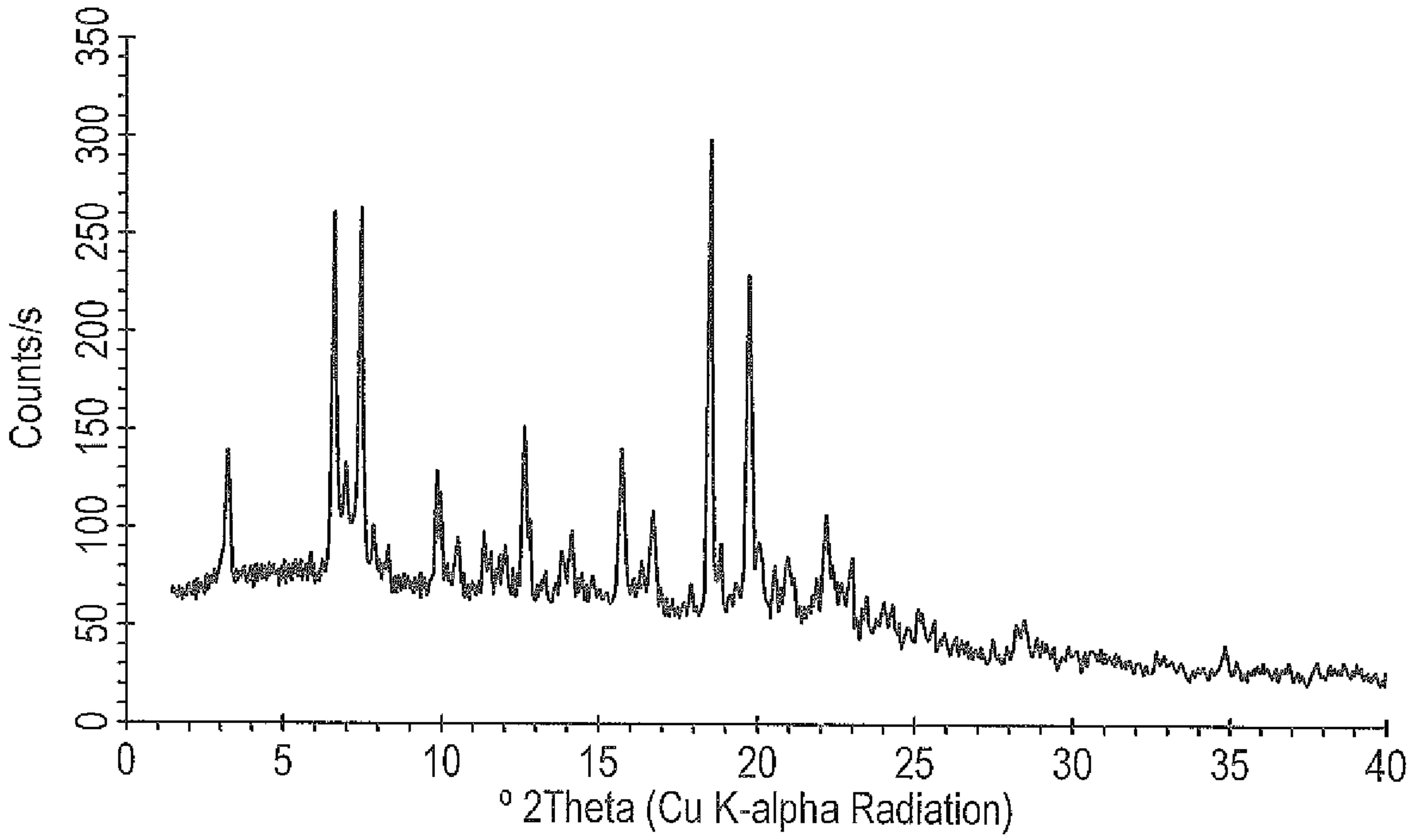


FIG. 6A

XRPD of the acetic acid crystal solvate in table format:

Angle [°2Θ]	d value [Å]	Intensity [Cps]	Intensity %
3.32	26.6	s	46.1
6.71	13.2	vs	87.1
7.07	12.5	s	44.3
7.57	11.7	vs	88.1
7.96	11.1	s	33.6
9.93	8.9	s	46.9
10.09	8.8	s	38.9
10.59	8.3	s	30.1
11.52	7.7	s	36.8
11.66	7.6	m	28.5
12.15	7.3	m	29.9
12.80	6.9	s	50.5
13.47	6.6	m	25.8
14.04	6.3	m	28.7
14.30	6.2	s	32.5
15.92	5.56	s	46.9
16.53	5.36	m	27.4
16.88	5.25	s	35.8
18.11	4.90	m	23.6
18.69	4.74	vs	100.0
19.01	4.67	s	30.4
19.89	4.46	vs	76.8
20.19	4.40	s	30.7
20.69	4.29	m	26.6
21.12	4.20	m	27.8
22.02	4.03	m	24.7
22.35	3.97	s	35.4
23.12	3.84	m	28.2
23.62	3.76	m	21.7
24.21	3.67	m	20.3
24.44	3.64	m	19.9
25.32	3.52	m	18.8
27.65	3.22	w	14.0
28.62	3.12	m	17.5

FIG. 6B

XRPD of high purity amorphous Tiacumicin material:

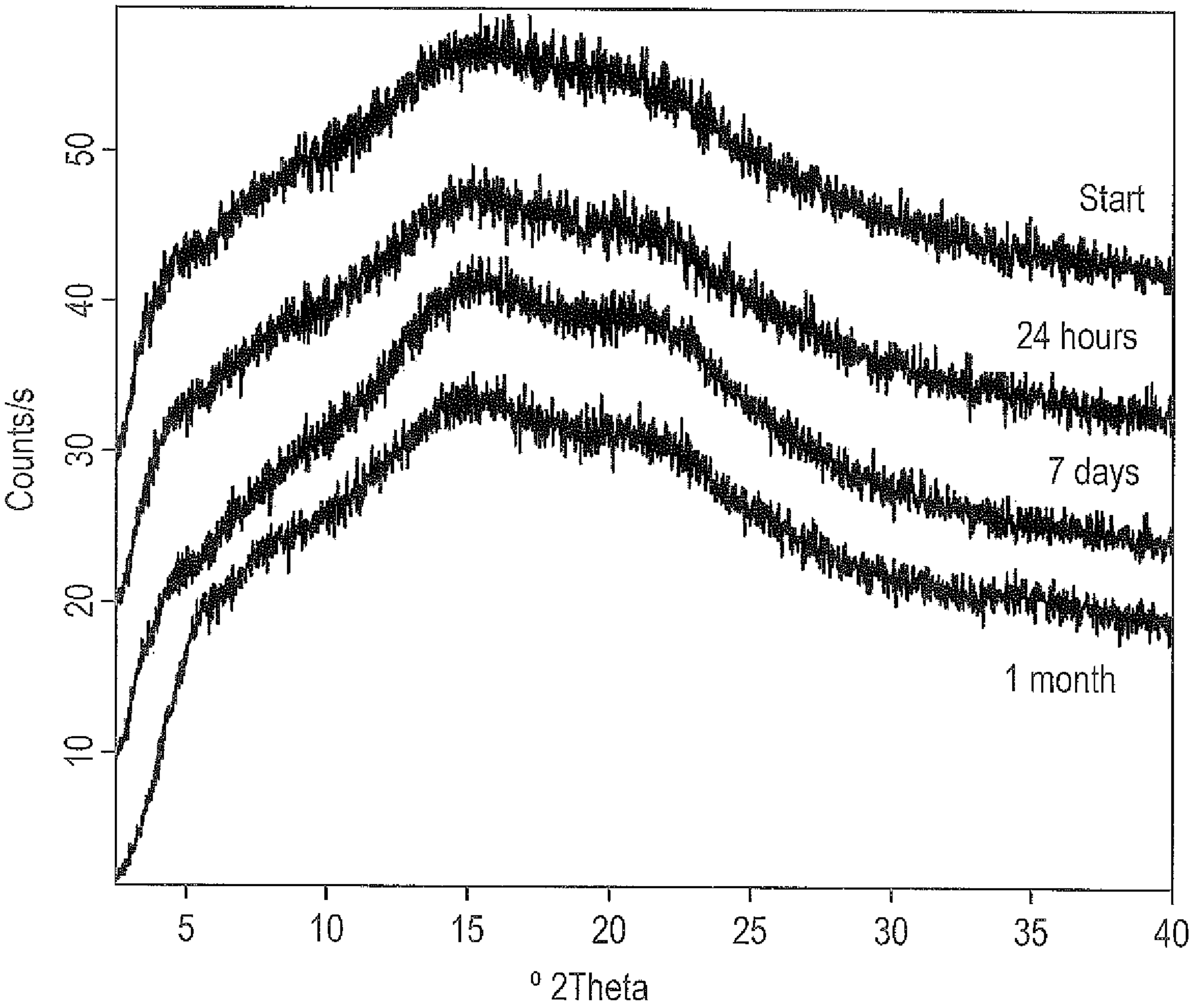


FIG. 7A

XRPD of high purity amorphous Tiacumicin material:

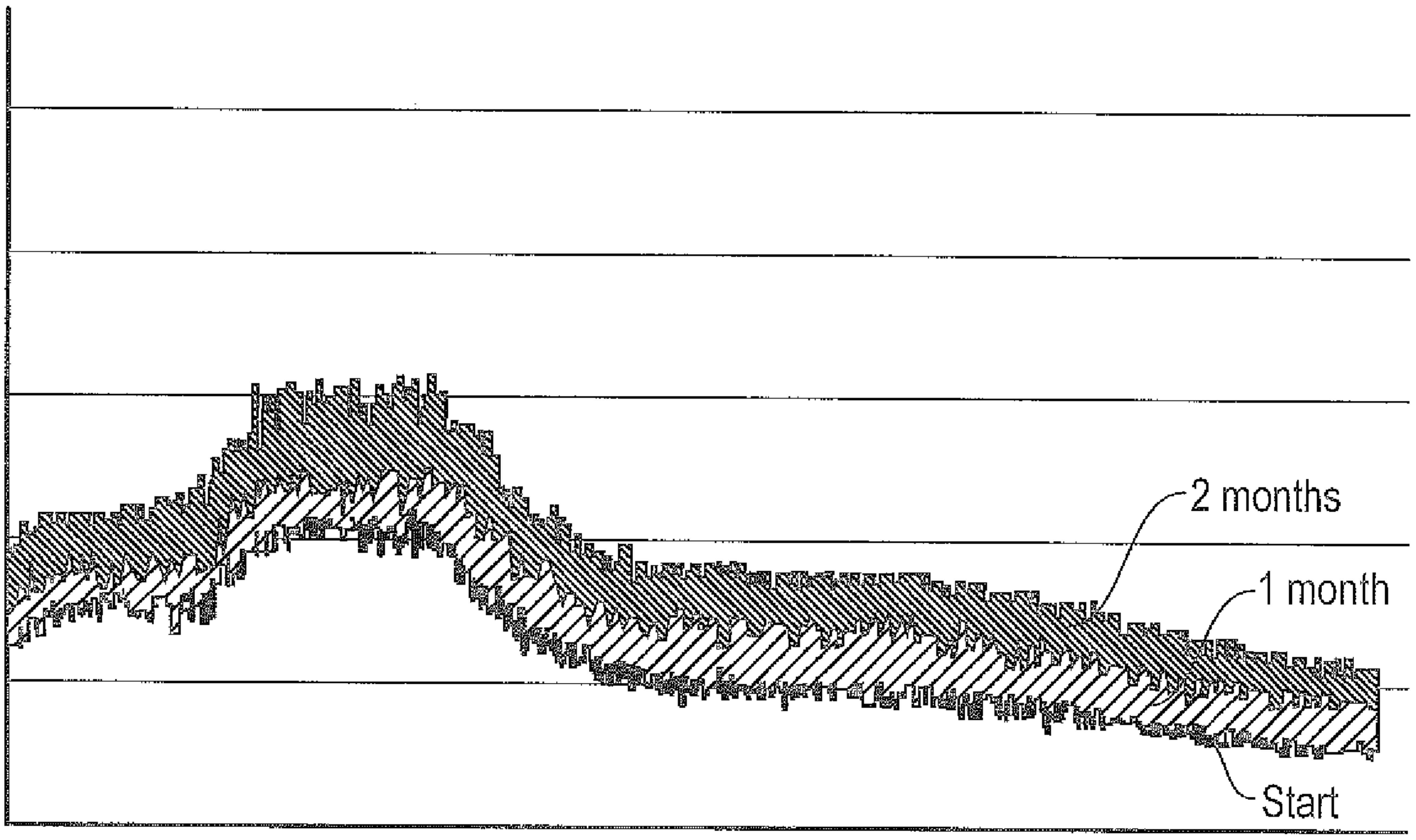


FIG. 7B

Glass transition temperature of high purity amorphous Tiacumicin material by using Fox Equation:

%H2O	Tg (°C)	Tg(K)	RH(%)
0.0%	113	386	0%
0.5%	108	381.4	6%
1.0%	104	376.9	13%
1.5%	100	372.5	19%
2.0%	95	368.2	26%
2.5%	91	364.0	32%
3.0%	87	359.9	39%
3.5%	83	355.9	45%
4.0%	79	352.0	51%
4.5%	75	348.2	58%
5.0%	71	344.4	64%
5.5%	68	340.7	71%
6.0%	64	337.1	77%
6.5%	61	333.6	84%
7.0%	57	330.2	90%
7.5%	54	326.8	96%
8:0%	50	323.5	103%

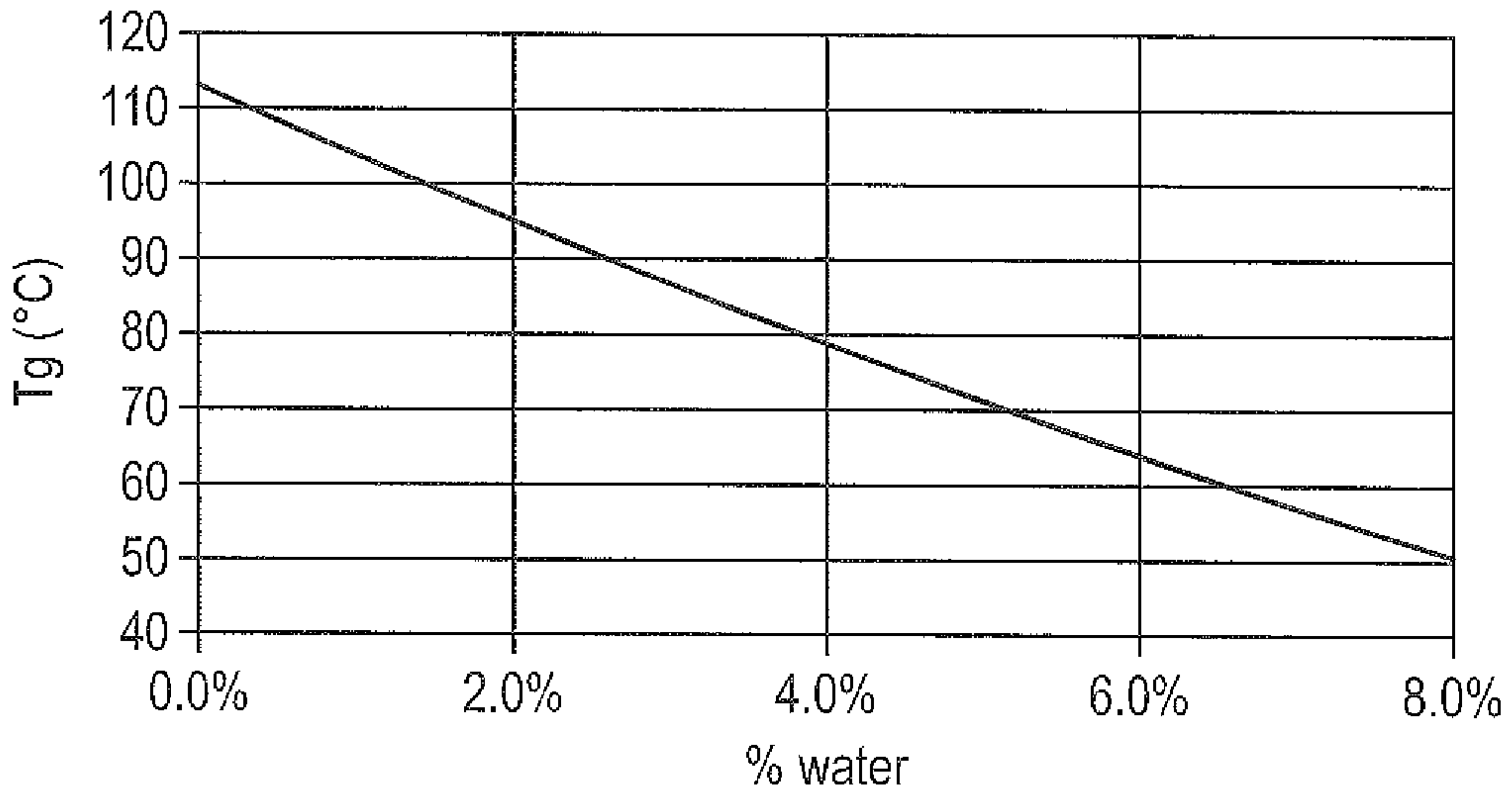


FIG. 7C

XRPD of the Tiacumicin solvate form α:

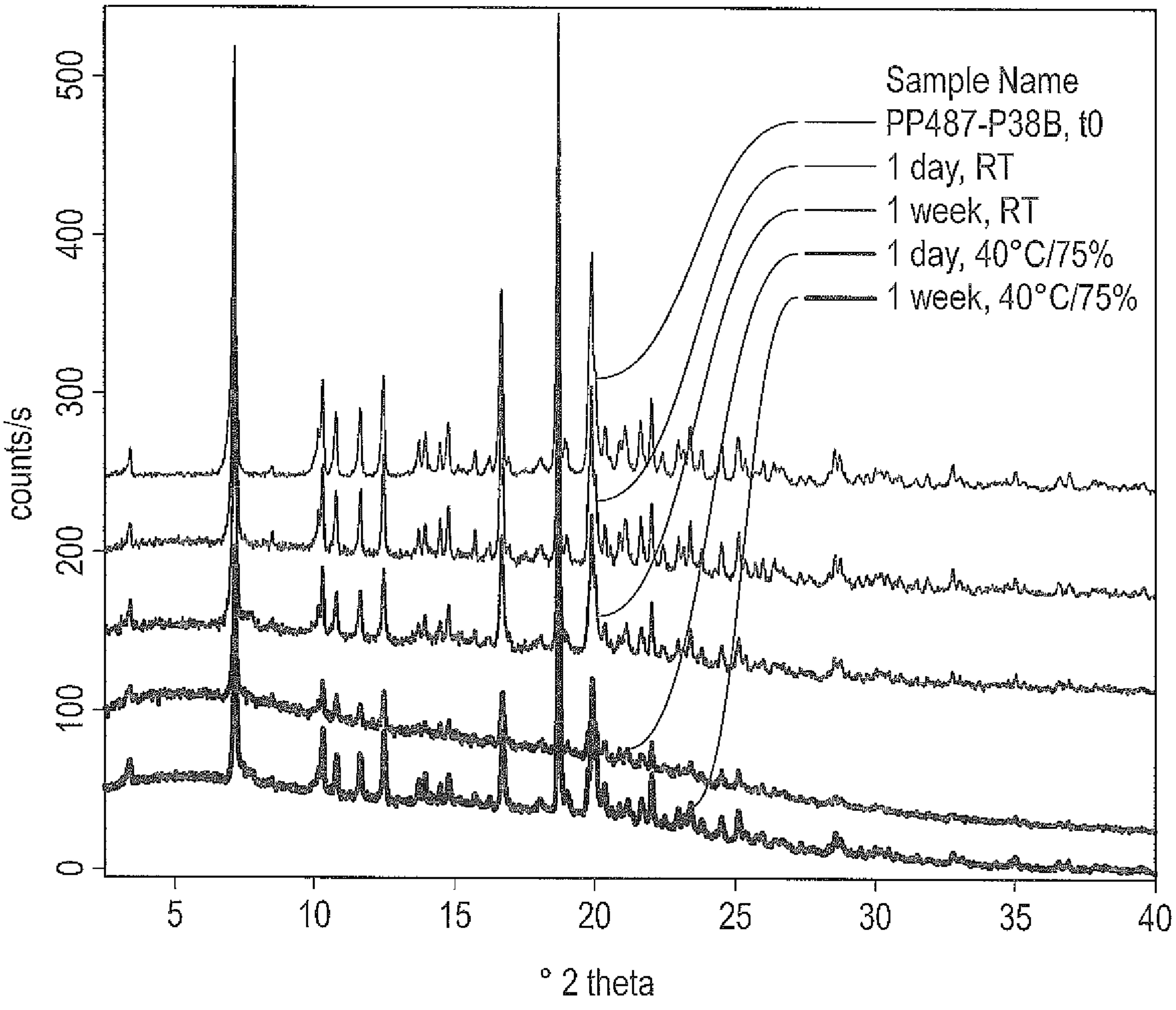
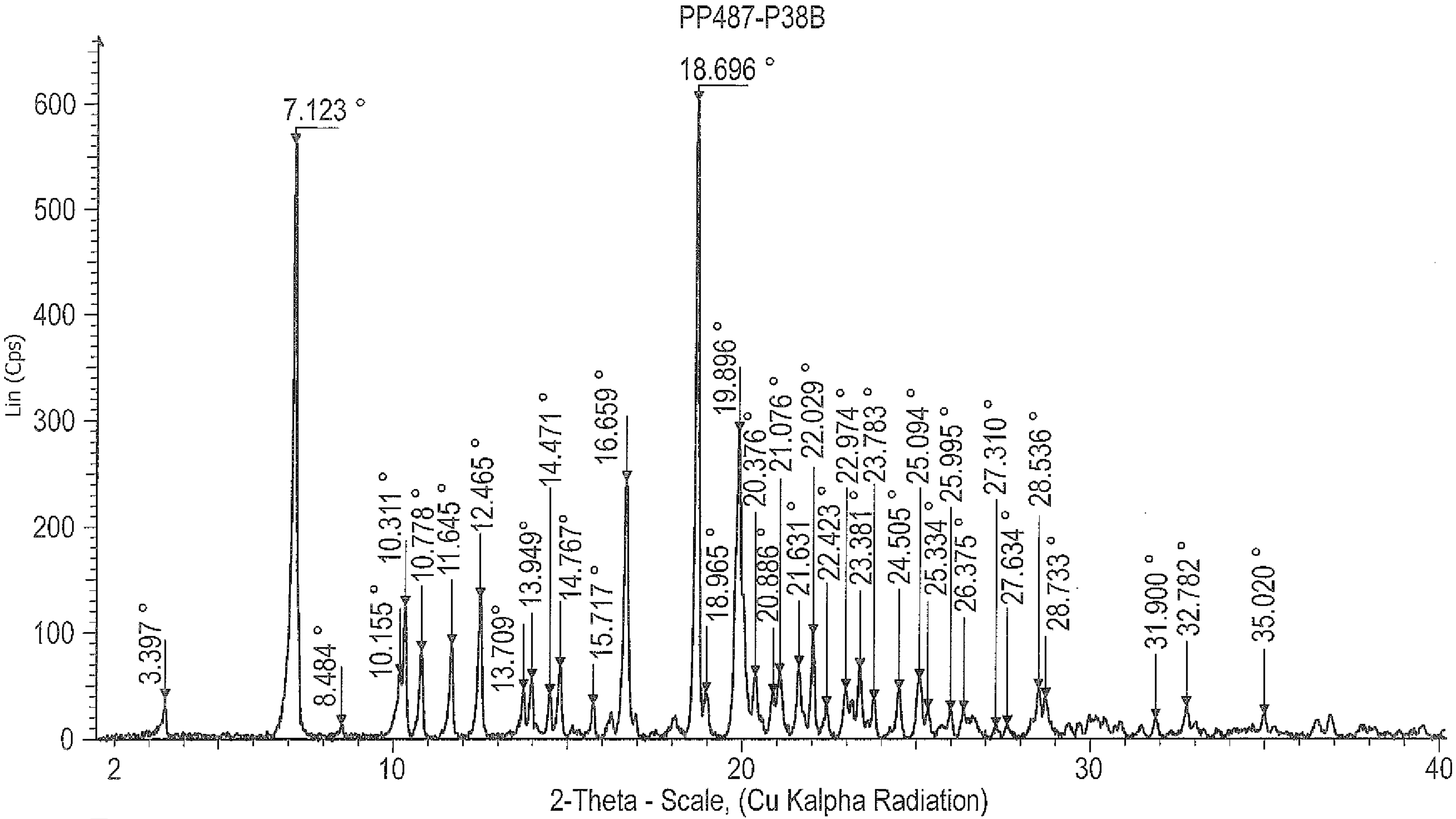


FIG. 8A

XRPD of the Tiacumicin solvate form α with peaks assigned with 2θ values:



File: 528e_n14-11432_pp487.raw - PP487-P38B - Step: 0.020 ° - Step time: 12. s - Creation: 03.06.2014 11:51:00
Operations: Background 1.000,1.000 | Import

FIG. 8B

XRPD in table format of Tiacumicin solvate form α.

Angle 2-Theta °	d value Angstrom	Intensity rel	Intensity % %
3,40	26,0	w	6,2
7,12	12,4	vs	93,3
8,48	10,4	vw	2,0
10,16	8,7	w	10,0
10,31	8,6	m	20,7
10,78	8,2	w	13,6
11,65	7,6	w	14,6
12,47	7,1	m	21,9
13,71	6,5	w	7,6
13,95	6,3	w	9,2
14,47	6,1	w	6,8
14,77	5,99	w	11,1
15,72	5,63	w	5,2
16,66	5,32	s	40,3
18,70	4,74	vs	100,0
18,97	4,68	w	7,2
19,90	4,46	s	47,9
20,38	4,36	w	9,7
20,89	4,25	w	6,9
21,08	4,21	w	10,1
21,63	4,11	w	11,3
22,03	4,03	m	16,1
22,42	3,96	vw	4,9
22,97	3,87	w	7,7
23,38	3,80	w	10,9
23,78	3,74	w	5,9
24,51	3,63	w	7,5
25,09	3,55	w	9,2
25,33	3,51	vw	4,4
26,00	3,42	vw	4,2
26,38	3,38	vw	4,2
27,31	3,26	vw	1,6
27,63	3,23	vw	1,9
28,54	3,13	w	7,6
28,73	3,10	w	6,2
31,90	2,80	vw	2,9
32,78	2,73	vw	4,9
35,02	2,56	vw	3,6

FIG. 8C

XRPD of the Tiacumicin solvate form β during storage:

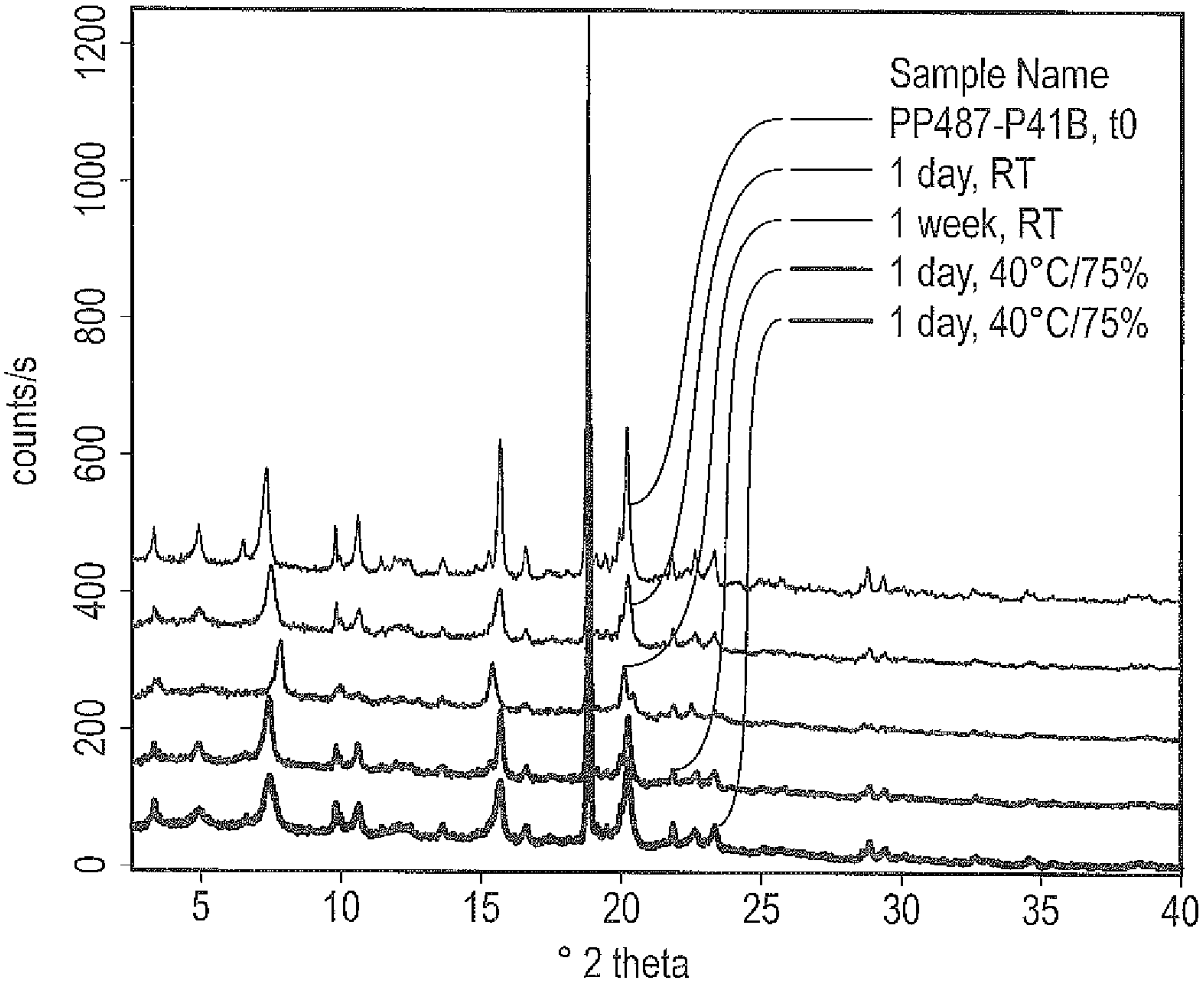
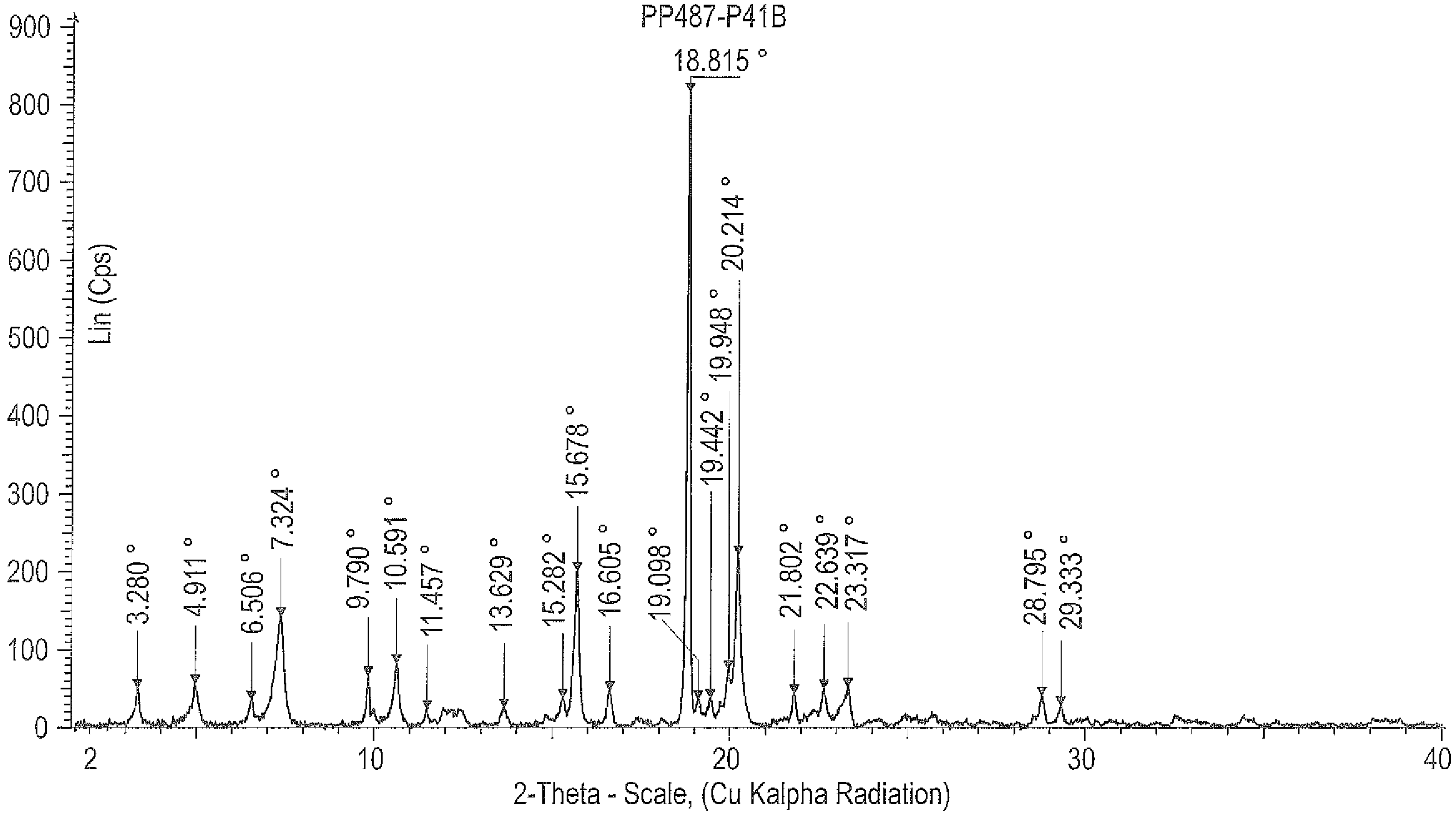


FIG. 9A

XRPD of the Tiacumicin solvate form β with peaks assigned with 2θ values:



▩ File: 531e_n14-11432_pp487.raw - PP487-P41B -Step: 0.020 ° - Step time: 12. s - Creation: 03.06.2014 12:33:00
Operations: Background 1.000,1.000 | Import

FIG. 9B

XRPD in table format of Tiacumicin solvate form β.

Angle 2-Theta °	d value Angstrom	Intensity rel.	Intensity % %
3,28	26,9	w	5,9
4,91	18,0	w	6,6
6,51	13,6	vw	4,1
7,32	12,1	m	17,2
9,79	9,0	w	7,9
10,59	8,3	w	9,8
11,46	7,7	vw	2,6
13,63	6,5	vw	2,8
15,28	5,79	vw	4,3
15,68	5,65	m	24,4
16,61	5,33	w	5,5
18,82	4,71	vs	100,0
19,10	4,64	vw	4,1
19,44	4,56	vw	4,3
19,95	4,45	w	8,8
20,21	4,39	m	26,9
21,80	4,07	w	5,0
22,64	3,92	w	5,8
23,32	3,81	w	6,1
28,80	3,10	vw	4,7
29,33	3,04	vw	3,2

FIG. 9C

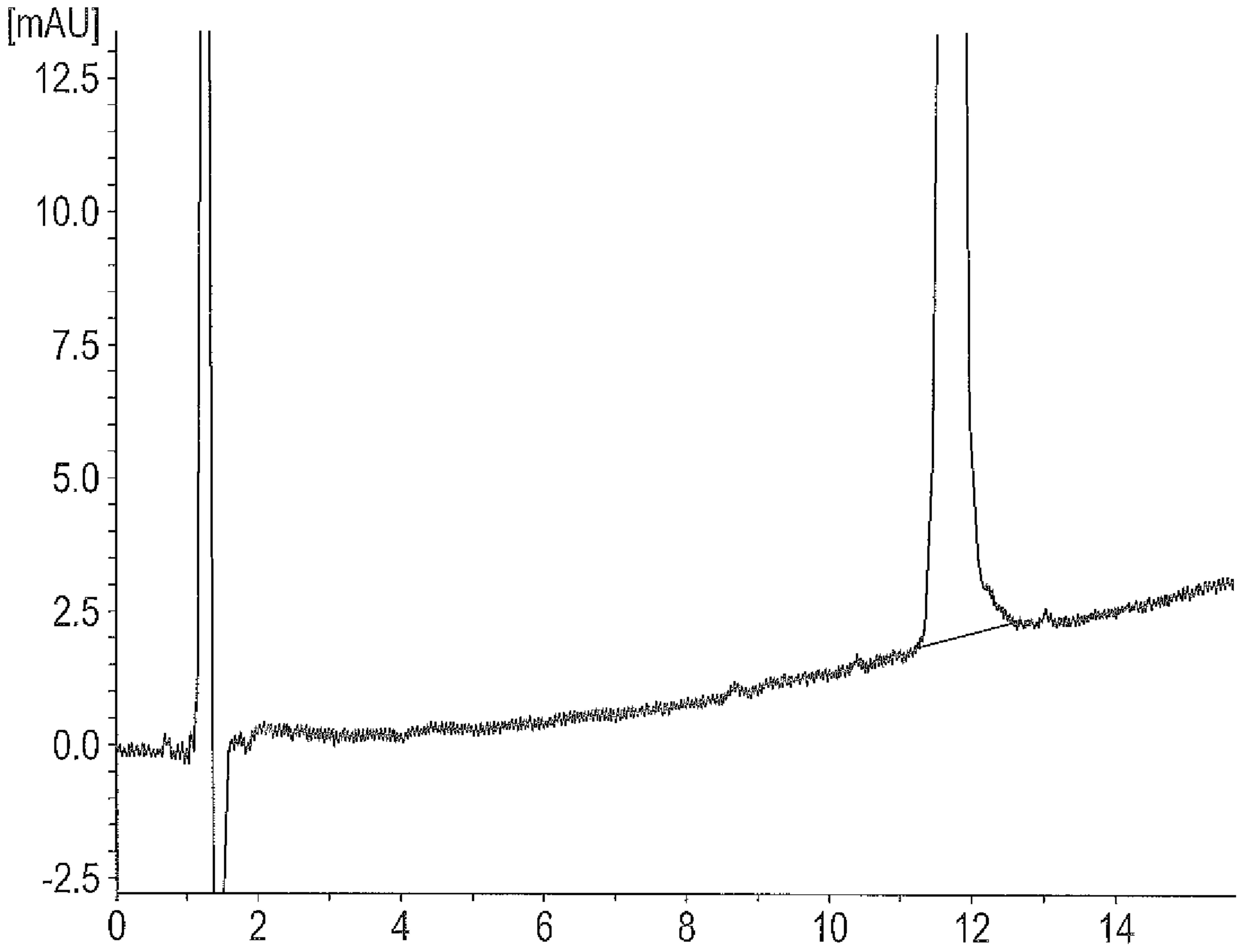


FIG. 10

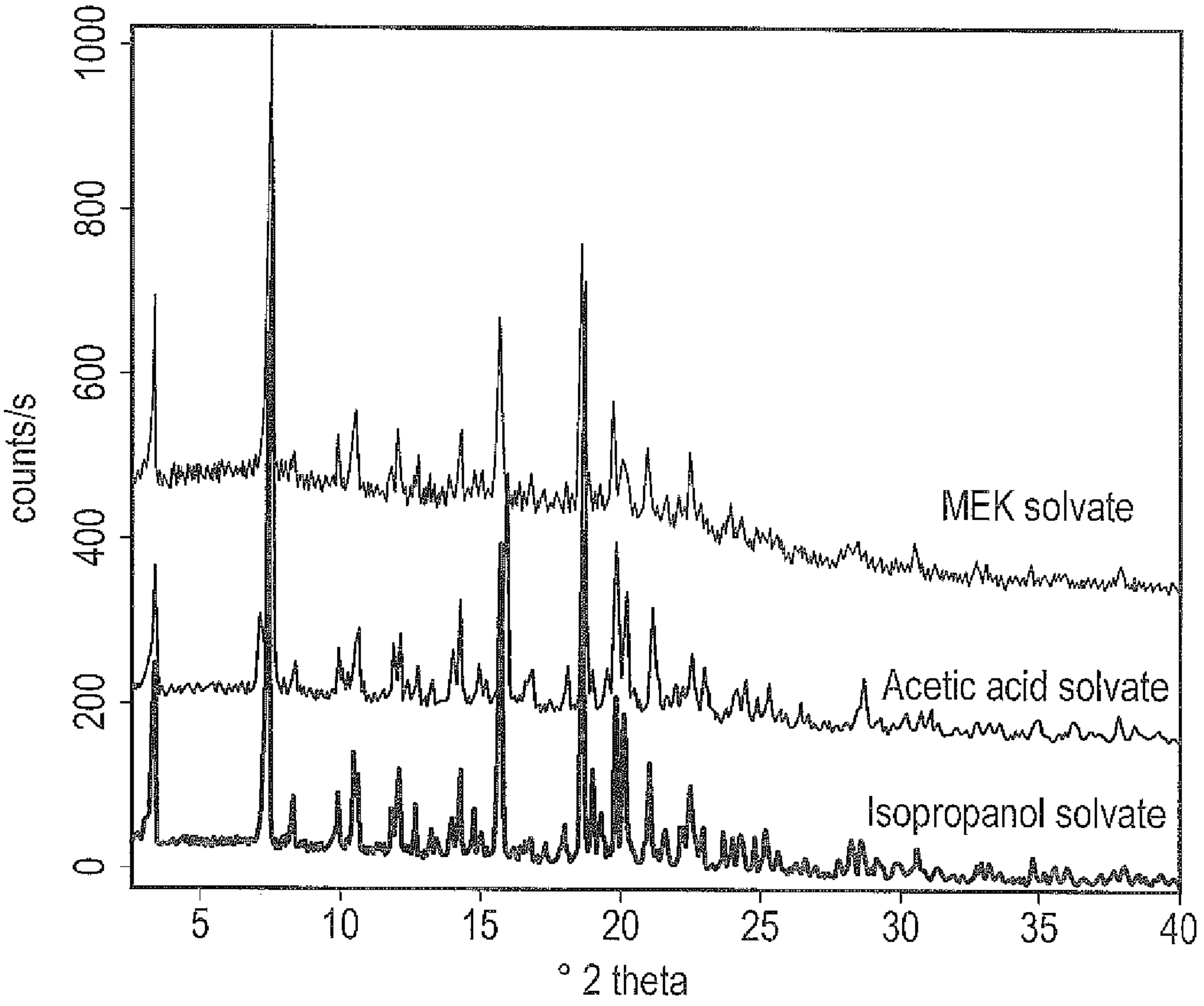

1000
800
600
400
200
0
counts/s
5
10
15
20
25
30
35
40
° 2 theta
MEK solvate
Acetic acid solvate
Isopropanol solvate

FIG. 11