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(54) **HYDRATED POLYMORPHS OF 2'-O-FUCOSYLLACTOSE AND METHODS FOR THEIR PRODUCTION**

HYDRIERTE POLYMORPHE VON 2'-O-FUCOSYLLACTOSE UND VERFAHREN ZU DEREN HERSTELLUNG

POLYMORPHES HYDRATÉS DU 2'-O-FUCOSYLLACTOSE ET LEURS PROCÉDÉS DE PRODUCTION

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The file contains technical information submitted after the application was filed and not included in this specification

Description

FIELD OF THE INVENTION

[0001] This invention relates to the field of new hydrate compounds and one anhydrous polymorph of 2'-O-fucosyllactose (2'FL) and their methods of preparation, and in particular refers to two new hydrate and anhydrous polymorphs.

STATE OF THE ART

[0002] In recent years, many efforts have been made to synthesise oligosaccharides, given their involvement in numerous biological processes. Much importance, from a commercial point of view, has been given to oligosaccharides secreted in human milk, both for their food value and for their therapeutic role. One of the main ones, present in the highest concentrations, is 2'FL (Diagram 1 shows the structural formula) not only important from a nutritional and probiotic point of view, but also due to its anti-adhesiveness, immunostimulation, neuronal development etc...

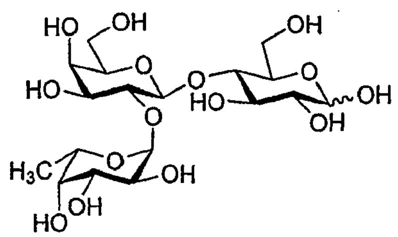


Diagram 1

[0003] An interesting commercial objective is to use this oligosaccharide in baby foods such as infant formula, nutritional supplements, or cosmetic and pharmaceutical formulations. To do this one would need to have a simple and cheap method of isolation and purification, such as crystallisation.

[0004] The first samples of oligosaccharides secreted in human milk, were obtained by chromatography of the milk itself, with purities that were not very high, given the large number of structural isomers present. In the specific case of 2'-O-Fucosyllactose, the presence of the structural isomer 3-O-Fucosyllactose made its isolation difficult for a long time.

[0005] Note that the various crystalline or amorphous phases of an active molecule (such as 2'FL), may possess chemical, physical, and mechanical properties that may be quite different from one another (e.g. solubility and bioavailability, hygroscopicity, thermal and electrical conductivity, chemical stability, hardness, etc.), with considerable consequences on their use, handling, and purification. Furthermore, the possibility of interconversion between the various forms can have very serious consequences on the life of a product and on the maintenance of the desired properties (therapeutic efficacy in the case of a drug, chromatic properties in the case of a pigment, etc.). More specifically, it should be considered that, depending on variables such as temperature, pressure, and relative humidity, a metastable form can be transformed into a thermodynamically more stable form, or else an anhydrous crystalline form can be transformed into a hydrate crystalline form by the absorption of water vapour from the environment, and a solvate crystalline form can, in turn, be transformed into an anhydrous crystalline form or one with a different degree of solvation due to solvent loss.

[0006] Regarding 2'FL the first crystalline form is given in the literature by Kuhn et al. (Chem. Ber. 1955, 88, 1135; ibid., Chem. Ber. 1956, 89, 2513), which describe the crystallisation of the oligosaccharide isolated from human milk. They obtain a crystal that has no hydration water, which they believe to be in α anomeric form and which has a melting point (with decomposition) of 230-231°C. As described by Kuhn this crystalline form can be obtained from the 2'FL isolated from human milk (as syrup or amorphous) dissolving it in 75% hot methanol to which, in the presence of seed crystals, absolute ethanol is gradually added. The seed crystals were obtained because, after prolonged storage, they were found to be precipitated on the walls of the flasks containing the 2'FL syrup or else precipitated from a solution of aqueous methanol, n-butanol, and n-hexanol at 4°C for several weeks.

[0007] After Kuhn, crystalline forms are disclosed in patent application WO/2011 150939 where there are reported two polymorphs of 2'-O-Fucosyllactose therein named Polymorph I and Polymorph II.

[0008] Polymorph I has an X-ray powder diffraction with characteristic peaks at angles 2θ 21.34°, 20.92°, 18.37°, 16.70°, 9.91°, 13.13°, 7.87° and 8.90°. Polymorph I is described as being predominantly in α anomeric form, free of crystallisation water or solvent and with an endothermic DSC curve whose maximum peak is at 260$\pm$1°C or 246$\pm$1°C as the β anomer percentage decreases (from 20$\pm$3% to 12$\pm$3%). This Polymorph I is described as being obtainable by crystallisation, in the absence of seed crystals, from an aqueous mixture of methanol or ethanol. Polymorph II has an X-ray powder diffraction with characteristic peaks at angles 2θ 16.98°, 13.65°, 18.32°, 21.70°, 15.22°, 20.63° and 11.94°. Polymorph II is described as anhydrous and non-solvate. It can be a mixture of the two anomers or else just one of the two. This, in DSC, presents an endothermic curve which has a maximum peak at 259.5±2°C. This Polymorph II is described as being obtainable by crystallisation, in the presence of seed crystals, from an aqueous mixture of methanol, or alternatively can be obtained by crystallisation, without seed, from Polymorph I from ethyl acetate.

[0009] For the production of 2FL the need is still felt to gain more knowledge relating to its possible crystalline forms so as to improve the characteristics of the product and improve long-term possibilities for production, purification, and shelf-life.

[0010] The purpose of this invention is to provide further polymorphs of 2'FL and their preparation methods, which would preferably be industrially exploitable.

DEFINITIONS AND ABBREVIATIONS

[0011]

XRPD: X-ray powder diffraction
IR: infrared
DSC: differential scanning calorimetry
NMR: nuclear magnetic resonance
TGA: thermogravimetric analysis
DTA: differential thermal analysis
LOD = loss on drying
KF: Karl Fischer

SUMMARY OF THE INVENTION

[0012] This invention solves the above problems through the isolation and identification of new hydrate compounds and a new anhydrous polymorph of 2'FL:

- Hydrate compounds with general formula $2'FL \cdot nH_2O$ where n is a number between 1 and 3.

[0013] In particular the following two new hydrate compounds have been identified:

- $2'FL \cdot 3/2\ H_2O$, preferably in the crystalline form of Polymorph A with characteristic XRPD peaks at 18.86±0.20, 9.89±0.20, 17.05±0.20 2θ,
- $2'FL \cdot 5/2\ H_2O$, preferably in the crystalline form of Polymorph B with characteristic XRPD peaks at 9.96±0.20, 20.48±0.20, 11.90±0.20 2θ,

[0014] This invention also relates to methods of preparing these polymorphs

[0015] Surprisingly the isolation of the polymorphs of 2'-O-Fucosyllactose according to the invention is easily feasible with excellent yields on an industrial scale. In particular, in fact, Polymorphs A and B are isolated, at the end of the 2'FL synthesis process, with high purity, and can therefore be used as ingredients in various types of pharmaceutical or food formulations.

BRIEF DESCRIPTION OF FIGURES

[0016]

Figure 1 Shows the X-ray powder diffraction spectrum of the 2'-O-Fucosyllactose Polymorph A of Example 1
Figure 2 Shows the IR spectrum of the 2'-O-Fucosyllactose Polymorph A of Example 2
Figure 3 Shows the differential scanning calorimetry analysis curve of the 2'-O-Fucosyllactose Polymorph A of Example 2
Figure 4 Shows the X-ray powder diffraction spectrum of the 2'-O-Fucosyllactose Polymorph A of Example 2
Figure 5 Shows the ORTEP diagram of the single crystal structure of the 2'-O-Fucosyllactose Polymorph A of Example 2
Figure 6 Shows the thermogravimetric and differential thermal analysis of Polymorph A of Example 2
Figure 7 Shows the X-ray powder diffraction spectrum of the 2'-O-Fucosyllactose Polymorph A of Example 4
Figure 8 Shows the X-ray powder diffraction spectrum of the 2'-O-Fucosyllactose Polymorph A of Example 6

Figure 9 Shows the IR spectrum of the 2'-O-Fucosyllactose Polymorph A of Example 6
Figure 10 Shows the X-ray powder diffraction spectrum of the 2'-O-Fucosyllactose Polymorph A of Example 7
Figure 11 Shows the IR spectrum of the 2'-O-Fucosyllactose Polymorph A of Example 7
Figure 12 Shows the IR spectrum of the 2'-O-Fucosyllactose Polymorph A of Example 8
Figure 13 Shows the X-ray powder diffraction spectrum of the 2'-O-Fucosyllactose Polymorph A of Example 8
Figure 14 Shows the X-ray powder diffraction spectrum of the 2'-O-Fucosyllactose Polymorph A of Example 9
Figure 15 Shows the X-ray powder diffraction spectrum of the 2'-O-Fucosyllactose Polymorph A of Example 10
Figure 16 Shows the ^{1}HNMR spectrum of the 2'-O-Fucosyllactose Polymorph A of Example 11
Figure 17 Shows the X-ray powder diffraction spectrum of the 2'-O-Fucosyllactose Polymorph A of Example 13
Figure 18 Shows the X-ray powder diffraction spectrum at different temperatures of the 2'-O-Fucosyllactose Polymorph A and B of Example 14
Figure 19 Shows the IR spectrum of the 2'-O-Fucosyllactose Polymorph B of Example 15
Figure 20 Shows the X-ray powder diffraction spectrum of the 2'-O-Fucosyllactose Polymorph B of Example 15
Figure 21 Shows the IR spectrum of the 2'-O-Fucosyllactose Polymorph B of Example 17
Figure 22 Shows the differential scanning calorimetry analysis curve of the 2'-O-Fucosyllactose Polymorph B of Example 17
Figure 23 Shows the thermogravimetric and differential thermal analysis of Polymorph B of Example 17
Figure 24 Shows the X-ray powder diffraction spectrum of the 2'-O-Fucosyllactose Polymorph B of Example 17
Figure 25 Shows the ^{1}HNMR spectrum of the 2'-O-Fucosyllactose Polymorph B of Example 18

DETAILED DESCRIPTION OF THE INVENTION

[0017] For the purposes of this invention X-ray powder diffractometry refers to measurements made using Cu-Ka radiation.

[0018] To obtain the new hydrate compounds of this invention, the 2'FL could have been obtained by way of extraction, enzymatically, chemically (for example see synthesis procedures described in WO 2010/070616, WO 2010/115934, WO 2010/115935), or by a combination of these ways.

[0019] This invention also relates to a method for obtaining Polymorph A which is obtained by crystallisation from a mixture comprising water and at least one other solvent. Said solvent may be selected from alcohols, ketones (e.g. acetone), nitriles (e.g. acetonitrile), organic acids (for example glacial acetic acid), or esters (e.g. ethyl acetate). The preferred solvents are C1-C3 alcohol and in particular C2 alcohol and crystallisation can be conducted either with or without seeding.

[0020] The water to solvent ratio can vary between 40 and 10% (v/v), and the preferred amount is between 25 and 15% (v/v).

[0021] Polymorph A is 3/2 hydrate, and with a solvent content of less than 1100 ppm, preferably less than 550 ppm, still more preferably less than 200 ppm and in particular less than 100 ppm.

[0022] Polymorph A obtained, surprisingly, in the solid state, presents itself exclusively in β anomeric form as shown by the single crystal structure (See Figure 5). The anomeric percentage was also determined from the ^{1}HNMR spectrum recorded in deuterated solvent at dissolution (See details in the experimental section). The spectra recorded in these conditions show a percentage of α anomer of less than 15%, preferably less than 10%.

[0023] The polymorph that is obtained can reach very high purity, with total impurities of less than 5%, preferably less than 3%, more preferably less than 1%, still more preferably less than 0.5%. Residual impurities can be small percentages of residual solvents, simple sugars (lactose, galactose, glucose, fucose), reaction intermediates, unreacted intermediates, and products of secondary reactions depending on the synthesis route employed.

[0024] To obtain Polymorph A, the 2'FL (amorphous, as a crystal or already in solution) is prepared as an aqueous solution at a concentration by weight between 53% and 75% and then charged with solvent.

[0025] Even more preferably, for the purposes of this invention and this description, the 2'-O-fucosyllactose is that in solution coming directly from the hydrogenation reaction, the final step of the synthesis as shown in WO2010/070616, which is brought to a concentration by weight of between 53% and 75% and then charged with the solvent, as mentioned above for obtaining Polymorph A.

[0026] The preferred concentration of the aqueous solution is between 60 and 65% by weight, and to this is added the solvent which can be absolute or have a variable water content, of up to 20%, preferably up to 15%, still more preferably up to 10%, and even more preferably up to 4%.

[0027] In the production of Polymorph A one of the methods involves concentrating 2'-O-Fucosyllactose to a syrup, maintaining the solution warm between 35 and 70°C, preferably between 45 and 67°C, still more preferably between 50 and 60°C, and additing the solvent warm or at boiling temperature. The solvent is added gradually and the mixture is left under agitation warm for between 1 and 15 hours. Then the mixture is allowed to return spontaneously to a temperature between 13 and 28°C, preferably between 20 and 25°C, filtered, washed with a water/solvent mixture, and the obtained solid dried.

[0028] The crystallisation mixture is maintained, during the addition of the solvent, at a temperature between 35 and 70°C, preferably between 45 and 67°C, still more preferably between 50 and 60°C. Typically one can observe the formation of a few crystallisation nuclei during or at the end of the addition of the solvent. Alternatively, the crystallisation can be initiated with a small quantity of crystals of Polymorph A.

[0029] The crystallisation mixture after the addition of the solvent, is maintained for 1-24 hours, preferably for 1-16 hours, still more preferably for 2-8 hours, at a temperature between 40 and 70°C, preferably between 45 and 67°C, and even more preferably between 55 and 65°C. Then the mixture is cooled or allowed to cool to room temperature in 1-24 hours. The mixture is maintained at room temperature (20-25°C) for a time ranging from a few hours to a few days, preferably between 1 and 24 hours, still more preferably between 8 and 18 hours. Optionally the mixture, before being filtered to collect the crystalline solid, may be cooled further to 2-18°C, preferably to 5-15°C.

[0030] The operations for collecting the crystalline solid are those well known to experts in the field with usual means of filtration and suitable washing with aliquots of the water/solvent system used for crystallisation.

[0031] This invention also provides a method for preparing Polymorph A starting from Polymorph B. This phase change is made possible by drying Polymorph B in a desiccator with anhydrous silica gel for 12-48 hours, preferably 18-36 hours. Even more preferably drying can be conducted at 46-65°C, preferably at 48-55°C at ambient pressure or lower and subsequent cooling to room temperature. With the latter procedure, Polymorph B, before transforming into A passes through a new anhydrous phase with characteristic XRPD peaks at 9.96±0.20, 18.88±0.20, 1.17±0.20, 11.79±0.2, 16.09±0.20, 12.62±0.2, 15.70±0.2, 14.20±0.2 2θ.

[0032] Polymorph A exhibits characteristic IR peaks at 3338±4 cm^{-1}, 2938±4 cm^{-1}, 1655±4 cm^{-1}, 1344±4 cm^{-1}, 1021±4 cm^{-1}, 819±4 cm^{-1}, 760±4 cm^{-1}, preferably at 3338±4 cm^{-1}, 2938±4 cm^{-1}, 1655±4 cm^{-1}, 1344±4 cm^{-1}, 1237±4 cm^{-1}, 1171±4 cm^{-1}, 1131±4 cm^{-1}, 1021±4 cm^{-1}, 819±4 cm^{-1}, 760±4 cm^{-1}, 687±4 cm^{-1}. In particular the peak at 1655±4 cm^{-1} is present, much more intense than in the anhydrous polymorphs.

[0033] Under DSC analysis it shows a trend characterised by three endothermic peaks. The first has the maximum peak at the temperature of 61±3°C, the second at 182±3°C and the third at 247±3°C. The first peak is attributable to dehydration, the second one to melting, and the third one to the decomposition of the sample.

[0034] The loss on drying of Polymorph A, carried out with Method 1 (See experimental section), varies from 1.5% by weight to 3% by weight, preferably from 1.1% by weight to 2.5% by weight.

[0035] Polymorph A shows characteristic Karl-Fischer values between 4.0% and 6.0% by weight and preferably between 4.4% and 5.2% by weight.

[0036] In particular Polymorph A, was subjected to studies of stability both accelerated at 40°±2°C; 75±5% RH and at 25°±2°C, 60±5% RH. From these studies it was observed that the sample tends to adsorb humidity immediately (1-3 months) showing, compared to the start, an increase in the KF value that remains constant over time.

[0037] In particular, the percentage of water takes values between 5% and 7%, preferably between 5.5% and 6.5%, still more preferably between 5.7% and 6.2% (See Table 1). This extra water detected by KF, however, is not crystallisation water because the XRPD spectrum of these samples continues to show the characteristic peaks of Polymorph A. What changes is the loss on drying which passes to values between 2.5% by weight to 4.8% by weight, preferably between 3.0% by weight to 4.3% by weight, and more preferably between 3.2% by weight and 4.0% by weight. See Figure 7

Table 1: accelerated stability (40°±2 °C and 75±5% RH) Polymorph A

Time (months)	0	1	2	3	4	5	6
KF%	4.2	6.0	6.0	5.7	5.9	6.0	6.0

[0038] Polymorph A has characteristic XRPD peaks at 18.86±0.20, 9.89±0.20, 17.05±0.20 2θ, preferably at 18.86±0.20, 9.89±0.20, 17.05±0.20, 21.65±0.2, 14.20±0.20 2θ, more preferably at 18.86±0.20, 9.89±0.20, 17.05±0.20, 21.65±0.2, 14.20±0.20, 21.80±0.2, 20.36±0.2 2θ, still more preferably at 18.86±0.20, 9.89±0.20, 17.05±0.20, 21.65±0.2, 14.20±0.20, 21.80±0.2, 20.36±0.2, 15.55±0.2 2θ. The list of peaks with the relative intensities and the d (interplanar distances) of Polymorph A are shown in Table 2.

Table 2: List of XRPD peaks for Polymorph A

Pos. [°2Th.]	d-spacing [A]	Rel. Int. [%]	Pos. [°2Th.]	d-spacing[Å]	Rel. Int. [%]
9.5494	9.25416	16.40	24.7910	3.58848	16.98
9.8946	8.93207	87.81	24.9682	3.56342	10.54
11.7744	7.50996	31.79	25.5128	3.48857	14.14

(continued)

Pos. [°2Th.]	d-spacing [A]	Rel. Int. [%]	Pos. [°2Th.]	d-spacing[Å]	Rel. Int. [%]
12.7465	6.93932	26.09	25.9598	3.42950	5.68
13.8212	6.40205	21.77	26.1113	3.40996	5.77
14.2037	6.23051	58.63	26.6579	3.34126	2.62
14.5352	6.08914	14.69	27.5941	3.22999	12.98
15.5499	5.69399	31.30	28.6108	3.11747	15.62
15.8620	5.58265	5.89	29.3651	3.03909	12.50
16.1664	5.47824	49.80	30.1835	2.95853	9.56
16.8455	5.25888	23.05	30.6994	2.90998	1.15
17.0532	5.19528	78.13	31.4016	2.84649	1.86
18.8562	4.70239	100.00	32.1642	2.78072	6.24
19.8596	4.46702	12.50	32.6782	2.73814	5.35
20.1831	4.39615	14.08	33.8253	2.64787	2.14
20.3621	4.35790	42.32	34.0448	2.63129	3.35
20.8790	4.25116	11.55	34.4040	2.60464	7.45
21.6483	4.10181	74.01	34.6906	2.58377	4.41
21.7996	4.07366	54.07	35.3942	2.53400	2.66
22.2975	3.98382	3.66	36.3277	2.47100	4.54
22.9174	3.87744	4.57	36.5727	2.45501	5.22
23.9457	3.71322	14.66	37.0702	2.42319	3.37
24.4818	3.63310	6.05			

[0039] Polymorph A as a single crystal has a spatial group of the type $P2_12_12_1$. It is arranged in an orthorhombic crystal system and the unit cell has the following parameters: a = 12.4098(8) Å, b = 12.737(2) Å, c = 13.756(2) Å and a volume of 2212.5 $Å^3$.

[0040] The asymmetric unit of the crystal of the sample contains a molecule of 2'-O-Fucosyllactose and two sites containing water molecules. The site corresponding to the oxygen atom O26 (See Figure 5) is fully occupied, whilst in the case of the site of the oxygen atom O27 the calculated occupancy is equal to 50%. From this we clearly see that the chemical composition of the crystal is such that for every molecule of polysaccharide there are 1.5 water molecules or 2'-O-fucosyllactose.1.5H_2O.

[0041] The single crystal X-ray analysis also shows that Polymorph A is present exclusively in β form (See Figure 5).

[0042] This invention also relates to a method for preparing Polymorph B which is obtained by crystallisation from water. Crystallisation can be conducted with or without a primer.

[0043] Polymorph B is 2.5 hydrous and surprisingly occurs predominantly in β anomeric form, with an α anomer percentage less than 10%. The anomeric percentage is determined from the ^{1}HNMR spectrum recorded in deuterated solvent at dissolution (See experimental section).

[0044] The polymorph obtained has a very high purity, greater than 95%, preferably greater than 97%, still more preferably greater than 99%. Impurities, depending on the method of obtaining 2'FL, may be residual solvents, simple sugars (lactose, galactose, glucose, fucose), reaction intermediates, unreacted intermediates, products of secondary reactions, 2'-O-Fucosyllactose in polymorphic form, or other amorphous phases (for example that of Polymorph A).

[0045] To obtain Polymorph B one of the methods involves concentrating it (or preparing an aqueous solution) to a weight percentage between 50 and 80%. Precipitation from the aqueous solution may optionally take place by seeding.

[0046] The preferred concentration of the syrup is between 74% and 77% by weight.

[0047] In the production of Polymorph B one of the methods involves concentrating 2'FL to a syrup, maintaining it warm between 30 and 60°C, preferably between 35 and 50°C, more preferably between 38 and 48°C, still more preferably between 40 and 45°C in the aqueous solution, and the optional addition of Polymorph B as seeding. At the end of concentration the syrup is kept hot for 1-12 hours, preferably for 1-8 hours, still more preferably for 1-4 hours. Typically one can observe the formation of crystals, or alternatively the crystallisation can be seeded with the addition of a small quantity of Polymorph B.

[0048] The crystallisation mixture is maintained at a temperature between 20 and 30°C, preferably between 20 and 25°C for a time ranging from 10 hours to 10 days, preferably for about 12-120 hours, still more preferably for 12-24 hours.

[0049] The mixture is then cooled to between 2 and 18°C, preferably between 10 and 15°C before collecting the crystal.

[0050] The crystal is filtered and washed with successive aliquots of water cooled to 4-15°C, preferably between 4 and 10°C.

[0051] Polymorph B shows characteristic IR peaks at 3340±4 cm^{-1}, 2938±4 cm^{-1}, 1655±4 cm^{-1}, 1348±4 cm^{-1}, 1023±4 cm^{-1}, 819±4 cm^{-1}, 762±4 cm^{-1}, preferably at 3340± cm^{-1}, 2938± cm^{-1}, 1655±4 cm^{-1}, 1348±4 cm^{-1}, 1239±4 cm^{-1}, 1173±4 cm^{-1}, 1133±4 cm^{-1}, 1023±4 cm^{-1}, 819±4 cm^{-1}, 760±4 cm^{-1}, 687±4 cm^{-1}. Even Polymorph B, as with Polymorph A, is distinguished from the anhydrous phases due to the presence of an intense peak at 1655±4 cm^{-1}.

[0052] The DSC analysis of Polymorph B shows three intense endothermic peaks, the first having the maximum peak at 83±3°C, the second at 182±3°C, and the third at 242±3°C. The first peak is attributable to dehydration, the second one to melting, and the third one to the decomposition of the sample.

[0053] Polymorph B is characterised by Karl-Fischer values of between 8.0% and 8.9% by weight, as well as losses on drying, measured by Method 1, between 5.2% by weight and 6.4% by weight.

[0054] Polymorph B has characteristic XRPD peaks at 9.96±0.20, 20.48±0.20, 11.90±0.20 2θ, preferably at 9.96±0.20, 20.48±0.20, 11.90±0.20, 19.84±0.2, 22.20±0.20, 2θ, more preferably at 9.96±0.20, 20.48±0.20, 11.90±0.20, 19.84±0.2, 22.20±0.20, 14.03±0.2, 9.18±0.2 2θ, still more preferably at 9.96±0.20, 20.48±0.20, 11.90±0.20, 19.84±0.2, 22.20±0.20, 14.03±0.2, 9.18±0.2, 9.26±0.20 2θ. The list of peaks with relative intensities and the d (interplanar distances) of Polymorph B are shown in Table 3.

Table 3: List of XRPD peaks for Polymorph B

Pos. [°2Th.]	d-spacing [Å]	Rel. Int. [%]	Pos. [°2Th.]	d-spacing[Å]	Rel. Int. [%]
9.1778	9.62805	25.75	22.1968	4.00166	41.97
9.2632	9.53951	25.64	22.7019	3.91376	9.34
9.9612	8.87255	100.00	22.9013	3.88014	13.27
11.6236	7.60706	19.71	23.3036	3.81406	15.83
11.9041	7.42844	43.84	23.9677	3.70985	16.31
13.8934	6.36897	16.00	24.5568	3.62217	16.76
14.0269	6.30864	37.88	25.5249	3.48695	11.44
14.2108	6.22742	47.68	25.9384	3.43228	19.42
15.1931	5.82692	8.30	26.3721	3.37682	4.65
15.5341	5.69975	34.04	27.0745	3.29079	2.96
15.8480	5.58757	35.04	28.0196	3.18190	12.44
16.7859	5.27742	39.87	28.8292	3.09436	15.78
18.5609	4.77655	85.09	29.2610	3.04966	11.39
19.6786	4.50770	18.05	29.7297	3.00265	5.36
19.8445	4.47039	43.36	30.1664	2.96017	3.63
20.4789	4.33330	47.79	30.8471	2.89638	2.04
20.8612	4.25475	20.12	31.4947	2.83829	5.55
21.6447	4.10248	8.12	31.6945	2.82085	9.83

[0055] The unit cell obtained from the single crystal collection of Polymorph B is orthorhombic (a=14.762, b=12.622, c=12.3540) and perfectly in accordance with that calculated from the powder spectrum (a=14.905 (7), b=12.663 (6), c=12.447 (5)). The spatial group in which it crystallises is $P2_12_12_1$ and it has a cell volume of 2349.4 $Å^3$ against the 2212.5 $Å^3$ of Polymorph A.

[0056] The operations for collecting the crystalline solid are those well known to experts in the field with usual means of filtration and suitable washing with aliquots of the solvent used for crystallisation.

[0057] A further subject-matter of this invention is a Polymorph A or B as described above for use as a medicament, as a dietary supplement, or an ingredient in cosmetics.

[0058] The scope of the invention therefore also includes pharmaceutical, food, or cosmetics compositions comprising any one of Polymorphs A or B as described above. Particularly preferred food compositions are infant formulas. This invention will be better understood in light of the following embodiments.

EXPERIMENTAL SECTION

Analytical methods:

1) DSC analysis

[0059] The differential calorimetric analyses were carried out using a Mettler T STAR^e system instrument (Mettler Toledo, Switzerland) equipped with a DSC cell. Samples weighed with a Mettler MX5 microbalance in suitable A1 containers, subsequently sealed and perforated, underwent analysis, carried out at a scan rate of 10°C/min in the temperature range 30 to 300°C in static air.

[0060] The instrument was previously calibrated with indium as a standard (purity 99.98%, melting point 156.61°C, melting enthalpy 28.71 J/g).

2) Karl-Fischer analysis

[0061] Determination of water content in the product based on a specific oxidation-reduction reaction between the water present in the sample and the Karl Fischer reagent.

LIST OF EQUIPMENT AND REAGENTS

[0062]

- Metrohm automatic titrator mod. 701KF Titrino.
- Analytical balance
- Hydranal composite 2 Riedel de Haen or equivalent
- Hydranal solvent Riedel de Haen or equivalent

MODES OF OPERATION

[0063] The parameters used are as follows:

I(pol)	12 μA
EP	250 mV
Stop crit.	Drift
Drift	20μl/min
Max rate	30 mL/min
Min. volume incr.	9 μ

[0064] About 200 mg of the 2'-FL were weighed into the appropriate container, the balance was set to zero and the substance transferred into the previously dehydrated cell. The empty container was placed on the pan of the balance to determine the exact value of the substance transferred into the cell. Set the measured weight into the formula:

$$\% H_2O = V \times T \times F / PC$$

Where:

V = volume of Karl Fischer reagent used at the equivalent point;
T = Titre of the reagent in mg/mL;
PC = Weight of the sample analysed;

F = multiplication factor = 100.

[0065] The table below shows the theoretical percentages by weight of water compared to the degree of hydration of the molecule.

Type	2'FL.1H_2O	2'FL.1.5H_2O	2'FL.2H_2O	2'FL.2.5H_2O	2'FL.3H_2O
%H_2O (p/p)	3.6	5.2	6.9	8.4	10

3) LOD

[0066] Gravimetric method with heating of the sample using a halogen lamp Ohaus themobalance mod. 45 MB

Method 1

[0067] The conditions of Method 1 are as follows:

Profile: phases
Step 1: 50°C for 1 minute
Step 2: 60°C for 1 minute
Final: 90°C
Power off: alib (<1 mg in 120 seconds)
Unit. Per.: OFF
Target weight: none

Method 2

[0068] The conditions of Method 2 are as follows:

Profile: phases
Step 1: 50°C for 1 minute
Step 2: 50°C for 1 minute
Final: drying temperature 56 °C
Power off: alib (<1 mg in 60 seconds)
Unit. Per.: OFF
Target weight: none

4) ^{1}HNMR analyses

[0069] The ^{1}HNMR analyses were carried out on a Varian VXR300 or Gemini 200 dissolving 10 mg of product in 0.75ml of D_2O or DMSO in a tube for NMR spectra. The spectra were recorded at dissolution and at equilibrium.

[0070] In the first case the tube is prepared with the product and deuterated solvent and the spectrum immediately recorded.

[0071] In the second case the product is dissolved in deuterated solvent, left to equilibrate at least one night, and then the spectrum is recorded.

[0072] For the spectra in D_2O, in order to calculate the α/β ratio, reference is made to the following peaks:

a) peak at 5.3 ppm (H-1 fucose)
b) peak at 5.2 ppm (H-1 α glucose)

[0073] The calculation of the percentage of α compared to β is as follows:

$$(\text{Integral of signal b/Integral of signal}) * 100$$

[0074] For the spectra in DMSO reference is made to the following peaks:

c) peak at 6.7 ppm (OH glucose β)
d) peak at 6.3 ppm (OH glucose α)

[0075] The calculation of the percentage of α compared to β is as follows:

$$[\text{Integral of signal d/ (integral of signal c+ integral of signal d)}]^{*}\ 100$$

5) Melting point

[0076] The melting point analyses were carried out with a BUCHI Melting Point B-540.

[0077] An aliquot of the solid is placed into a melting point capillary tube and inserted into the appropriate slot on the instrument. A preliminary analysis is carried out with fast heating to check the approximate melting temperature. The analysis is then repeated by setting a rapid heating to 10-15°C below the melting temperature and then a heating of 2°C/ minute until the melting/ decomposition of the sample is observed.

6) Powder spectra

[0078] An aliquot of the substance, between 10 and 20 mg was ground and placed on a zero-background sample holder. For each sample, spectra were recorded on an X'PERT PRO diffractometer with Cu-Ka radiation (λ = 1.5418) in the range 2θ 4-60°.

[0079] Spectra at various temperatures have been recorded with the same instruments. In situ thermodiffractometry was carried out in an Anton Paar HTK1200N high-temperature chamber. Powder spectra were recorded at the temperatures 25, 50, 75, 100, 125 and 150°C with a heating ramp of 5°C/min. Similarly the spectra were recorded at 125, 100, 75, 50, 25°C whilst cooling.

7) X-Ray

[0080] The data were collected at 100K using an Oxford Diffraction Xcalibur 3 diffractometer equipped with Cu-Ka radiation and a CCD detector. Data were collected using the CrysAlis CCD programme whilst the data reduction was carried out with the CrysAlis CCD programme. Absorption correction was applied with the ABSPACK programme. The structure was solved by direct methods using the SIR97 programme and refined through the F2s with the full-matrix least-squares techniques with the SHELXL programme. All atoms excepting hydrogen atoms were refined anisotropically. The hydrogen atoms bonded to the carbon atoms and the hydroxyl groups were placed in calculated positions and with an isotropic factor of 20% or 50% greater than that of the atoms to which they are bonded.

8) TGA and DTA

[0081] Thermogravimetric analysis (TGA) and the coupled differential thermal analysis (DTA) were carried out on a Seiko EXSTAR 6200 Thermogravimetric Analyser (TGA/DTA) under a flow of nitrogen (50 mL/min) with a heating ramp of 5°C min^{-1}.

9) IR determination

EQUIPMENT USED

[0082] Perkin Elmer IR mod. BX2

MODES OF OPERATION

[0083] In an agate mortar, a mixture made up of 1 to 2 mg of product and 100 to 150 mg of infrared grade KBr previously dried at 105°C overnight, is ground thoroughly. The mixture is transferred into the appropriate holder and maintained under vacuum and under a pressure of 100 Kg/m^2 (10 $Tons/ft^2$) for at least 30 minutes.

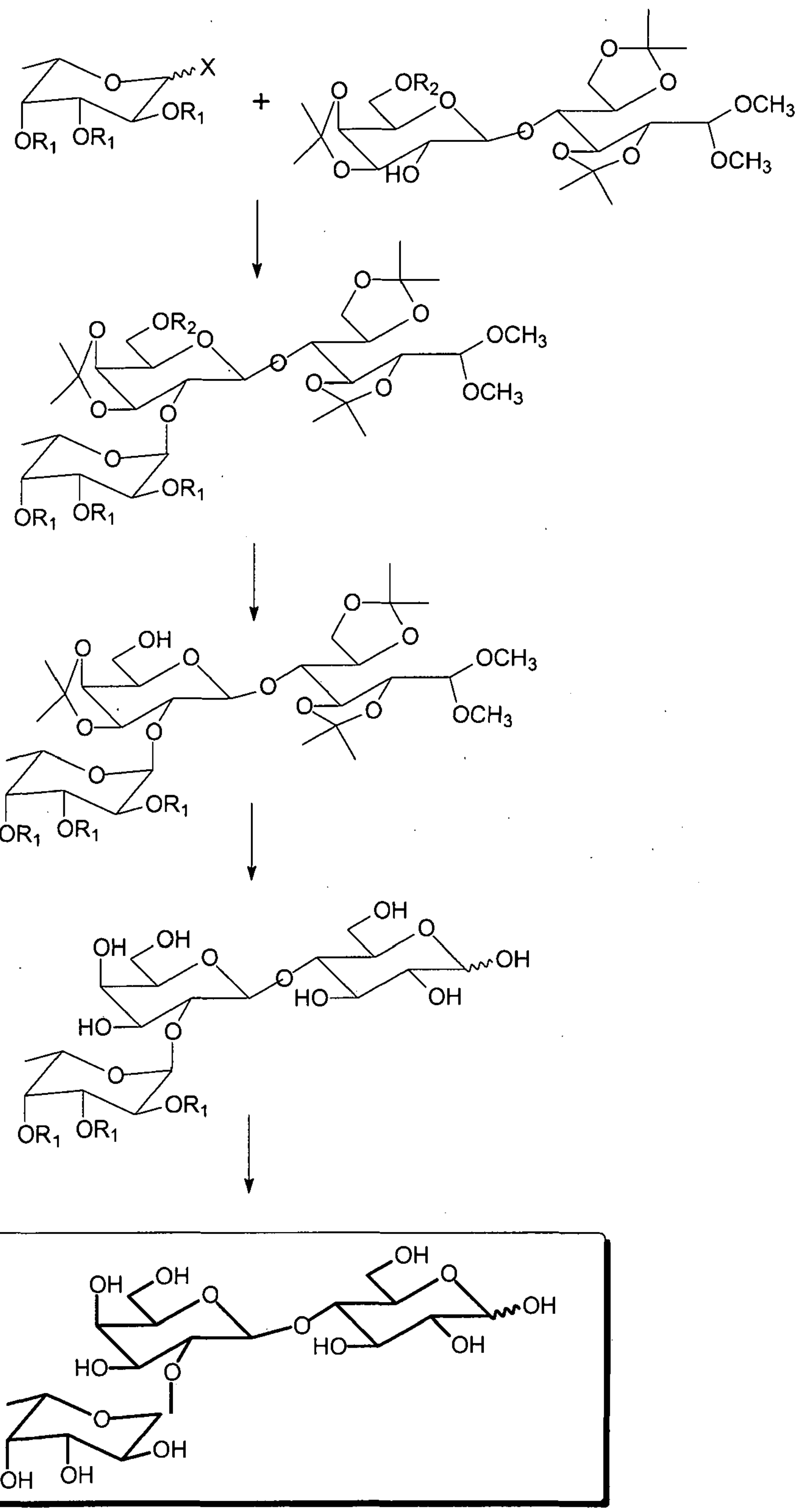
[0084] The spectrum is recorded in the range 4000 ÷ $600cm^{-1}$

10) HPLC

[0085] The HPLC analyses are undertaken for comparison with a standard weighed with the following equipment:

HPLC line with thermostated cell refractive index detector, isocratic pump and autosampler.
300 mm long stainless steel column filled with Transgenomic IC-SEP ICE-ION-300 stationary phase (Stepbio cod. ICE-99-9850) equipped with a similar guard cartridge (code ICE-99-2364).
Column temperature: 45°C.
Injections: 20μl.
Flow: 0.4mL/minute.
Integrator: Perkin Elmer Totalchrom workstation

[0086] The experimental section shows examples where the starting fucosyllactose was obtained as described in patent WO2010/070616 for which below is the scheme of the synthesis and the last step in detail:



R_1 = benzyl groups substituted with chlorine, bromine, alkoxy, or nitro groups
R_2 = a group selected from acyl, alkyl or aryl, benzyl, P, trityl, silyl-derived; preferably R3 is acyl, and more preferably acetyl or benzoyl, optionally mono- or di-, substituted by chlorine, bromine, alkoxy, or nitro groups.

[0087] 65.00 g of O-[2,3,4-Tri-O-(4-Chlorobenzyl)-6-Deoxy-α-L-Galactopyranosyl]-(1→2)-O-β-D-Galactopyranosyl-(1→4)-D-Glucose, 20g of sodium acetate, 9.3 g of unreduced 5% Pd/C, and 880 g of methanol were loaded into a reactor. The solution, kept under agitation, after 3 cycles of vacuum/nitrogen, had hydrogen blown into it up to 1.5 bar. The mixture was heated to 60°C in 3 hours and maintained at temperature at 1.8 bar for 16 hours. The TLC control (Toluene, methanol, AcOH 10:10:1) shows that the reaction is complete.

[0088] The suspension was then filtered over dicalite, concentrated to a syrup and reconstituted with 150 mL of demineralised water. The aqueous solution was extracted with ethyl acetate (3 x 30ml), concentrated to 20-25° Brix and deionised on a pair of ion exchange resins (IR 120($H^{+)}$ and FPA 55).

[0089] In the product obtained after deionisation, there is an impurity, 2'-Fucosyllactose methyl glycoside, in a variable percentage up to 5%.

[0090] The solution of 2'-O-fucosyllactose thus obtained can be brought to a certain saccharometric concentration in order to be crystallised or dried in a vacuum (80 mbar) and hot (50°C) to produce an amorphous solid.

POLYMORPH A

Example 1

[0091] 24 Kg of 2'FL are brought to a concentration of 64° Brix and heated to 55-60°C. Over the period of 70 minutes, 112 L of 96% ethanol were added, maintaining the temperature at 45±10°C. The mixture, mid-addition, self-primed at 37°C. The suspension was left under agitation for 4 hours at 55°C and was then brought to room temperature in 13 hours. After about 72 hours under agitation at room temperature it was cooled to 10°C in 3 hours. The solid was filtered, washed with 1 volume of water/ethanol = 9/1 and dried under vacuum at 60°C for 10 hours and then one night in static vacuum without heating.

[0092] The solid obtained (20.7 Kg dry) presents:

KF = 5.0%;
LOD = 1.98% (Method 1)
PF = 177-186
^{1}HNMR (D_2O) at dissolution (300 MHz; nt = 1): α anomer/β anomer = 10/90;
Powder diffraction spectrum = Shows the phase of Polymorph A (2'FL.1.5H_2O) (See Figure 1)

Example 2

[0093] 50 measured Kg of 2'FL were brought to a concentration of 63° Brix. The internal temperature was increased and maintained at 55°C during the addition of 222 L of 96% ethanol (35 minutes). The mixture was seeded with 200 g of Polymorph A and maintained, after crystallisation occurred, for 6 hours at 56°C, then brought down to 25°C in 4 hours. The suspension, after 18 hours at 23°C, was cooled to 11°C in 3 hours and then filtered. The solid was washed with 1.5 volumes of ethanol/water mixture = 9/1.

[0094] After drying in a vacuum at 60°C, 49.5 kg of dry solid were obtained.

KF = 4.54%
LOD = 0.73% (Method 2); 1.86% (Method 1)
PF = 175-184°C
^{1}HNMR(D_2O) at dissolution (300 MHz; nt = 1): α anomer/β anomer = 13/87
IR = 3337, 9; 2978, 44; 2938, 35; 1655, 55; 1388, 36; 1342, 33; 1237, 40; 1171, 19; 1132, 16; 1072, 8; 1023, 6; 877, 47; 818, 37; 760, 29; 722, 38; 686, 30; 644, 30. (See Figure 2)
DSC = Initial broadened endothermic effect, ranging between 45 and 85°C, desolvation phenomena index, followed by an intense endothermic peak (T_{onset} = 178.10°C; T_{peak} = 184.68) attributable to the melting of the sample, followed, at higher temperatures by an intense endothermic band with a peak at 247.8°C due to phenomena of thermal decomposition. (See Figure 3)
Powder XRPD spectrum = Shows the phase of Polymorph A (2'FL.3/2H_2O). See Figure 4 Single crystal refinement XRD:

Table 5. Crystallographic data and details of structure refinement

Empirical formula	2x($C_{18}H_{32}O_{15}$) 3xH_2O
Formula weight	1030.92
Temperature	100(2) K

(continued)

Wavelength	1.54184 Å
Crystal system	Orthorhombic
Space group	$P\ 2_1\ 2_1\ 2_1$
Unit cell dimensions	a = 12.4098(8) Å
b = 12.737(2) Å	
c = 13.756(2) A	
Volume	2174.3(5) $Å^3$
Z	2
Density (calculated)	1.575 Mg/m^3
Absorption coefficient	1.225 mm^{-1}
F(000)	1100
Crystal size	0.1 x 0.05 x 0.05 mm^3
Theta range for data collection	4.73 to 70.96°.
Index ranges	-14<=h<=6, -10<=k<=15, -11<=l<=16
Reflections collected	6449
Independent reflections	3634 [R(int) = 0.0439]
Completeness to = 65°	99 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	1 and 0.0916
Refinement method	Full-matrix least-squares on F2
Data / restraints / parameters	3634 / 0 / 328
Goodness-of-fit on F2	1.064
Final R indices [I>2(I)]	R_1 = 0.0726, wR_2 = 0.1779
R indices (all data)	R_1 = 0.1082, wR_2 = 0.2124
Extinction coefficient	0.0069(8)
Largest diff. peak and hole	0.855 and -0.436 e·$Å^{-3}$

[0095] The asymmetric unit of the crystal of the sample contains a molecule of 2'-O-fucosyllactose and two sites containing water molecules. The site corresponding to the oxygen atom O26 is fully occupied, whilst in the case of the site of the oxygen atom O27 we have a calculated occupancy of 50%. From this we clearly see that the chemical composition of the crystal is such that for every molecule of polysaccharide there are 1.5 water molecules or 2'-O-fucosyllactose.3/2H_2O (See Figure 5). This ratio between water molecules and 2'-O-fucosyllactose is confirmed by thermogravimetry (TGA).

Table 6 Atomic co-ordinates

	x	y	z		U(eq)
C(1)	7641(5)	3409(6)	-2842(8)		43(2)
C(2)	7757(5)	2328(7)	-2411(7)		43(2)
C(3)	6657(5)	1798(6)	-2310(5)		28(2)
C(4)	5842(4)	2514(5)	-1839(6)		24(2)
C(5)	5850(4)	3599(6)	-2253(6)		27(2)

(continued)

	x	y	z		U(eq)
C(6)	5086(5)	4363(6)	-1763(5)		29(2)
C(11)	4238(4)	1796(5)	-1154(5)		21(1)
C(12)	3157(4)	1295(6)	-1416(5)		23(2)
C(13)	2594(4)	985(6)	-480(5)		23(2)
C(14)	3317(5)	307(5)	184(5)		23(2)
C(15)	4417(4)	841(6)	301(5)		22(2)
C(16)	5211(5)	191(6)	869(6)		32(2)
C(21)	2073(4)	1755(6)	-2811(5)		24(2)
C(22)	1256(5)	2552(5)	-3149(5)		26(2)
C(23)	1782(5)	3552(7)	-3511(6)		36(2)
C(24)	2681(5)	3340(6)	-4229(6)		30(2)
C(25)	3440(4)	2528(5)	-3806(5)		28(2)
C(26)	4320(5)	2185(6)	-4481(5)		32(2)
O(1)	8611(4)	3946(5)	-2815(5)		47(2)
O(2)	8367(4)	1787(5)	-3161(4)		49(2)
O(3)	6800(3)	848(4)	-1782(4)		34(1)
O(4)	4804(3)	2021(4)	-2000(3)		25(1)
O(5)	6941(3)	4006(4)	-2160(4)		29(1)
O(6)	5153(3)	4320(4)	-724(3)		29(1)
O(12)	2532(3)	2092(4)	-1917(3)		24(1)
0(13)	1622(3)	422(4)	-726(4)		31(1)
O(14)	3498(4)	-703(4)	-226(4)		32(1)
0(15)	4864(3)	1026(4)	-629(3)		26(1)
O(16)	6192(3)	765(4)	1053(4)		39(1)
O(22)	523(3)	2793(4)	-2372(3)		32(1)
O(23)	983(4)	4217(5)	-3929(5)		55(2)
O(24)	2165(3)	2983(5)	-5099(4)		37(1)
O(25)	2850(3)	1585(4)	-3536(4)		27(1)
O(26)	3115(4)	3949(4)	-75(4)		41(1)
O(27)	367(5)	5832(7)	-3078(7)		24(2)

= TGA shows a loss of water of 5.45%, consistent with a type hydrated 1.5 times. See Figure 6

Example 3

[0096] 1371.6 measured g of 2'FL were concentrated to 58° Brix and heated to 55°C. 5.5 litres of absolute ethanol, heated to 56°C, were added over the course of 1 hour. The solution, already opalescent, was seeded and left hot, always under agitation, for several minutes, and then 1 litre of absolute ethanol heated to 50°C was added. The suspension was left at 57°C for 1 hour and 30 minutes, and then a cooling ramp was started that brought the temperature to 26°C in 3 hours. The mixture was left overnight under agitation at room temperature, then filtered and washed with 1 volume of ethanol/water mixture = 9/1. The solid was dried under vacuum (80 mbar) at 50°C for 21 hours.

[0097] 1412 grams were obtained with a titre of 92.6%.

KF = 4.37%
^{1}HNMR (D_2O) at dissolution (300 MHz, nt = 12): α anomer/β anomer = 12/88
LOD = 1.13% (Method 1)

Example 4

[0098] 210 measured g of 2'-O-fucosyllactose were concentrated under vacuum and hot, at 70° Brix. The syrup was heated to 60°C and, under agitation, 250 mL of 96% ethanol was added. The suspension was seeded, again with Polymorph A, and after 10 minutes under agitation and hot, a further 50 mL of 96% ethanol were added. The mixture was kept under agitation for 2 hours and 30 minutes at 60°C, and was then allowed to return slowly and spontaneously to r.t.. After a few days (5) under agitation at room temperature, it was cooled to 10°C and maintained at this temperature for 1 hour. The cooled solid was filtered, washed with 1 volume of ethanol/water = 9/1 and 0.5 volumes of 96% ethanol, both cold. 206.8 grams of solid were obtained after drying at 55°C under vacuum (80 mbar).

KF = 4.54%
KF after 6 months = 6.00%
LOD = 2.17% after 6 months (Method 2)
PF = 174-176
^{1}HNMR (DMSO) at dissolution (300 MHz; nt = 1): α anomer/β anomer = 14/86 Powder spectrum after 6 months: See Figure 7
HPLC = purity 98.4%

Example 5

[0099] 8 measured Kg of the 2'-O-Fucosyllactose were concentrated to 71° Brix. Over the course of 40 minutes, maintaining the temperature between 50-58°C, 17 litres of 96% ethanol were added. The solution was seeded and allowed to cool to room temperature over the course of 18-20 hours. The mixture was cooled to 15°C in 4 hours and the solid was filtered, washed with 1 volume of ethanol/water mixture = 9/1 and dried in a vacuum (80 mbar) at 60°C. 7.4 kg of dry solid were obtained.

KF = 4.4
LOD = 1.1% (Method 1)
PF = 175-181°C
^{1}HNMR (D_2O) at dissolution (300 MHz, nt = 4): α anomer/β anomer = 11/89
^{1}HNMR (D_2O) at equilibrium after one night at room temperature (300 MHz; nt = 1): α anomer/β anomer = 39/61

Example 6

[0100] 10 g of the 2'-O-Fucosyllactose were dissolved hot at 60° Brix. The solution, weighing 16.5 g, was maintained at 55°C during the addition of 32.5 mL of Propanol heated to 60°C. Mid-addition opalescence was noted. At full addition the suspension was left for 1 hour at 55-60°C and then, again under strong agitation, it was allowed to return spontaneously to room temperature. After about 12 hours at room temperature the suspension was cooled to 15°C over 1 hour. The solid was filtered, washed with 1 volume of propanol/water = 9/1 and dried in a vacuum (80 mbar) at 55°C for the night and stored in a desiccator with silica gel for 18 hours. 8.95 g of dry solid were obtained that presents:

KF = 5.11%
LOD = 1.30% (Method 2); 2.14% (Method 1)
PF = 175-186
^{1}HNMR (DMSO) at dissolution (200 MHz, nt = 24): α anomer/β anomer = 8/92
Powder XRPD spectrum = Shows the phase of Polymorph A (2'FL.1.5 H_2O) See Figure 8
IR = 3340, 1.62; 2977, 29; 2940, 22; 1656, 40; 1343, 19; 1237, 27; 1171, 8; 1132, 7; 1072, 1; 1023, 1; 875, 36; 819, 25; 762, 19; 722, 38; 688, 18; 646, 19. See Figure 9
HPLC = purity 99.5%

Example 7

[0101] 50 g 2'FL were brought to a syrup of 72.5° Brix. At 30°C it was diluted with 70 mL of methanol heated to 35°C and primed. The mixture was allowed to return to room temperature under agitation. The suspension was heated again to 40°C and 70 mL of methanol heated to 45°C were added. The suspension was allowed to return to room temperature and kept under agitation for a few days.

[0102] The solid was filtered, washed with 50 mL of cold methanol and dried at ambient pressure and temperature. 42 g of dry product were obtained

KF = 5.16%
LOD = 2.22% (Method 1); 1.30 (Method 2)
PF = 176-186
^{1}HNMR (DMSO) at dissolution (200 MHz; nt = 12): α anomer/β anomer = 8/92
^{1}HNMR (DMSO) at equilibrium after one night at room temperature (200 MHz, nt = 12): α anomer/β anomer = 24/76
^{1}HNMR (D2O) at dissolution (200 MHz, nt = 20): α anomer/β anomer = 12/88; Powder spectrum = Shows the phase of the Polymorph (2'FL·3/2H_2O). See Figure 10
IR = 3334, 1; 2976, 27; 2939, 17; 1657, 42; 1386, 20; 1344, 14; 1237, 25; 1172, 4; 1132, 3; 1072, 1; 1025, 1; 875, 36; 819, 21; 762, 14; 724, 22; 689, 13; 646, 14. See Figure 11

Example 8

[0103] 10 g of 2'FL of Polymorph A were dissolved in water (5.6 g), whilst hot, 64° Brix. The solution was maintained at 60°C during the addition of acetone heated to 50°C. The addition was completed in thirty minutes. After a further 30 minutes under agitation whilst hot, the mixture was allowed to return spontaneously to room temperature. After 10 hours under agitation at room temperature the suspension was cooled to 10°C in 1 hour, filtered and washed with 10 mL of cold acetone. The solid was dried in a stove under a vacuum (80 mbar) at 50°C and stored in a desiccator for 20 hours. 9.29 grams of dry product were obtained.

KF = 4.7%
LOD = 1.39 (Method 2), 2.21 (Method 1)
^{1}HNMR (D_2O) at dissolution (300 MHz, nt = 20) α anomer/β anomer = 13/87
PF= 175-179
IR= 3340, 10; 2978, 42; 2940, 34; 2875, 35; 1655, 53; 1343, 32; 1237, 40; 1172, 19; 1132, 17; 1072, 8; 1023, 6; 875, 46; 818, 36; 761, 30; 687, 30; 645, 30. See Figure 12
Powder spectrum: Shows the phase of Polymorph A (2'FL.3/2H_2O). Figure 13

Example 9

[0104] 5 g of 2'FL, dried from syrup, were dissolved in water until solubilisation. Totally 5.3 g of water were used. Under agitation, at 60°C, 20 mL of glacial acetic acid were added. At full addition slight opalescence was noticed. The suspension was maintained a further 1 hour at 60°C and then the heating was stopped and it was left under agitation overnight. After about 10 hours at room temperature, the solid was filtered, washed with 1 volume of 80% acetic acid and dried under vacuum (80 mbar) at 55°C for 16 hours and in a desiccator for 6 hours. 2.87 g of dry solid were obtained, presenting:

KF = 4.37%
LOD = 0.74% (Method 2)
PF = 175-183°C
^{1}HNMR (D_2O) at dissolution (200 MHz, nt = 120): α anomer/β anomer = 12/88 Powder spectrum: see Figure 14

Example 10

[0105] 10 g of 2'FL, dried from syrup, were dissolved in water until solubilisation. The solution was brought to 64° Brix and 60 mL of acetonitrile at 50°C were added dropwise, whilst hot (60°C) and under energetic agitation. Opalescence was already noted mid-addition. The suspension was maintained 30 minutes under agitation at 60°C and then the heating was turned off and it was allowed to return, slowly and spontaneously to room temperature. After 10 hours at room temperature, the mixture was slowly cooled to 10°C. The solid was filtered, washed with 1 volume of acetonitrile cooled to 10°C, and dried under vacuum (80 mbar) at 55°C for 4 hours (9.3 g).

KF = 4.67%
LOD = 2.51 (Method 1); 0.93 (Method 2)
PF = 176-179
^{1}HNMR(D_2O) at dissolution (300 MHz, nt = 20): α anomer/β anomer = 12/88
^{1}HNMR (D_20) at equilibrium after one night at room temperature (200 MHz, nt = 16): α anomer/β anomer = 40/60
Powder spectrum: see Figure 15

Example 11

[0106] 20 g of 2'FL, dried from syrup, were dissolved at 64° Brix, heated to 55°C and 75 mL of a mixture of methanol/ethanol = 3/2 heated to 50°C was added. At the end of the dropwise addition the solution was brought to 62°C; it already had a suspension. After 1 hour at 62°C the temperature was lowered to 56°C, maintained for 3 hours and then allowed to return spontaneously to room temperature. After 12-15 hours under agitation at room temperature, the solid was cooled to 3°C, filtered, washed with 1 volume of a mixture of methanol/ethanol/water = 3/4/1 and dried in a vacuum (80 mbar) at 55°C for 2 hours.

KF = 4. 46%
LOD = 1.89%
^{1}HNMR (D_2O) at dissolution (300 MHz, nt = 20): α anomer/β anomer = 6/94. See Figure 16
^{1}HNMR (D_2O) at equilibrium after one night at room temperature. (300 MHz, nt = 20): α anomer/β anomer = 42/58

Example 12

[0107] 20 g of 2'FL Ex. 3 were dissolved in water and concentrated to 56° Brix. The solution was heated to 60°C and under agitation 75 mL of absolute ethanol heated to 57°C was added. The mixture was kept 1 hour at 66-64°C, 14 hours at 60°C, and then cooled to 5-8°C in 3-4 hours. The solid was filtered, washed with a water/ethanol mixture and dried both at ambient temperature and pressure (15 hours) and in a vacuum (80 mbar) at 55°C for 4 hours to obtain 16.33 g of solid.

KF = 4.61%
LOD = 1.24% (Method 1)
^{1}HNMR (D_2O) at dissolution (200 MHz, nt = 28): α anomer/β anomer = 9/91

Example 13

[0108] 6.59 g of 2'FL Polymorph B were placed on a petri dish placed in a desiccator with anhydrous silica gel for 46 hours. The solid was removed from the dryer and analysed.

KF = 4. 37%
LOD = 2.41% (Method 1)
Powder spectrum = Shows the phase of Polymorph A (2'FL.3/2H_2O). See Figure 17

Example 14

[0109] 50 mg of Polymorph B were subjected to powder thermodiffractometry. The powder was heated, with a heating ramp of 5°C/min, up to 150°C and then gradually cooled, with the same mode as for heating, at 25°C. Once at room temperature Polymorph A is obtained.
[0110] Powder spectrum = from Polymorph B by heating and subsequent cooling one obtains Polymorph A passing through a new crystalline phase. The spectrum of Polymorph A, immediately recorded after cooling still contains a small percentage of the anhydrous polymorph. See Figure 18

POLYMORPH B

Example 15

[0111] 22.9 kg of 2'FL were concentrated to 79° Brix. At 38°C the syrup self-seeded. 600 + 400 mL of water were added to the suspension and it was left under agitation at 38-48°C for 1 hour, then left to reach room temperature during the night. The suspension was kept under agitation at 23-28°C for one day, then cooled to 15±2°C in 6 hours and filtered. The solid was washed with 0.05V of cold water and dried in a stove under a vacuum, first at 30°C, then at 35°C for 15 hours.

18.4 kg of dry solid were obtained.
KF = 8.31%
LOD = 6.6% (Method 1)
PF = 176-181
[1]HNMR (DMSO) at dissolution (300 MHz; nt = 20): α anomer/β anomer = 4/96
[1]HNMR (DMSO) at equilibrium after one night at room temperature (300 MHz, nt = 12): α anomer/β anomer = 18/82
[1]HNMR (DMSO) at equilibrium after 5 days (300 MHz, nt = 12): α anomer/β anomer = 48/52
IR= 3342, 3; 2975, 28; 2939, 21; 2894, 23; 1657, 37; 1445, 27; 1370, 24; 1347, 20; 1318, 20; 1278, 31; 1238, 28; 1172, 10; 1132, 9; 1072, 3; 1023, 2; 932, 38; 895, 37; 874, 33; 819, 24; 762, 20; 690, 17; 647, 18. (See Figure 19)
Powder XRPD spectrum = Shows the phase of Polymorph B (2'FL.5/2H_2O). See Figure 20

Example 16

[0112] 24.8 g of 2'FL were concentrated at 77° Brix, and seeded with 200 mg of Polymorph B at 37°C. The suspension was left at 40±4°C for 4 hours and then, always under agitation, it was allowed to return spontaneously to room temperature. The suspension was maintained about 24 hours at 23°C then cooled and filtered at 18°C. The solid was washed with water at 4°C (400 mg x 3) and dried under vacuum (80 mbar) whilst hot (35-40°C) for 11 hours plus one night in static vacuum without heating.

17.7 g of solid were obtained.
KF = 7.99%
LOD = 5.18% (Method 1) 18.4% (Method 2)
PF = 178-184
[1]HNMR (D_2O) at dissolution (300 MHz, nt = 4): α anomer/β anomer = 8/92

Example 17

[0113] 67 Kg of Fucosyllactose Polymorph A were dissolved in water and concentrated to 74° Brix. The solution was brought to 40°C, seeded with Polymorph B and left 2 hours under agitation whilst hot. The heating was turned off and the suspension was allowed to return slowly to 21°C. The solid was kept under agitation at 20-22°C for 24 hours and then cooled to 15°C. The suspension was maintained for 6 hours at 15°C and cooled to 10°C. After 3 hours at 10°C the solid was centrifuged and washed with 0.10 volumes of water at 5°C. The solid was dried in a vacuum (80 mbar) at 35°C for 10 hours and in static vacuum without heating for 10 hours.

KF = 8.88
LOD = 6.4 (Method 1); 5.36 (Method 2)
[1]HNMR (DMSO) at dissolution (200 MHz; nt = 4): α anomer/β anomer = 6/94
IR= 3344, 31; 2939, 50; 1656, 62; 1348, 53; 1239, 58; 1173, 45; 1133, 44; 1072, 34; 1023, 31; 874, 57; 819, 53; 762, 51; 688, 48. (See Figure 21)
DSC: Initial broadened endothermic effect (55-100°C) attributable to phenomena of dehydration, followed by an intense endothermic peak (T_{start} = 173.92 °C; T_{peak} = 181.73°C) attributable to the melting of the sample. At higher temperatures it is followed by an intense endothermic band with a peak at 241.8°C, due to decomposition phenomena. (See Figure 22)
TGA = shows a loss of water of 7.90% compatible with the type hydrated 2.5 times (2'FL.5/2H_2O). See Figure 23
Powder XRPD spectrum: Shows the phase of Polymorph B (2'FL.5/2H_2O) and Polymorph A (less than 5%).

[0114] The spatial group in which it crystallises is $P2_12_12_1$. The cell is orthorhombic with volume of 2349.4 Å^3.

a (Å) = 14.905(7)
b (Å) = 12.663(6)
c (Å) = 12.447(5)
α(°) = 90
β(°) = 90
γ(°) = 90
See Figure 24

Example 18

[0115] 88 g of 2'FL were concentrated to 70° Brix. The syrup heated to 40°C crystallises spontaneously. After 1 hour under agitation at 40°C it was cooled to 30°C, maintained for 2 hours, and then allowed to return spontaneously to room temperature. After 10 hours at room temperature the suspension was cooled in 3 hours at 5°C. After being kept cool for 2 and a half hours the solid was filtered, washed with 0.1 volumes of chilled water, and dried in a vacuum (80mbar) at 35°C for 6 hours to obtain 51 g of product

KF = 8.10%
LOD = 6.14% (Method 1)
PF = 179-181
^{1}HNMR (D_2O) at dissolution (300 MHz, nt = 4): α anomer/β anomer = 7/93. See Figure 25
^{1}HNMR (D_2O) at equilibrium after one night at room temperature (300 MHz, nt = 4): α anomer/β anomer = 41/59
HPLC = purity 98.4%

Claims

1. 2'-O-Fucosyllactose (2'FL) hydrate with molecular formula

$$C_{18}H_{32}O_{15}*nH_2O$$

wherein n is a number in the range between 1 and 3;
in crystalline form of polymorph A, with n being 3/2, wherein said polymorph A is 2'FL-3/2 H_2O, having characteristic XRPD peaks at 9.89±0.20, 17.05±0.20, 18.86±0.20 2Θ; or
in crystalline form of polymorph B, with n being 5/2, wherein said polymorph B is 2'FL-5/2 H_2O, having characteristic XRPD peaks at 9.96±0.20, 11.90±0.20, 20.48±0.20 2Θ;
wherein the measurements are made using Cu-K-alpha radiation (lambda = 1,5418) in the range 2Θ 4 to 60 °.

2. The crystalline polymorph A according to claim 1, having characteristic XRPD peaks at 18.86±0.20, 9.89±0.20, 17.05±0.20, 21.65±0.2, 14.20±0.20 2Θ.

3. The crystalline polymorph A according to claim 2 having characteristic XRPD peaks as disclosed in table 2

Table 2: List of XRPD peaks for Polymorph A

Pos. [°2Th.]	d-spacing [Å]	Rel. Int. [%]	Pos. [°2Th.]	d-spacing[Å]	Rel Int. [%]
9.5494	9.25416	16.40	24.7910	3.58848	16.98
9.8946	8 93207	87.81	24.9682	3.56342	10.54
11.7744	7.50996	31.79	25.5128	3.48857	14.14
12.7465	6.93932	26.09	25.9598	3.42950	5.68
13.8212	6.40205	21.77	26.1113	3.40996	5.77
14.2037	6.23051	58.63	26.6579	3.34126	2.62
14.5352	6.08914	14.69	27.5941	3 22999	12.98
15.5499	5.69399	31.30	28.6108	3.11747	15.62
15.8620	5.58265	5.89	29.3651	3.03909	12.50
16.1664	5.47824	49.80	30.1835	2.95853	9.56
16.8455	5.25888	23.05	30.6994	2.90998	1.15
17.0532	5.19528	78.13	31.4016	2.84649	1.86
18.8562	4.70239	100.00	32.1642	2 78072	6.24
19,8596	4.46702	12.50	32.6782	2.73814	5.35

(continued)

Pos. [°2Th.]	d-spacing [Å]	Rel. Int. [%]	Pos. [°2Th.]	d-spacing[Å]	Rel Int. [%]
20.1831	4.39615	14.08	33.8253	2.64787	2.14
20.3621	4.35790	42.32	34.0448	2.63129	3.35
20.8790	4.25116	11.55	34.4040	2.60464	7.45
21.6483	4.10181	74.01	34.6906	2.58377	4.41
21.7996	4.07366	54.07	35.3942	2.53400	2.66
22.2975	3.98382	3.66	36.3277	2.47100	4.54
22.9174	3.87744	4.57	36.5727	2 45501	5 22
23.9457	3.71322	14.66	37.0702	2.42319	3.37
24.4818	3.63310	6.05			

4. The crystalline polymorph A according to any one of claims 1-3, wherein the single crystal has a $P2_12_12_1$ spatial group which is arranged within an orthorhombic crystalline system and the elementary cell has the following parameters: a = 12.4098(8) Angstrom, b = 12.737(2) Angstrom, c = 13.756(2) Angstrom and a volume of 2212.5 Angstrom^3.

5. The crystalline polymorph A according to any one of claims 1-4, having beta-anomeric conformation.

6. A method for obtaining the crystalline polymorph A, according to any one of claims 1-5, said method comprising the crystallization of 2'FL from a mixture comprising water and at least one solvent, wherein said solvent may be selected from alcohols, ketones, nitriles, organic acids, esters; wherein the 2'FL is prepared as an aqueous solution at a concentration by weight between 53 and 75 percent at a temperature between 35 and 70 °C and then charged with the solvent warm or at boiling temperature.

7. A method according to claim 6, wherein the solvent is selected from C1 -C3 alcohol.

8. A method according to claim 6 or 7, wherein the quantity of water with respect to the solvent may range between 40 and 10% (v/v).

9. The crystalline polymorph B according to claim 1, having characteristic XRPD peaks at 9.96±0.20, 20.48±0.20, 11.90±0.20, 19.84±0.2, 22.20±0.20 2Θ.

10. The crystalline polymorph B according to claim 9 having characteristic XRPD peaks as disclosed in table 3

Table 3: List of XRPD peaks for Polymorph B

Pos. [°2Th]	d-spacing [Å]	Rel. Int. [%]	Pos. [°2Th.]	d-spacing[Å]	Rel. Int. [%]	
9.1778	9.62805	25.75	22.1968	4.00166	41.97	
9.2632	9.53951	25.64	22.7019	3.91376	9.34	
9.9612	8.87255	100.00	22.9013	3.88014	13.27	
11.6236	7.60706	1971	23.3036	3.81406	15.83	
11.9041	7.42844	43.84	23.9677	3 70985	16.31	
13.8934	6.36897	16.00	24.5568	3.62217	16.76	
14.0269	6.30864	37.88	25.5249	3.48695	11.44	
14.2108	6.22742	47.68	25 9384	3.43228	19.42	
15.1931	5.82692	8.30	26.3721	3.37682	4.65	

(continued)

Pos. [°2Th]	d-spacing [Å]	Rel. Int. [%]	Pos. [°2Th.]	d-spacing[Å]	Rel. Int. [%]	
15.5341	5.69975	34.04	27.0745	3.29079	2.96	
15.8480	5.58757	35.04	28.0196	3.18190	1244	
16.7859	5.27742	39.87	28.8292	3.09436	15.78	
18.5609	4.77655	85.09	29.2610	3.04966	11.39	
19.6786	4 50770	18.05	29.7297	3.00265	5.36	
19.8445	4.47039	43.36	30.1664	2.96017	3.63	
20.4789	4.33330	47.79	30.8471	2.89638	2.04	
20.8612	4.25475	20.12	31.4947	2.83829	5.55	
21.6447	4.10248	8.12	31.6945	2.82085	983	

11. The crystalline polymorph B according to any one of claims 1 and 9-10, wherein the single crystal has a $P2_12_12_1$ spatial group which is arranged within an orthorhombic crystalline system and the elementary cell has the following parameters: a=14.905(7) Angstrom, b = 12.663(6) Angstrom, c = 12.447(5) Angstrom and a volume of 2349.4 Angstrom^3.

12. The crystalline polymorph B according to any one of claims 1 and 9-11, having beta-anomeric conformation.

13. A method for obtaining the crystalline polymorph B, according to any one of claims 1, 9-12, said method comprising the crystallization of 2'FL from an aqueous solution, wherein the method includes preparing a warm 2'FL-aqueous solution to a concentration 50-80% by weight, keeping the solution warm at 30 to 60 °C for 1-12 hours and optionally seeding with polymorph B.

14. The method according to claim 13, wherein the percentage by weight of 2'FL in aqueous solution is in the range between 50 and 80%.

15. 2'-O-Fucosyllactose (2'FL) hydrate according to any one of claims 1-5, 10-12 for use as a medicament or as a nutritional supplement or as a cosmetic ingredient.

16. A pharmaceutical or nutritional or cosmetic composition comprising 2'FL hydrate according to any one of claims 1-5, 10-12.

Patentansprüche

1. 2'-O-Fucosyllactose(2'FL)-hydrat mit der Summenformel

$$C_{18}H_{32}O_{15}*nH_2O,$$

wobei n für eine Zahl im Bereich von 1 bis 3 steht,
in der kristallinen Form der polymorphen Form A, wobei n für 3/2 steht, wobei es sich bei der polymorphen Form A um 2'FL-3/2 H_2O mit charakteristischen Röntgenpulverbeugungspeaks bei 9,89±0,20, 17,05±0,20, 18,86±0,20 2θ handelt, oder
in der kristallinen Form der polymorphen Form B, wobei n für 5/2 steht, wobei es sich bei der polymorphen Form B um 2'FL-5/2 H_2O mit charakteristischen Röntgenpulverbeugungspeaks bei 9,96±0,20, 11,90±0,20, 20,48±0,20 2θ handelt,
wobei die Messungen mit Cu-K-alpha-Strahlung (lambda = 1,5418) im Bereich von 2θ 4 bis 60° durchgeführt werden.

2. Kristalline polymorphe Form A nach Anspruch 1 mit charakteristischen Röntgenpulverbeugungspeaks bei

18,86±0,20, 9,89±0,20, 17,05±0,20, 21,65±0,2, 14,20±0,20 2θ.

3. Kristalline polymorphe Form A nach Anspruch 2 mit charakteristischen Röntgenpulverbeugungspeaks gemäß Tabelle 2

Tabelle 2; Liste von Röntgenpulverbeugungspeaks der polymorphen Form A

Pos. [2Th.]	d-Abstand [Å]	Rel. Int. [%]	Pos. [2Th.]	d-Abstand [Å]	Rel. Int. [%]
9,5494	9,25416	16,40	24,7910	3,58848	16,98
9,8946	8,93207	87,81	24,9682	3,56342	10,54
11,7744	7,50996	31,79	25,5128	3,48857	14,14
12,7465	6,93932	26,09	25,9598	3,42950	5,68
13,8212	6,40205	21,77	26,1113	3,40996	5,77
14,2037	6,23051	58,63	26,6579	3,34126	2,62
14,5352	6,08914	14,69	27,5941	3,22999	12,98
15,5499	5,69399	31,30	28,6108	3,11747	15,62
15,8620	5,58265	5,89	29,3651	3,03909	12,50
16,1664	5,47824	49,80	30,1835	2,95853	9,56
16,8455	5,25888	23,05	30,6994	2,90998	1,15
17,0532	5,19528	78,13	31,4016	2,84649	1,86
18,8562	4,70239	100,00	32,1642	2,78072	6,24
19,8596	4,46702	12,50	32,6782	2,73814	5,35
20,1831	4,39615	14,08	33,8253	2,64787	2,14
20,3621	4,35790	42,32	34,0448	2,63129	3,35
20,8790	4,25116	11,55	34,4040	2,60464	7,45
21,6483	4,10181	74,01	34,6906	2,58377	4,41
21,7996	4,07366	54,07	35,3942	2,53400	2,66
22,2975	3,98382	3,66	36,3277	2,47100	4,54
22,9174	3,87744	4,57	36,5727	2,45501	5,22
23,9457	3,71322	14,66	37,0702	2,42319	3,37
24,4818	3,63310	6,05			

4. Kristalline polymorphe Form A nach einem der Ansprüche 1-3, wobei der Einkristall eine $P2_12_12_1$-Raumgruppe aufweist, die in einem orthorhombischen Kristallsystem angeordnet ist, und die Elementarzelle die folgenden Parameter aufweist: a = 12,4098(8) Ångström, b = 12,737(2) Ångström, c = 13,756(2) Ängström und ein Volumen von 2212,5 Ångström^3.

5. Kristalline polymorphe Form A nach einem der Ansprüche 1-4 mit beta-anomerer Konformation.

6. Verfahren zum Erhalt der kristallinen polymorphen Form A nach einem der Ansprüche 1-5, wobei das Verfahren das Kristallisieren von 2'FL aus einer Wasser und mindestens ein Lösungsmittel umfassenden Mischung umfasst, wobei das Lösungsmittel aus Alkoholen, Ketonen, Nitrilen, organischen Säuren, Estern ausgewählt sein kann, wobei das 2'FL als eine wässrige Lösung in einer Gewichtskonzentration zwischen 53 und 75% bei einer Temperatur zwischen 35 und 70°C hergestellt und dann warm oder bei Siedetemperatur mit dem Lösungsmittel versetzt wird.

7. Verfahren nach Anspruch 6, wobei das Lösungsmittel aus C1-C3-Alkoholen ausgewählt ist.

8. Verfahren nach Anspruch 6 oder 7, wobei die Menge an Wasser, bezogen auf das Lösungsmittel, im Bereich von 40 bis 10 Vol.-% liegen kann.

9. Kristalline polymorphe Form B nach Anspruch 1 mit charakteristischen Röntgenpulverbeugungspeaks bei 9,96±0,20, 20,48±0,20, 11,90±0,20, 19,84±0,2, 22,20±0,20 2θ.

10. Kristalline polymorphe Form B nach Anspruch 9 mit charakteristischen Röntgenpulverbeugungspeaks gemäß Tabelle 3

Tabelle 3: Liste der Röntgenpulverbeugungspeaks der polymorphen Form B.

Pos. [2Th.]	d-Abstand [Å]	Rel. Int. [%]	Pos. [2Th.]	d-Abstand [Å]	Rel. Int. [%]
9,1778	9,62805	25,75	22,1968	4,00166	41,97
9,2632	9,53951	25,64	22,7019	3,91376	9,34
9,9612	8,87255	100,00	22,9013	3,88014	13,27
11,6236	7,60706	19,71	23,3036	3,81406	15,83
11,9041	7,42844	43,84	23,9677	3,70985	16,31
13,8934	6,36897	16,00	24,5568	3,62217	16,76
14,0269	6,30864	37,88	25,5249	3,48695	11,44
14,2108	6,22742	47,68	25,9384	3,43228	19,42
15,1931	5,82692	8,30	26,3721	3,37682	4,65
15,5341	5,69975	34,04	27,0745	3,29079	2,96
15,8480	5,58757	35,04	28,0196	3,18190	12,44
16,7859	5,27742	39,87	28,8292	3,09436	15,78
18,5609	4,77655	85,09	29,2610	3,04966	11,39
19,6786	4,50770	18,05	29,7297	3,00265	5,36
19,8445	4,47039	43,36	30,1664	2,96017	3,63
20,4789	4,33330	47,79	30,8471	2,89638	2,04
20,8612	4,25475	20,12	31,4947	2,83829	5,55
21,6447	4,10248	8,12	31,6945	2,82085	9,83

11. Kristalline polymorphe Form B nach einem der Ansprüche 1 und 9-10, wobei der Einkristall eine $P2_12_12_1$-Raumgruppe aufweist, die in einem orthorhombischen Kristallsystem angeordnet ist, und die Elementarzelle die folgenden Parameter aufweist: a = 14,905(7) Ångström, b = 12,663(6) Ångström, c = 12,447(5) Ängström und ein Volumen von 2349,4 Ångström^3.

12. Kristalline polymorphe Form B nach einem der Ansprüche 1 und 9-11 mit beta-anomerer Konformation.

13. Verfahren zum Erhalt der kristallinen polymorphen Form B nach einem der Ansprüche 1, 9-12, wobei das Verfahren das Kristallisieren von 2'FL aus einer wässrigen Lösung umfasst, wobei das Verfahren die Herstellung einer warmen wässrigen 2'FL-Lösung mit einer Konzentration von 50-80 Gew.-%, das 1-12-stündige Warmhalten der Lösung bei 30-60°C und gegebenenfalls das Animpfen mit der polymorphen Form B umfasst.

14. Verfahren nach Anspruch 13, wobei der Gewichtsprozentsatz von 2'FL in der wässrigen Lösung im Bereich zwischen 50 und 80% liegt.

15. 2'O-Fucosyllactose(2'FL)-hydrat gemäß einem der Ansprüche 1-5, 10-12 zur Verwendung als Medikament oder als Nahrungsergänzungsstoff oder als kosmetischer Inhaltsstoff.

16. Pharmazeutische oder alimentäre oder kosmetische Zusammensetzung, umfassend 2'FL-hydrat gemäß einem der Ansprüche 1-5, 10-12.

Revendications

1. Hydrate de 2'-O-fucosyllactose (2'FL) doté d'une formule moléculaire

$$C_{18}H_{32}O_{15}*nH_2O$$

n étant un nombre dans la plage entre 1 et 3 ;
sous forme cristalline de polymorphe A, n étant 3/2, ledit polymorphe A étant 2'FL-3/2H_2O, possédant des pics caractéristiques de XRPD à 9,89 ± 0,20, 17,05 ± 0,20, 18,86 ± 0,20 2θ ; ou
sous forme cristalline de polymorphe B, n étant 5/2, ledit polymorphe B étant 2'FL-5/2H_2O, possédant des pics caractéristiques de XRPD à 9,96 ± 0,20, 11,90 ± 0,20, 20,48 ± 0,20 2θ ;
les mesures étant faites à l'aide de rayonnement Cu-K-alpha (lambda = 1,5418) dans la plage 2θ de 4 à 60°.

2. Polymorphe cristallin A selon la revendication 1, possédant des pics caractéristiques de XRPD à 18,86 ± 0,20, 9,89 ± 0,20, 17,05 ± 0,20, 21,65 ± 0,20, 14,20 ± 0,20 2θ.

3. Polymorphe cristallin A selon la revendication 2 possédant des pics caractéristiques de XRPD tels que présentés dans le tableau 2

Tableau 2 : liste des pics de XRPD pour le polymorphe A.

Pos. [°2th.]	Espacemen t d [Å]	Int. rel. [%]	Pos. [°2th.]	Espacemen t d [Å]	Int. rel. [%]
9,5494	9,25416	16,40	24,791 0	3,58848	16,9 8
9,8946	8,93207	87,81	24,968 2	3,56342	10,5 4
11,774 4	7,50996	31,79	25,512 8	3,48857	14,1 4
12,746 5	6,93932	26,09	25,959 8	3,42950	5,68
13,821 2	6,40205	21,77	26,111 3	3,40996	5,77
14,203 7	6,23051	58,63	26,657 9	3,34126	2,62
14,535 2	6,08914	14,69	27,594 1	3,22999	12,9 8
15,549 9	5,69399	31,30	28,610 8	3,11747	15,6 2
15,862 0	5,58265	5,89	29,365 1	3,03909	12,5 0
16,166 4	5,47824	49,80	30,183 5	2,95853	9,56
16,845 5	5,25888	23,05	30,699 4	2,90998	1,15
17,053 2	5,19528	78,13	31,401 6	2,84649	1,86
18,856 2	4,70239	100,0 0	32,164 2	2,78072	6,24
19,859 6	4,46702	12,50	32,678 2	2,73814	5,35
20,183 1	4,39615	14,08	33,825 3	2,64787	2,14
20,362 1	4,35790	42,32	34,044 8	2,63129	3,35
20,879 0	4,25116	11,55	34,404 0	2,60464	7,45
21,648 3	4,10181	74,01	34,690 6	2,58377	4,41
21,799 6	4,07366	54,07	35,394 2	2,53400	2,66
22,297 5	3,98382	3,66	36,327 7	2,47100	4,54
22,917 4	3,87744	4,57	36,572 7	2,45501	5,22

(suite)

Pos. [°2th.]	Espacemen t d [Å]	Int. rel. [%]	Pos. [°2th.]	Espacemen t d [Å]	Int. rel. [%]
23,945 7	3,71322	14,66	37,070 2	2,42319	3,37
24,481 8	3,63310	6,05			

4. Polymorphe cristallin A selon l'une quelconque des revendications 1 à 3, le monocristal possédant un groupe spatial $P2_12_12_1$ qui est agencé dans un système cristallin orthorhombique et la maille élémentaire possédant les paramètres suivants : a = 12,4098(8) angströms, b = 12,737(2) angströms, c = 13,756(2) angströms et un volume de 2 212,5 angströms^3.

5. Polymorphe cristallin A selon l'une quelconque des revendications 1 à 4, possédant une conformation bêta-anomérique.

6. Procédé pour l'obtention du polymorphe cristallin A, selon l'une quelconque des revendications 1 à 5, ledit procédé comprenant la cristallisation de 2'FL d'un mélange comprenant de l'eau et au moins un solvant, ledit solvant pouvant être choisi parmi des alcools, des cétones, des nitriles, des acides organiques, des esters ; le 2'FL étant préparé en tant qu'une solution aqueuse à une concentration en poids comprise entre 53 et 75 pour cent à une température comprise entre 35 et 70 °C et ensuite chargé avec le solvant chaud ou à température d'ébullition.

7. Procédé selon la revendication 6, le solvant étant choisi parmi un alcool en C_{1-3}.

8. Procédé selon la revendication 6 ou 7, la quantité d'eau par rapport au solvant pouvant se situer dans la plage comprise entre 40 et 10 % (v/v).

9. Polymorphe cristallin B selon la revendication 1, possédant des pics caractéristiques de XRPD à 9,96 $\pm$ 0,20, 20,48 $\pm$ 0,20, 11,90 $\pm$ 0,20, 19,84 $\pm$ 0,2, 22,20 $\pm$ 0,20 2θ.

10. Polymorphe cristallin B selon la revendication 9 possédant des pics caractéristiques de XRPD tels que présentés dans le tableau 3

Tableau 3 : liste des pics de XRPD pour le polymorphe B.

Pos. [°2th.]	Espacemen t d [Å]	Int. rel. [%]	Pos. [°2th.]	Espacemen t d [Å]	Int. rel. [%]
9,1778	9,62805	25,75	22,196 8	4,00166	41,9 7
9,2632	9,53951	25,64	22,701 9	3,91376	9,34
9,9612	8,87255	100,0 0	22,901 3	3,88014	13,2 7
11,623 6	7,60706	19,71	23,303 6	3,81406	15,8 3
11,904 1	7,42844	43,84	23,967 7	3,70985	16,3 1
13,893 4	6,36897	16,00	24,556 8	3,62217	16,7 6
14,026 9	6,30864	37,88	25,524 9	3,48695	11,4 4
14,210 8	6,22742	47,68	25,938 4	3,43228	19,4 2
15,193 1	5,82692	8,30	26,372 1	3,37682	4,65
15,534 1	5,69975	34,04	27,074 5	3,29079	2,96
15,848 0	5,58757	35,04	28,019 6	3,18190	12,4 4
16,785 9	5,27742	39, 87	28,829 2	3,09436	15,7 8
18,560 9	4,77655	85,09	29,261 0	3,04966	11,3 9
19,678 6	4,50770	18,05	29,729 7	3,00265	5,36
19,844 5	4,47039	43,36	30,166 4	2,96017	3,63

(suite)

Pos. [°2th.]	Espacemen t d [Å]	Int. rel. [%]	Pos. [°2th.]	Espacemen t d [Å]	Int. rel. [%]
20,478 9	4,33330	47,79	30,847 1	2,89638	2,04
20,861 2	4,25475	20,12	31,494 7	2,83829	5,55
21,644 7	4,10248	8,12	31,694 5	2,82085	9,83

11. Polymorphe cristallin B selon l'une quelconque des revendications 1 et 9 et 10, le monocristal possédant un groupe spatial $P2_12_12_1$ qui est agencé dans un système cristallin orthorhombique et la maille élémentaire possédant les paramètres suivants : a = 14,905(7) angströms, b = 12,663(6) angströms, c = 12,447(5) angströms et un volume de 2 349,4 angströms^3.

12. Polymorphe cristallin B selon l'une quelconque des revendications 1 et 9 à 11, possédant une conformation bêta-anomérique.

13. Procédé pour l'obtention du polymorphe cristallin B, selon l'une quelconque des revendications 1, 9 à 12, ledit procédé comprenant la cristallisation de 2'FL à partir d'une solution aqueuse, le procédé comportant la préparation d'une solution aqueuse chaude de 2'FL à une concentration de 50 à 80 % en poids, le maintien de la solution chaude à une température de 30 à 60 °C pendant 1 à 12 heures et éventuellement l'ensemencement avec le polymorphe B.

14. Procédé selon la revendication 13, le pourcentage en poids de 2'FL dans la solution aqueuse se situant dans la plage comprise entre 50 et 80 %.

15. Hydrate de 2'-O-fucosyllactose (2'FL) selon l'une quelconque des revendications 1 à 5, 10 à 12 pour une utilisation en tant que médicament ou en tant que supplément nutritionnel ou en tant qu'ingrédient cosmétique.

16. Composition pharmaceutique ou nutritionnelle ou cosmétique comprenant de l'hydrate de 2'FL selon l'une quelconque des revendications 1 à 5, 10 à 12.

FIG. 1

XRPD Polymorph A (Example 1)

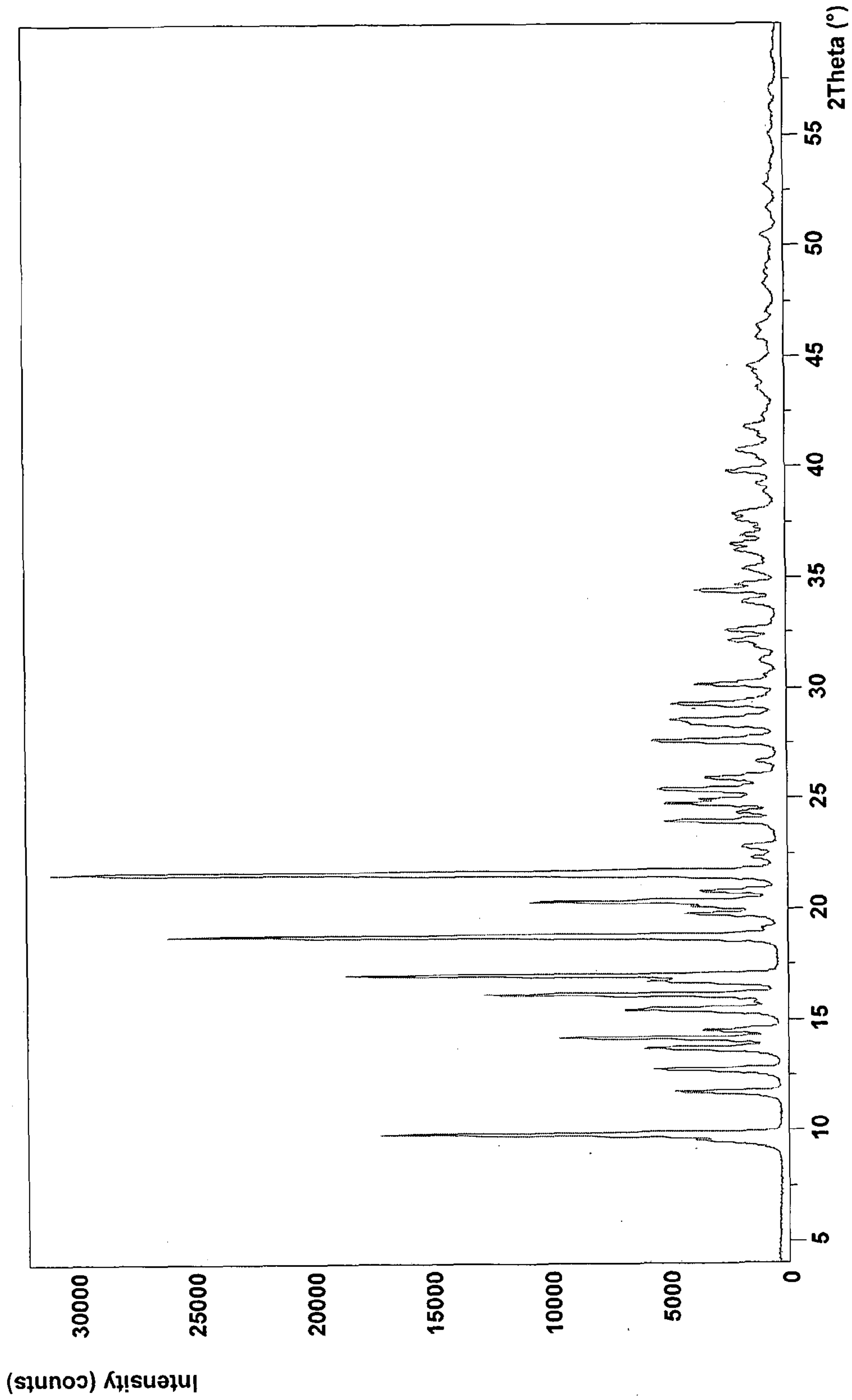


FIG. 2

IR spectrum Polymorph A (Example 2)

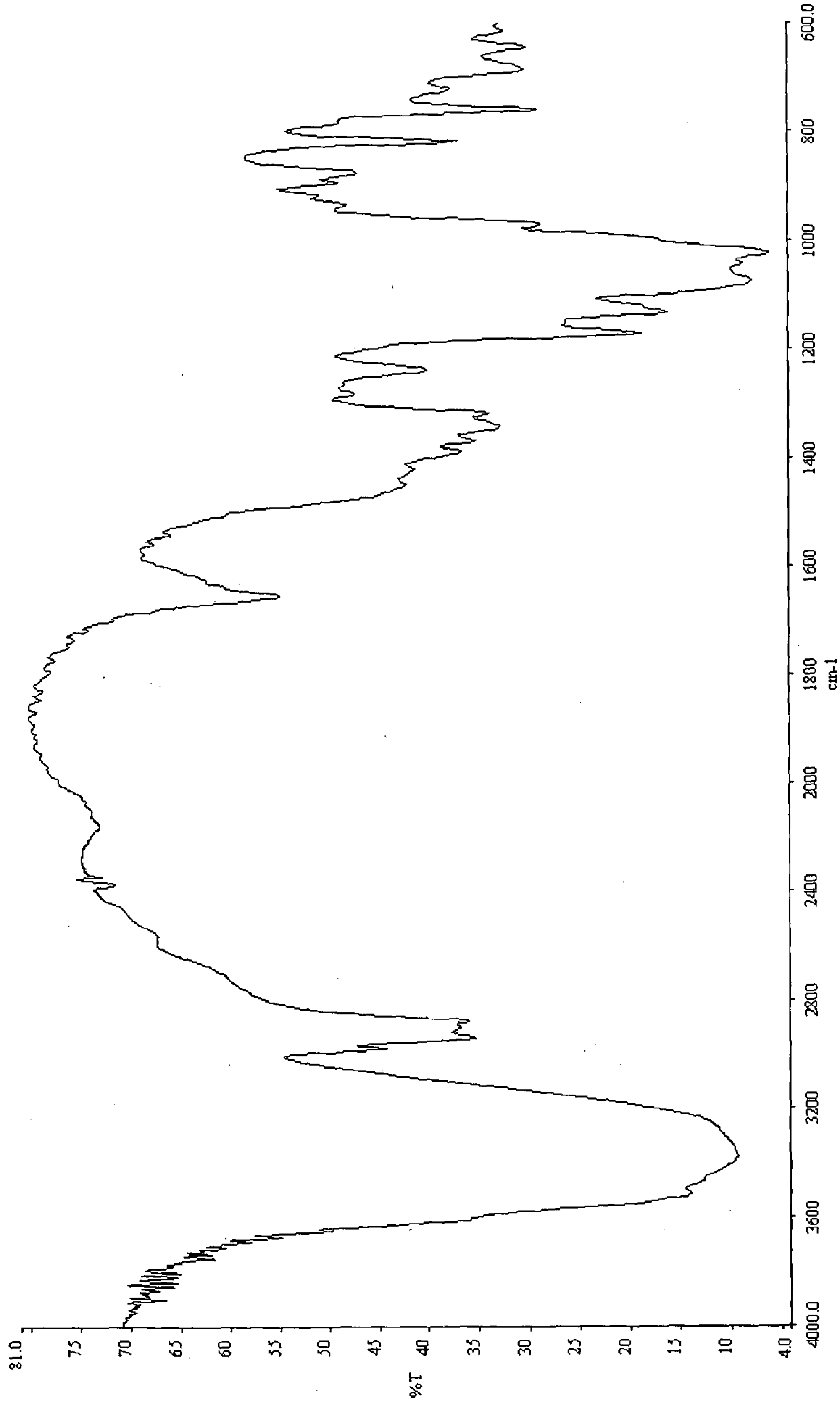


FIG. 3
DSC Polymorph A (Example 2)

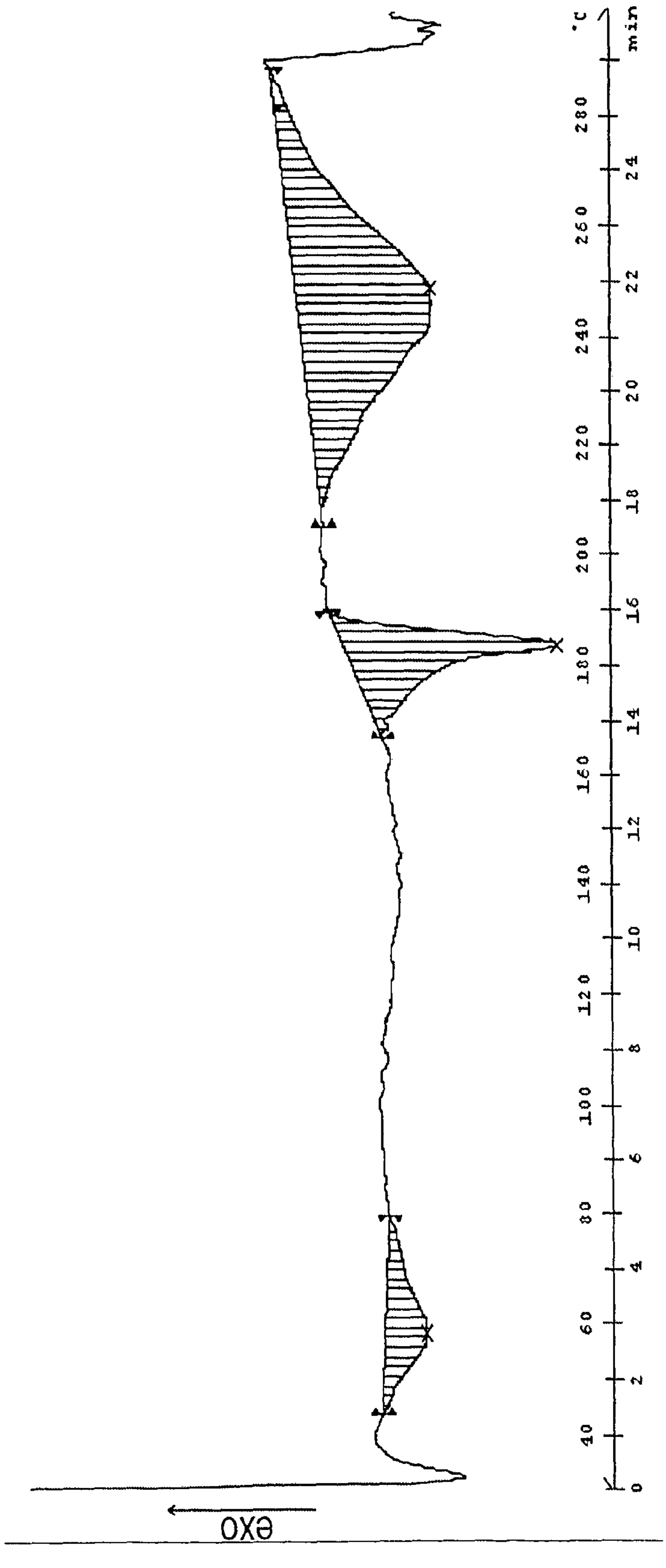


FIG. 4

XRPD Polymorph A (Example 2)

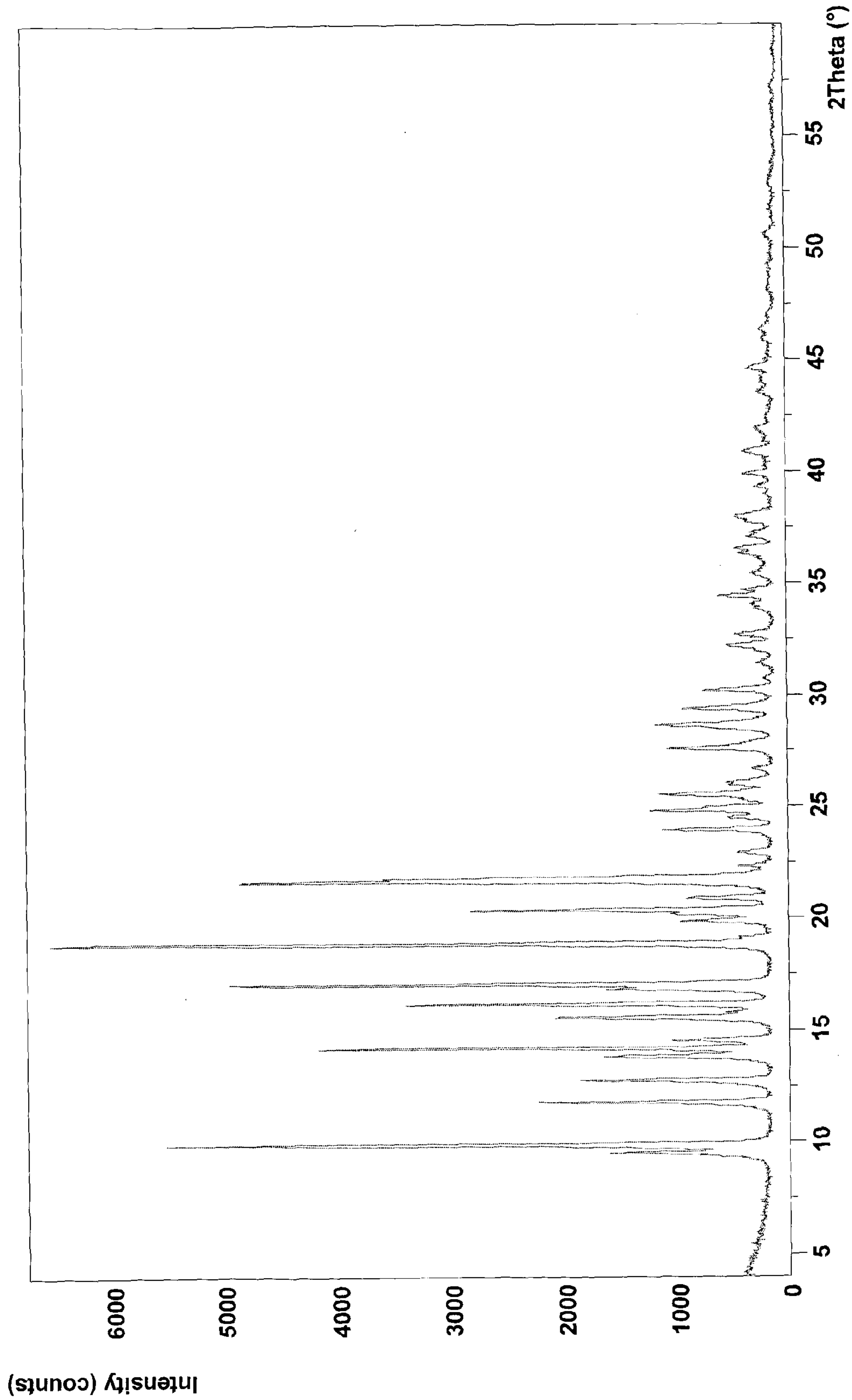


FIG. 5

ORTEP diagram of the structure from XRPD single crystal Polymorph A (Example 2)

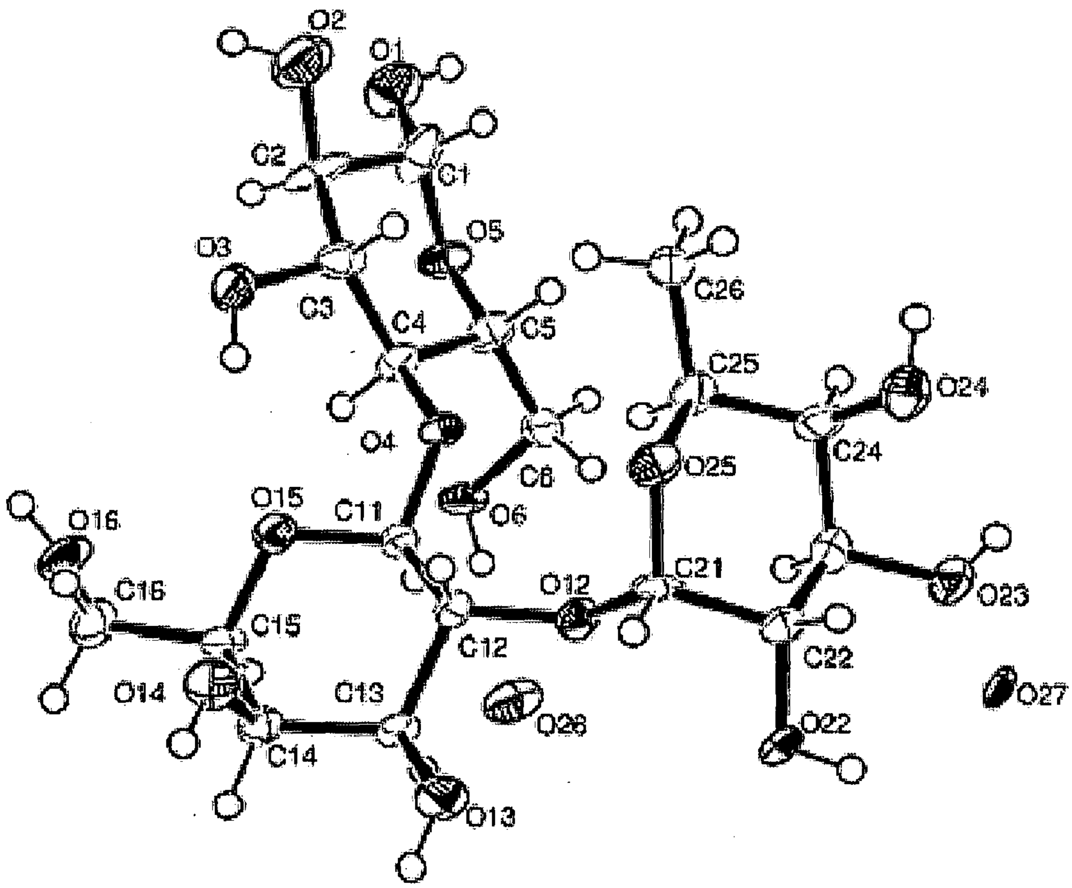


FIG. 6
TGA and DTA Polymorph A (Example 2)

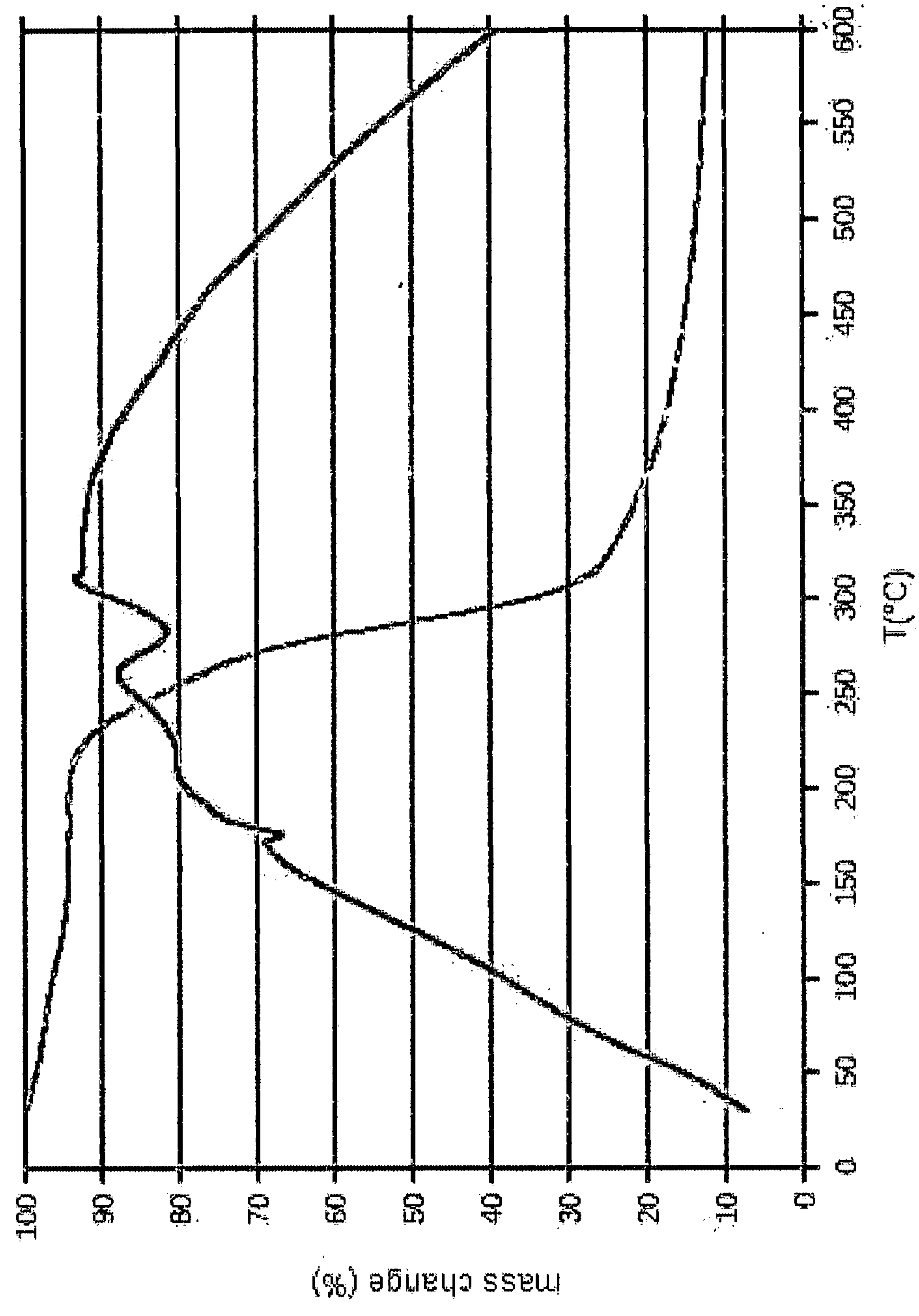


FIG. 7

XRPD Polymorph A (Example 4)

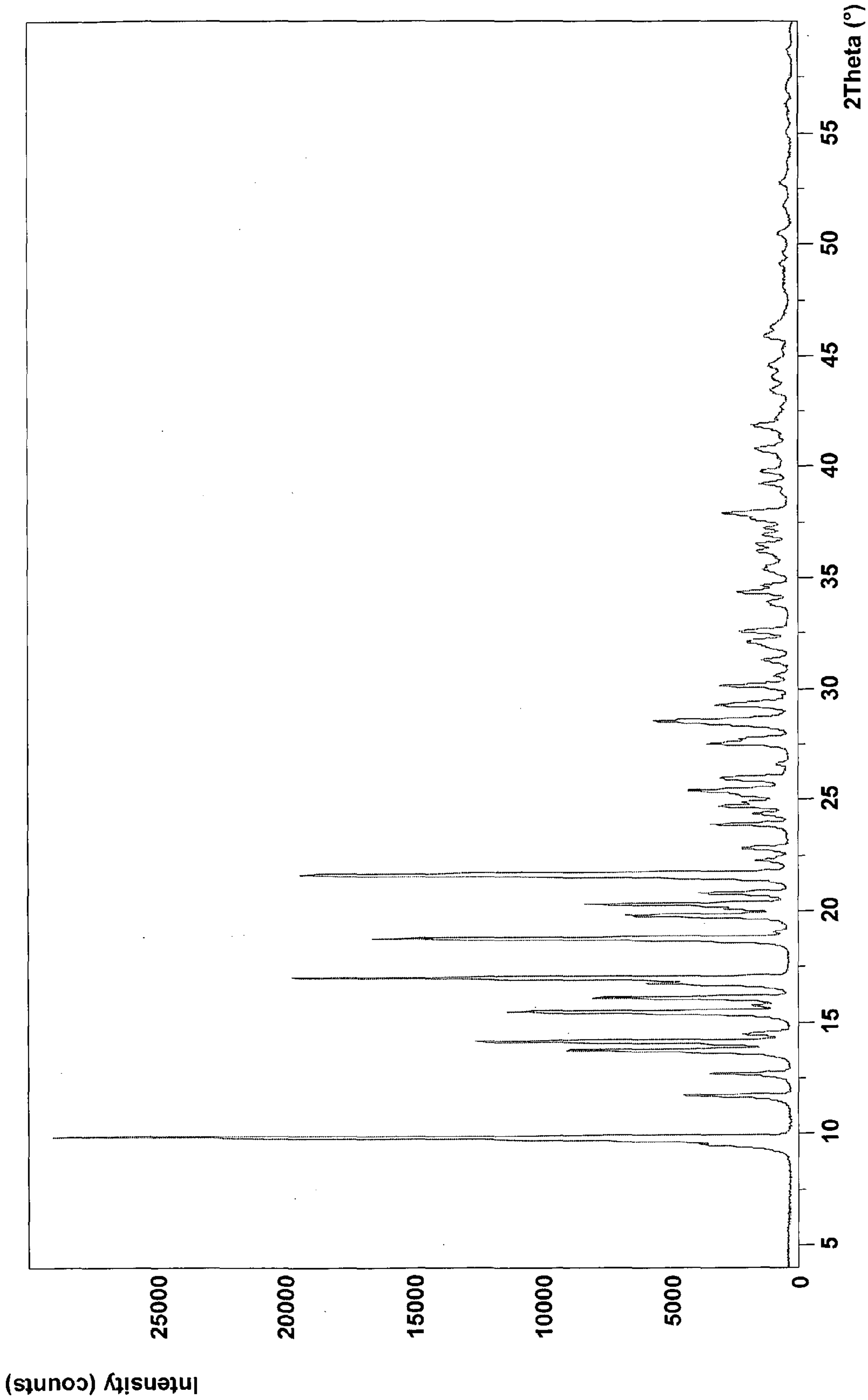


FIG. 8

XRPD Polymorph A (Example 6)

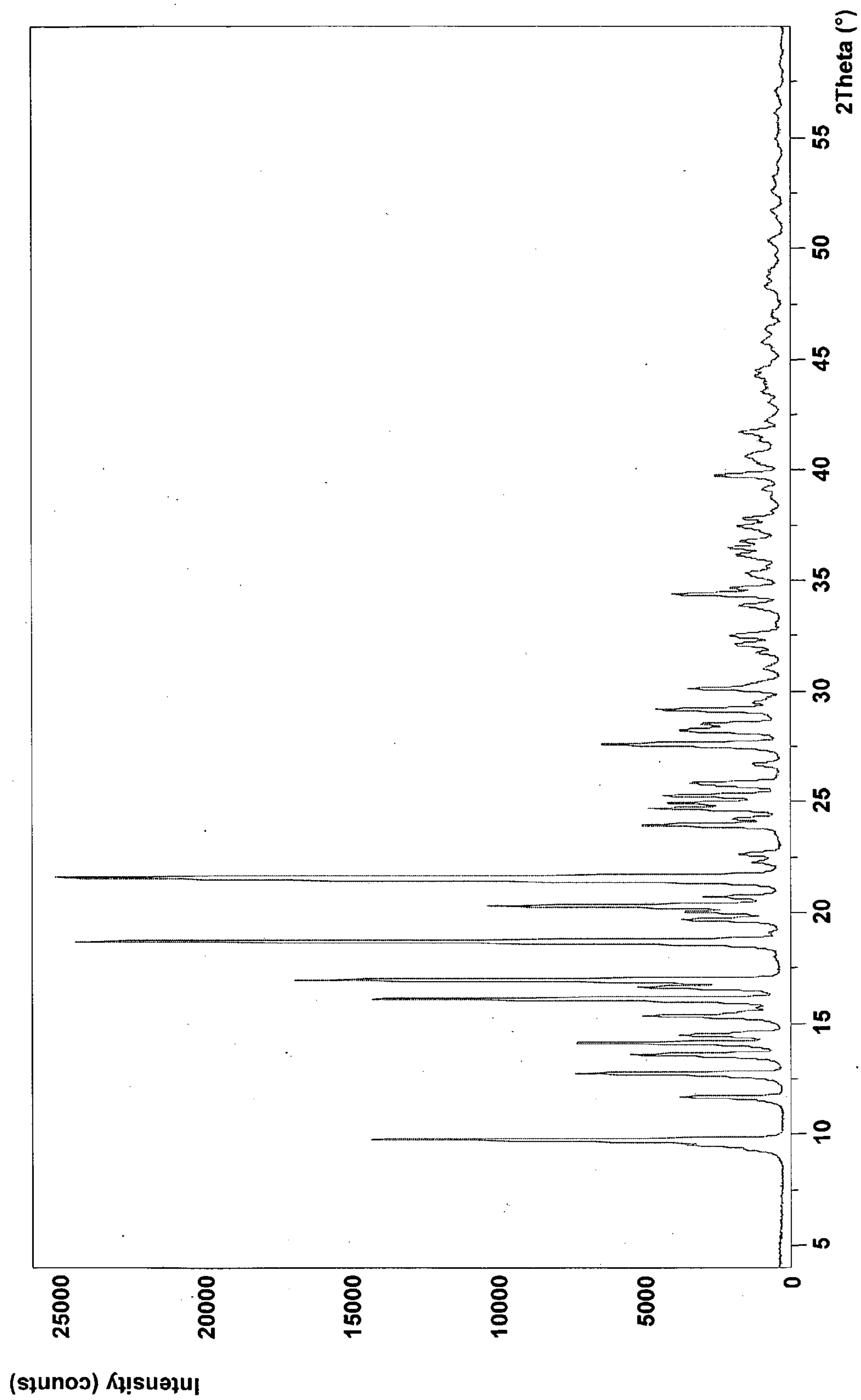


FIG. 9
IR spectrum Polymorph A (Example 6)

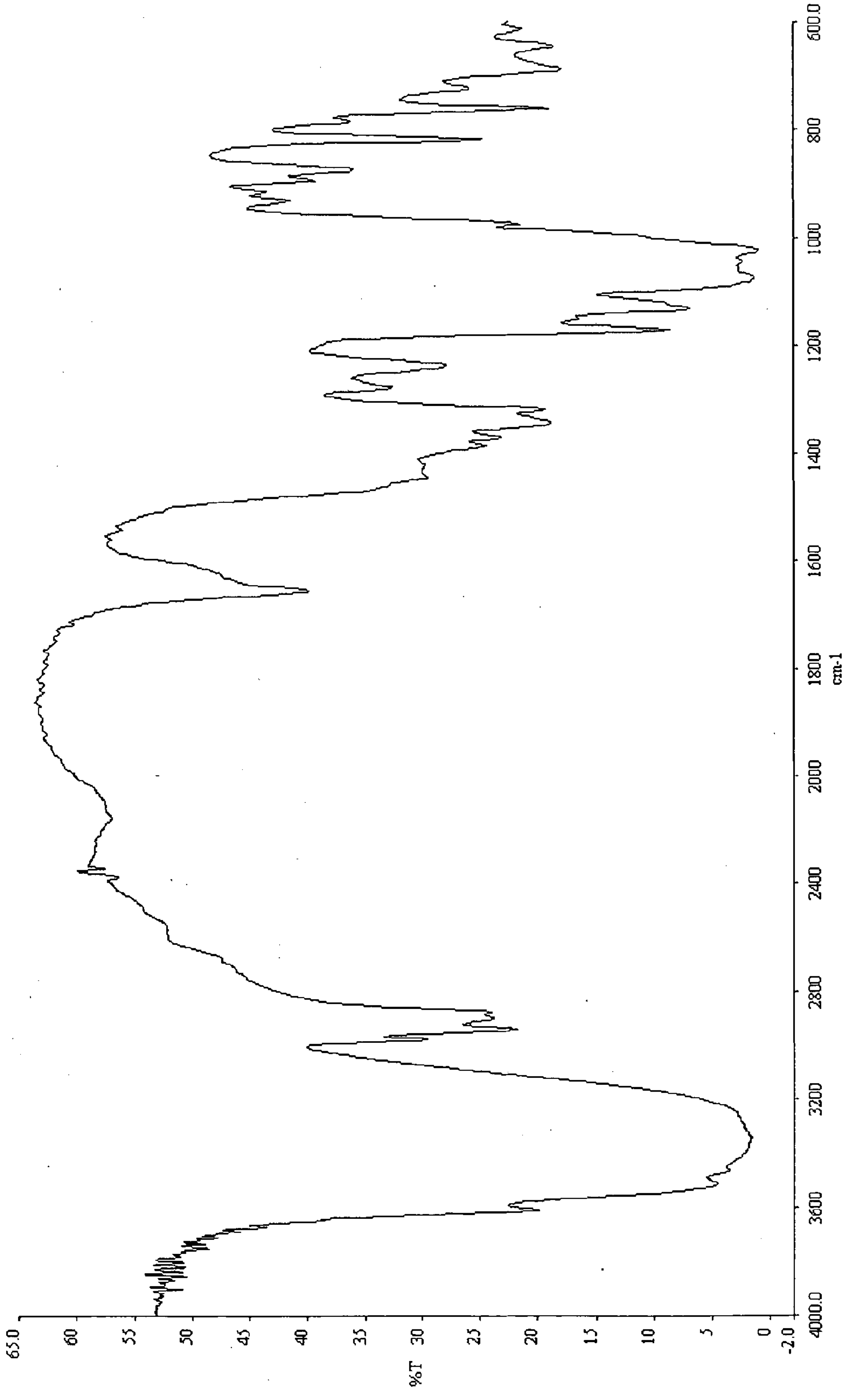


FIG. 10

XRPD Polymorph A (Example 7)

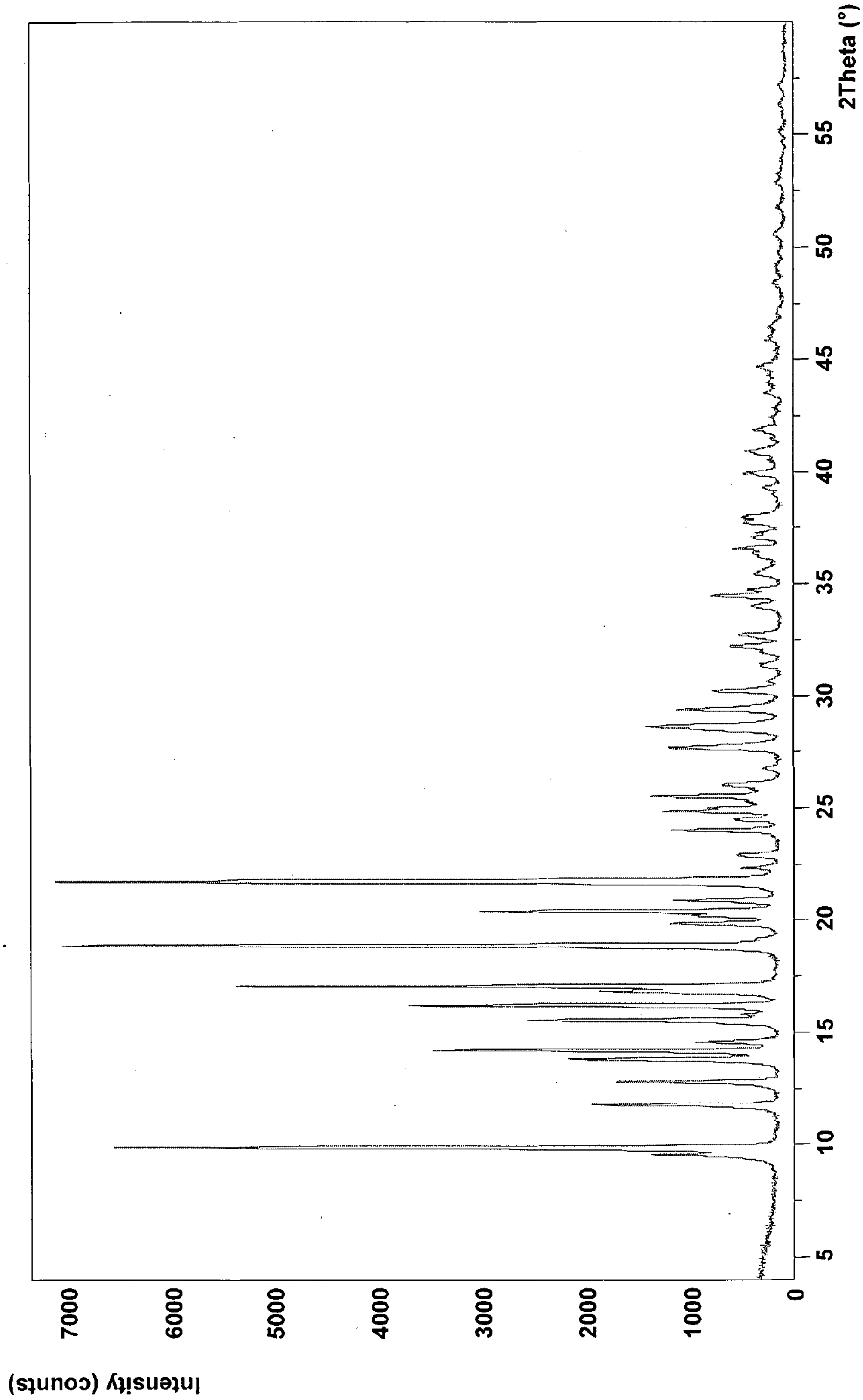


FIG. 11
IR spectrum Polymorph A (Example 7)

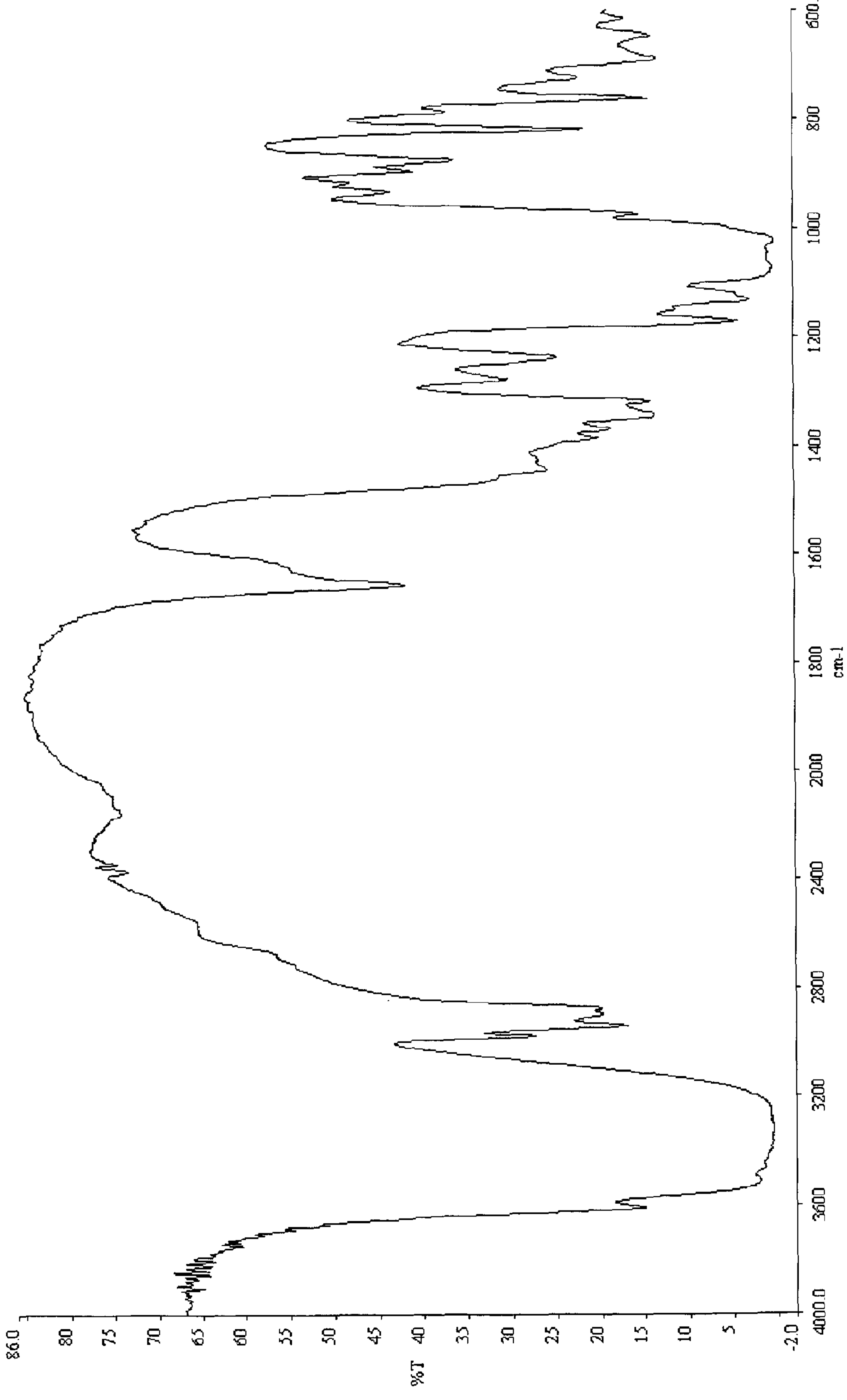


FIG. 12

IR spectrum Polymorph A (Example 8)

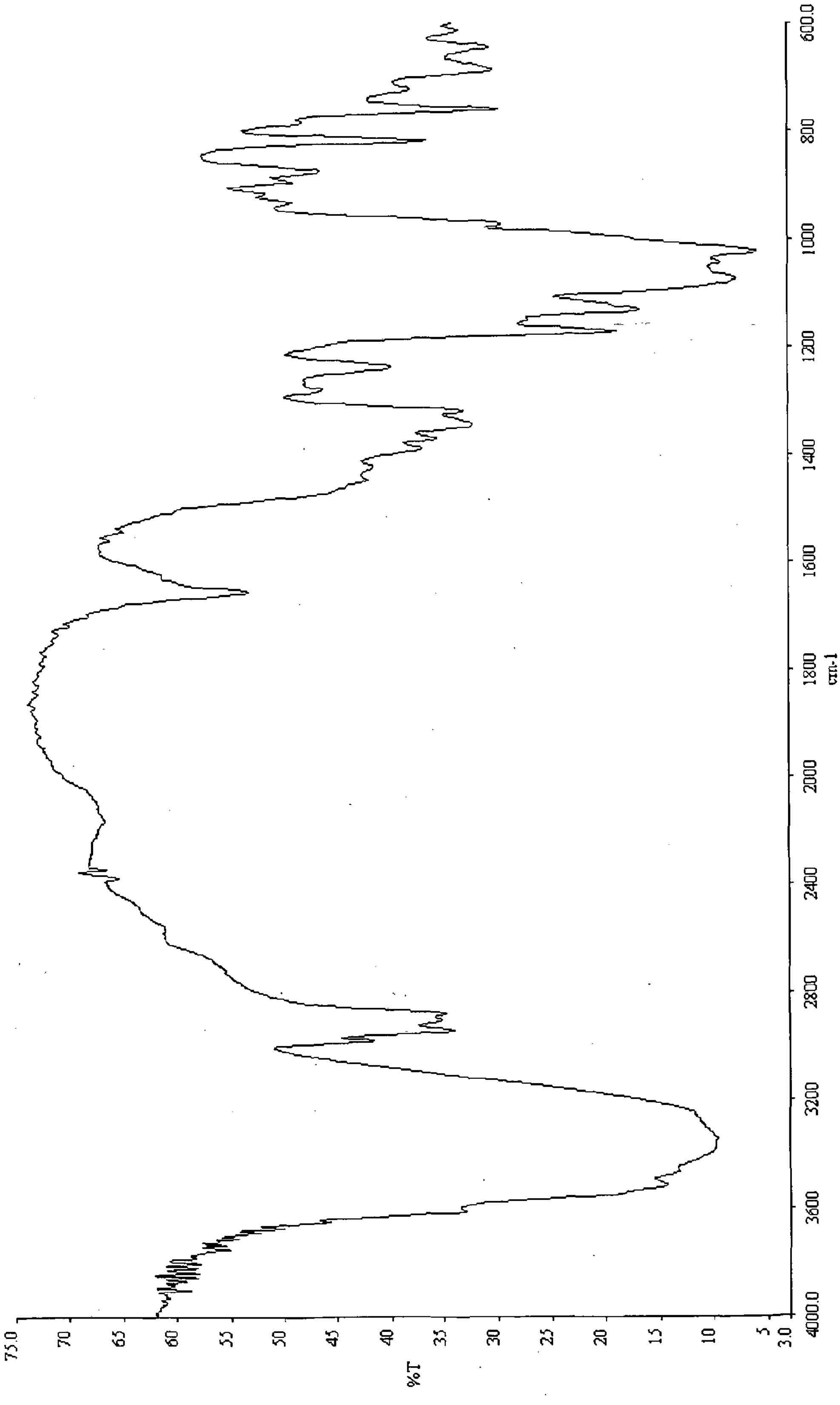


FIG. 13
XRPD Polymorph A (Example 8)

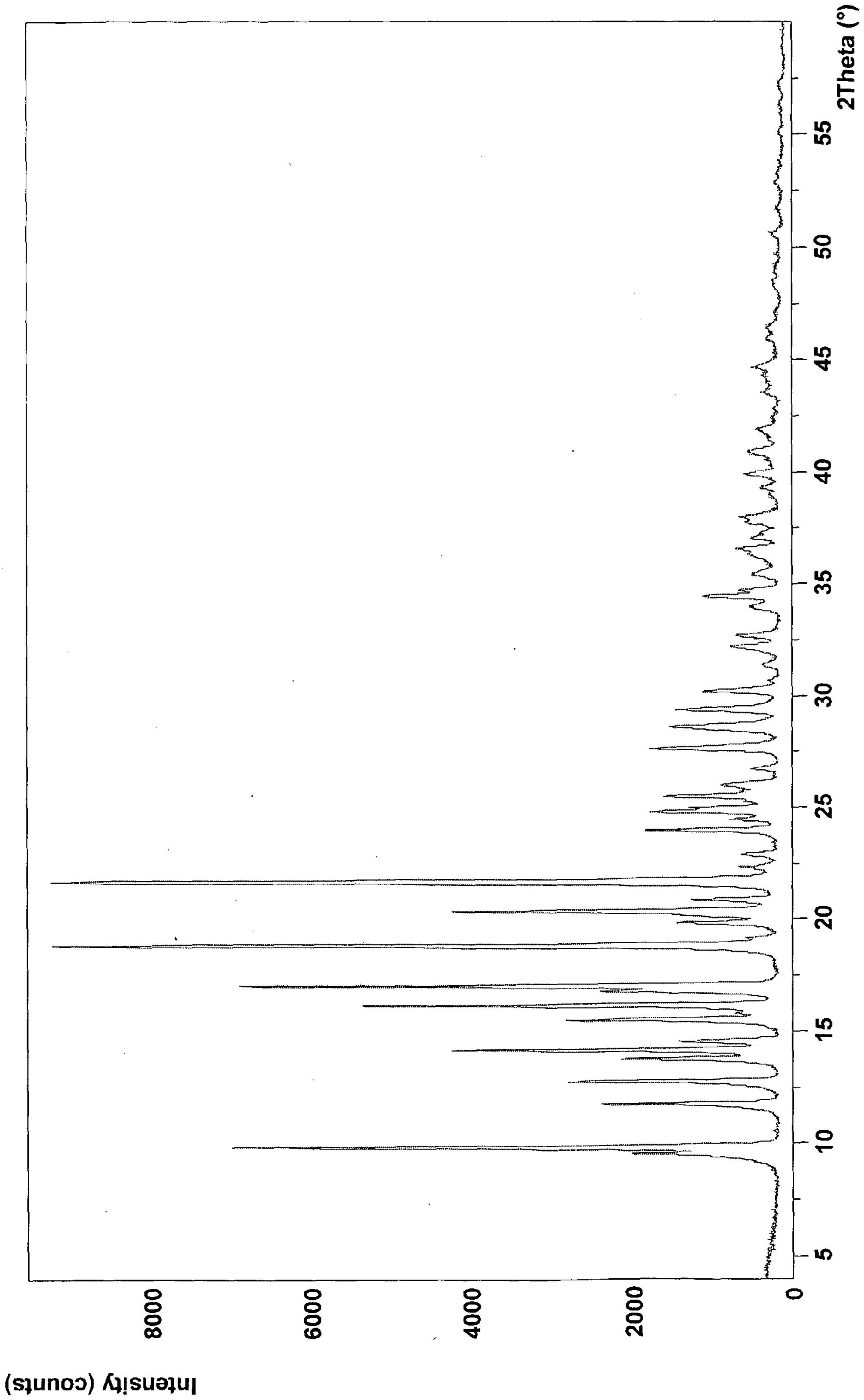


FIG. 14
XRPD Polymorph A (Example 9)

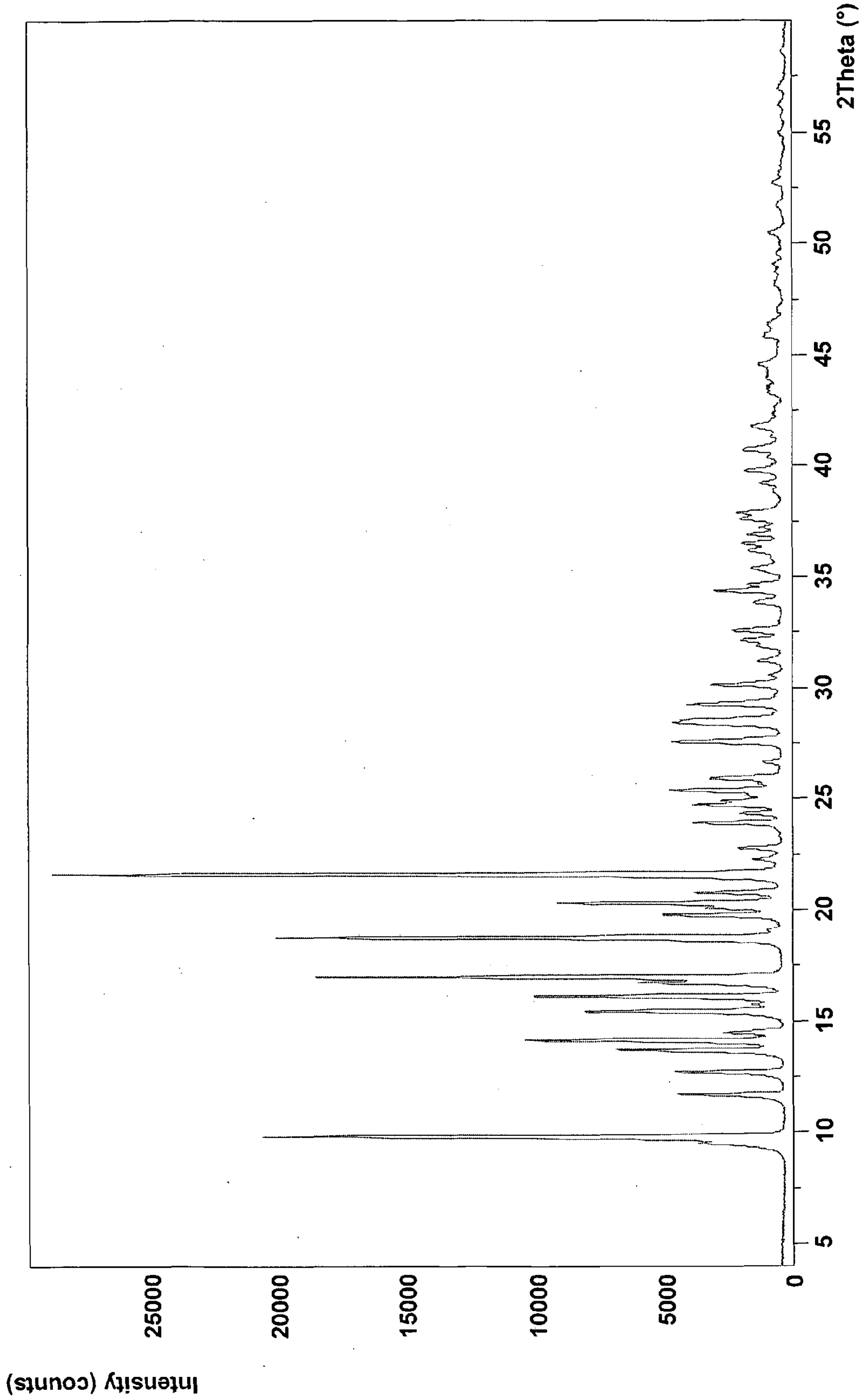


FIG.15
XRPD Polymorph A (Example 10)

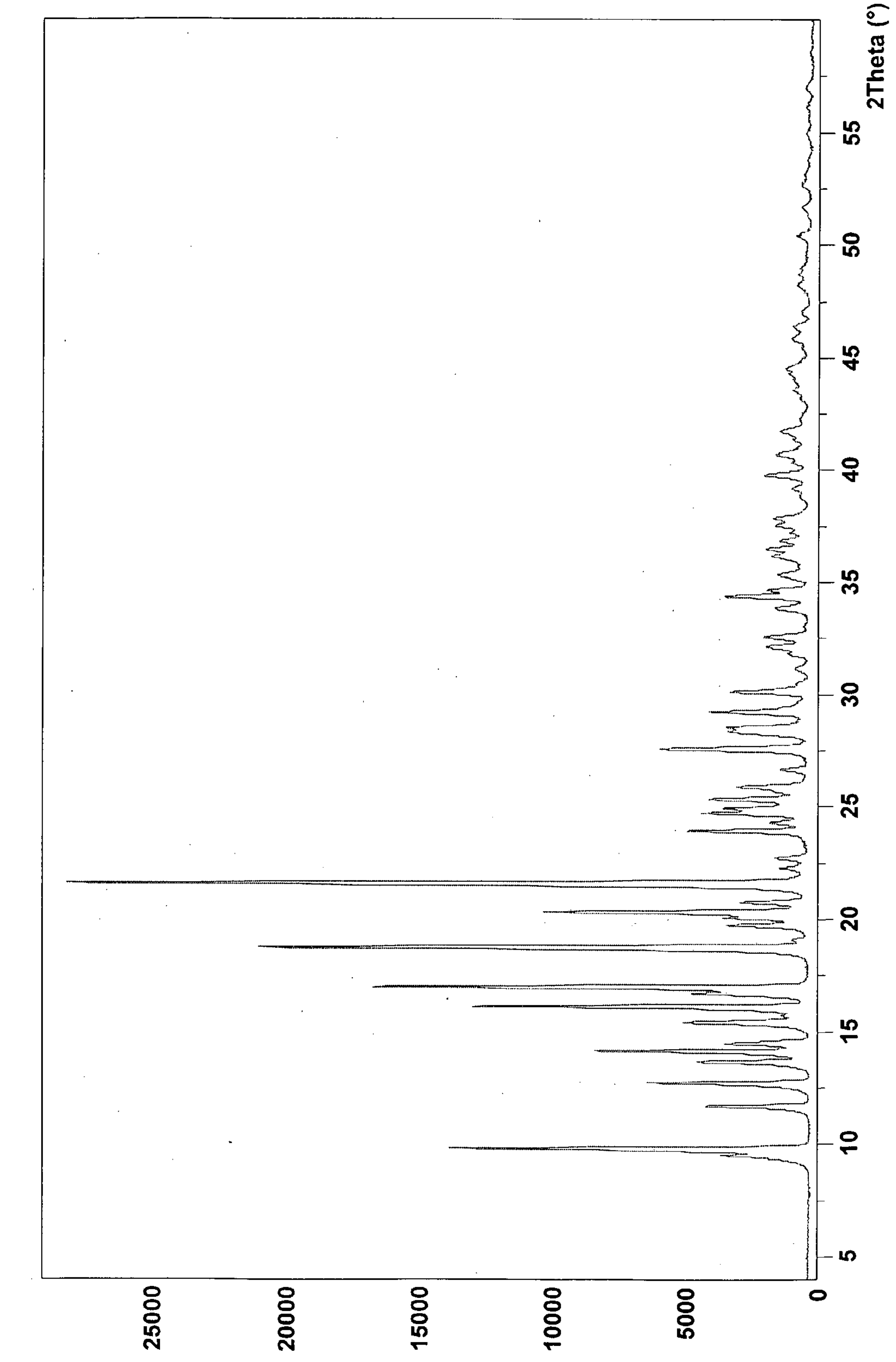


FIG.16

^{1}HNMR Polymorph A (Example 11)

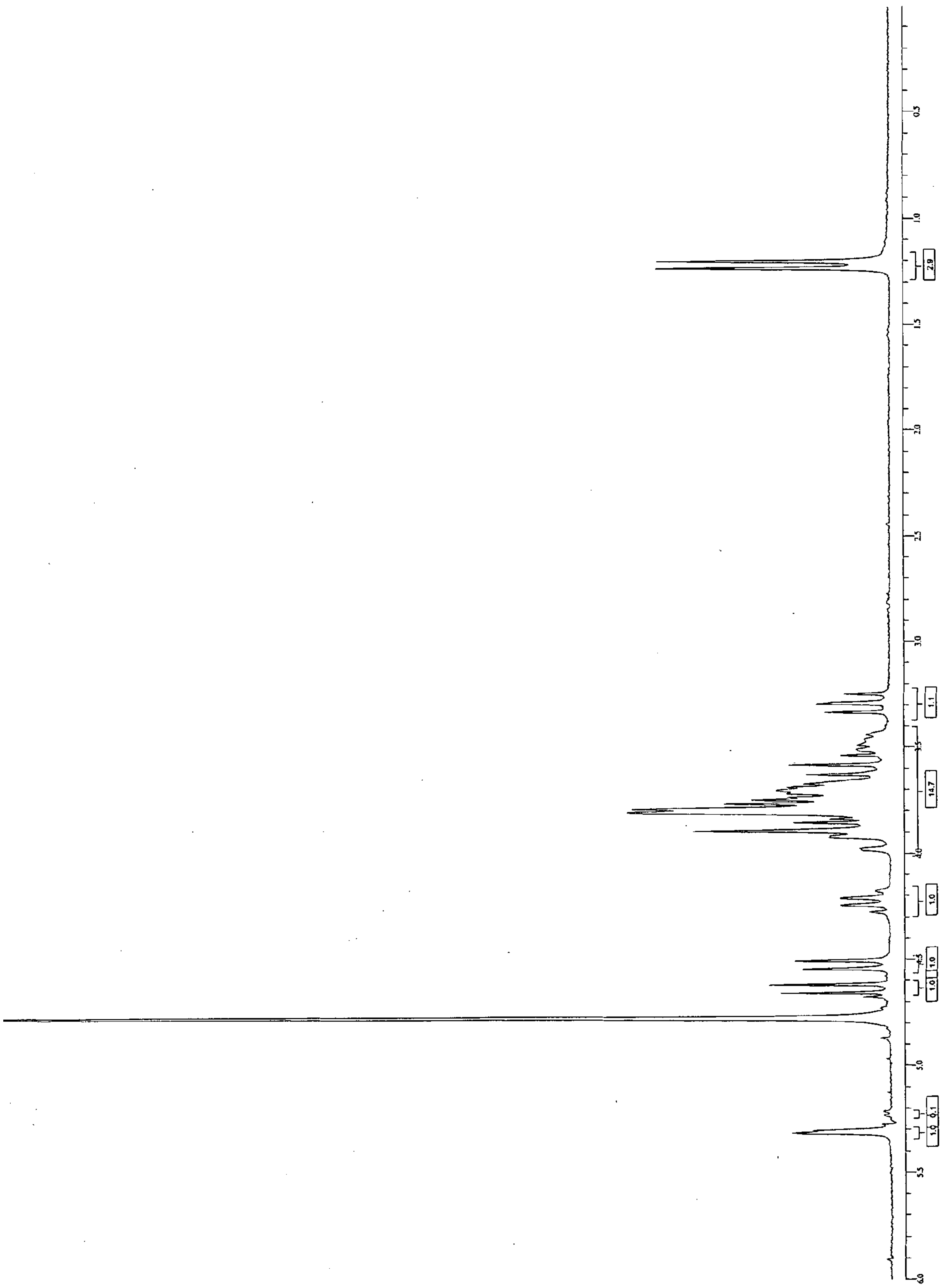


FIG. 17
XRPD Polymorph A (Example 13)

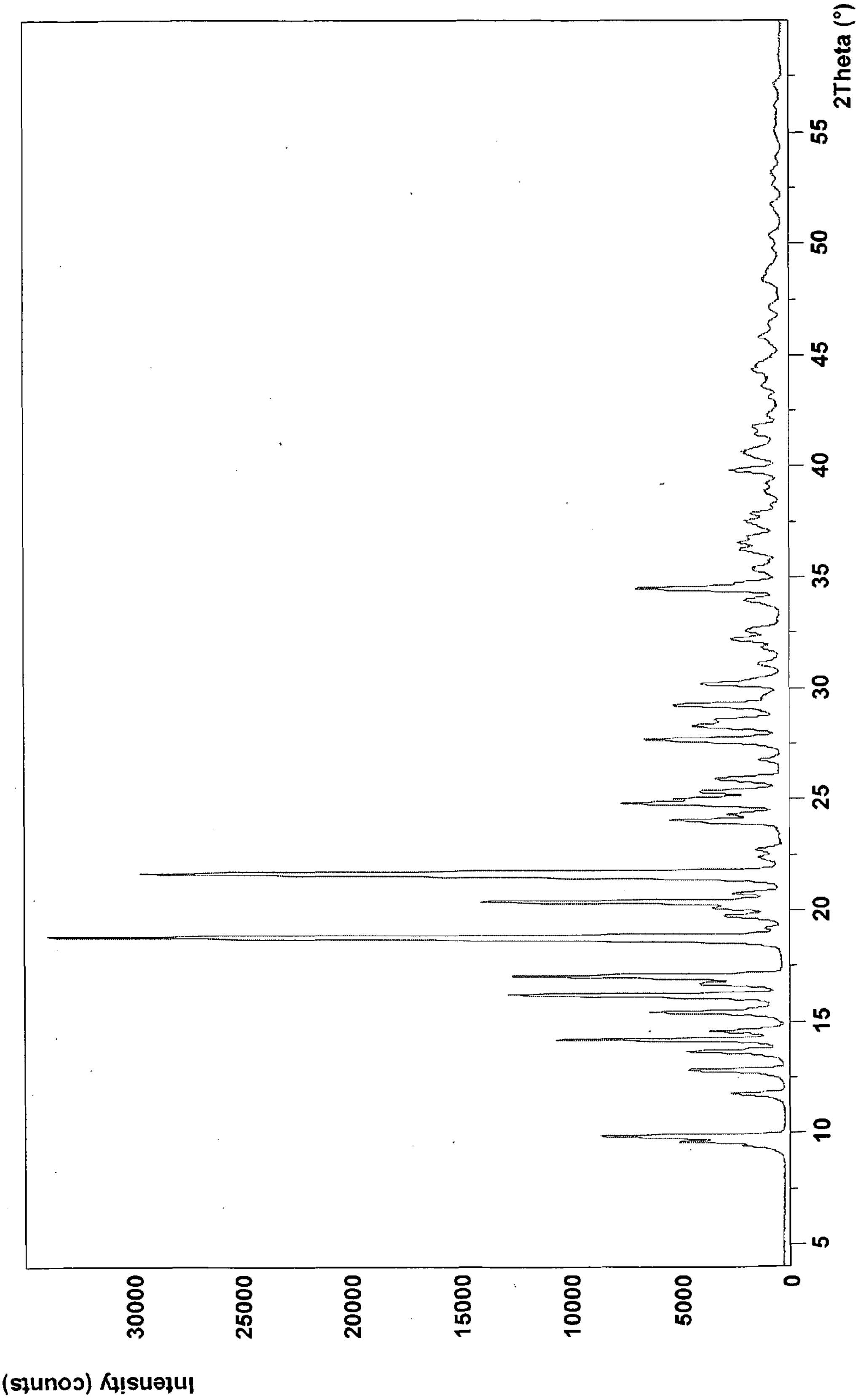


FIG. 18
Thermodiffractometry (Example 14)

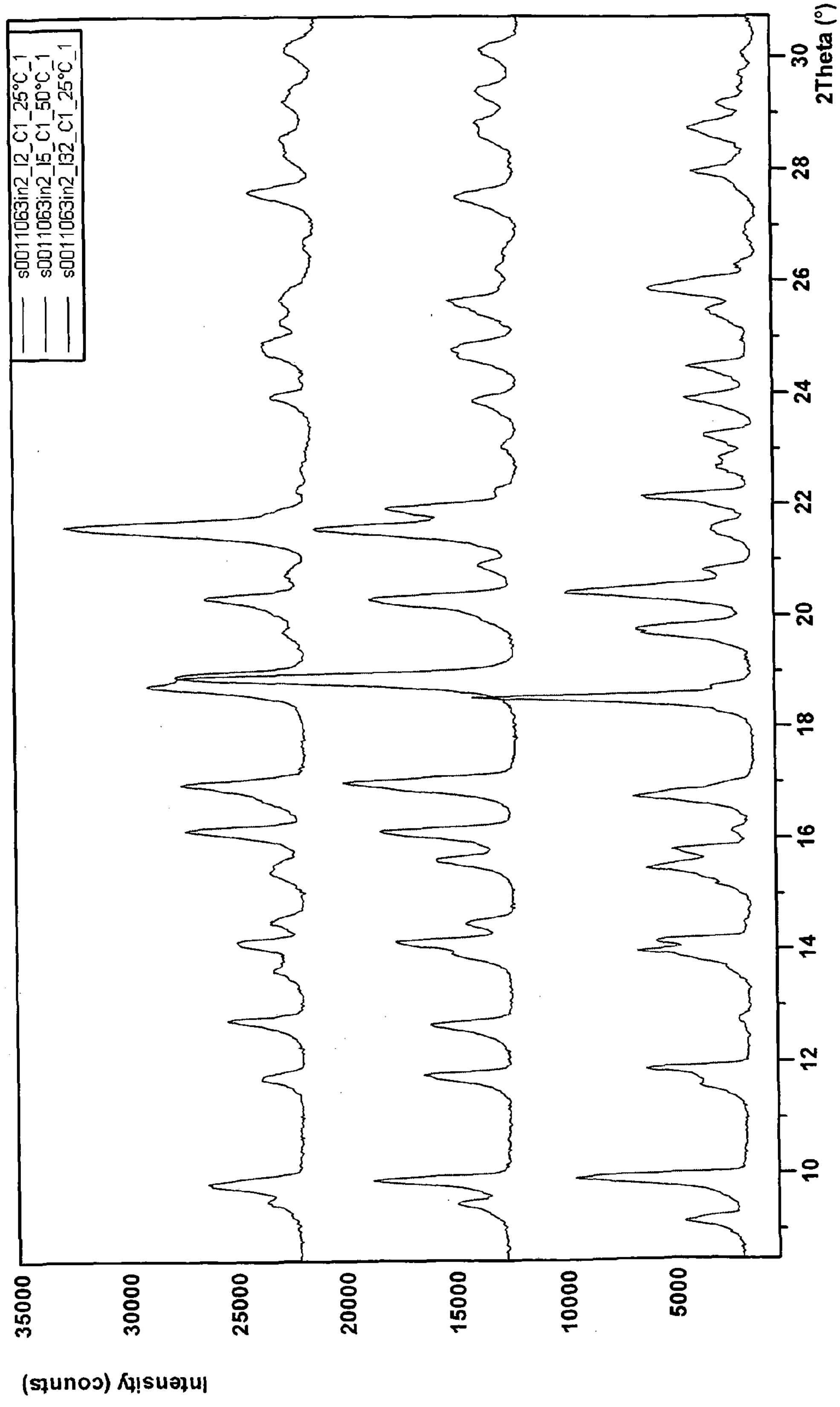


FIG. 19
IR spectrum Polymorph B (Example 15)

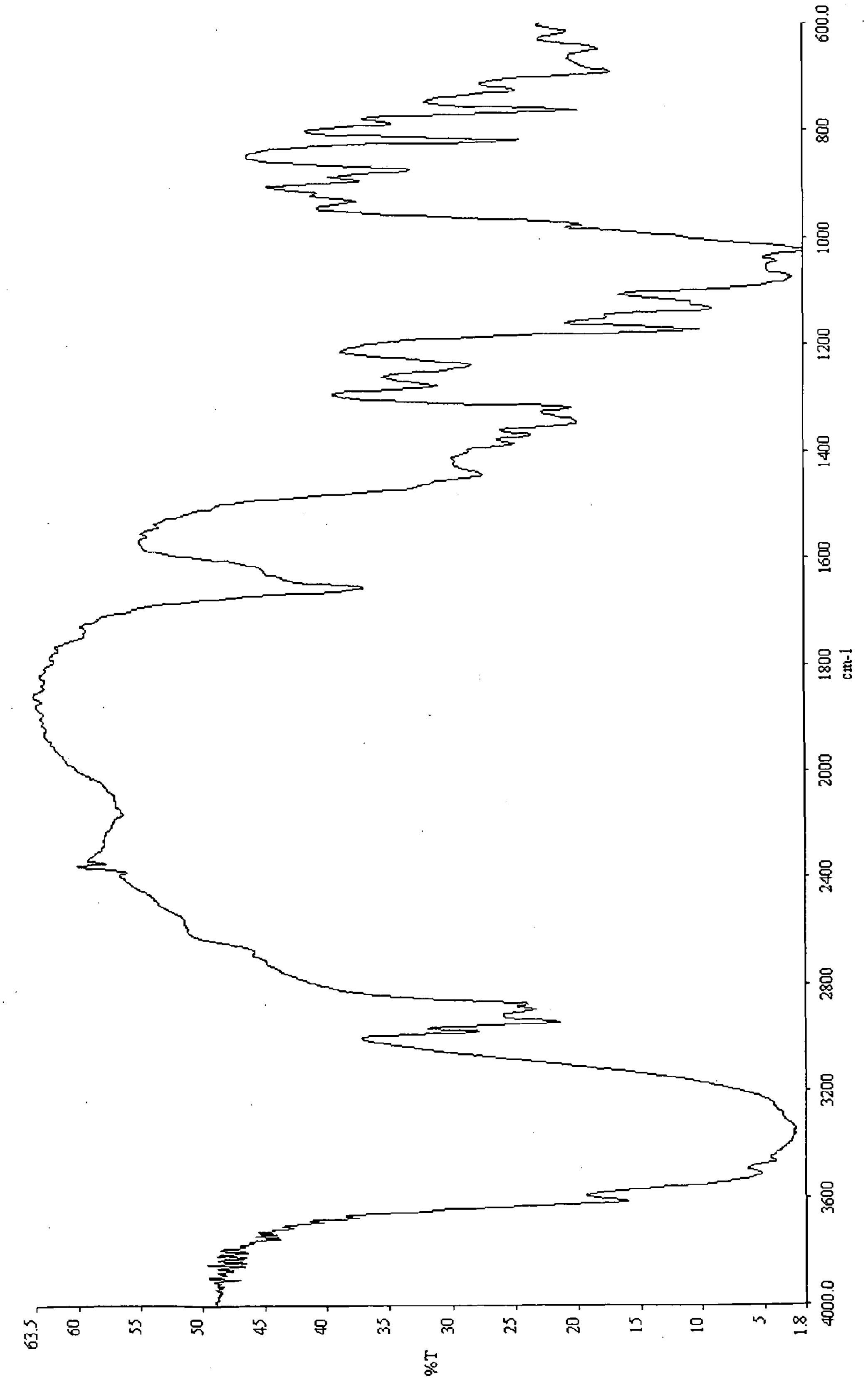


FIG. 20
XRPD Polymorph B (Example 15)

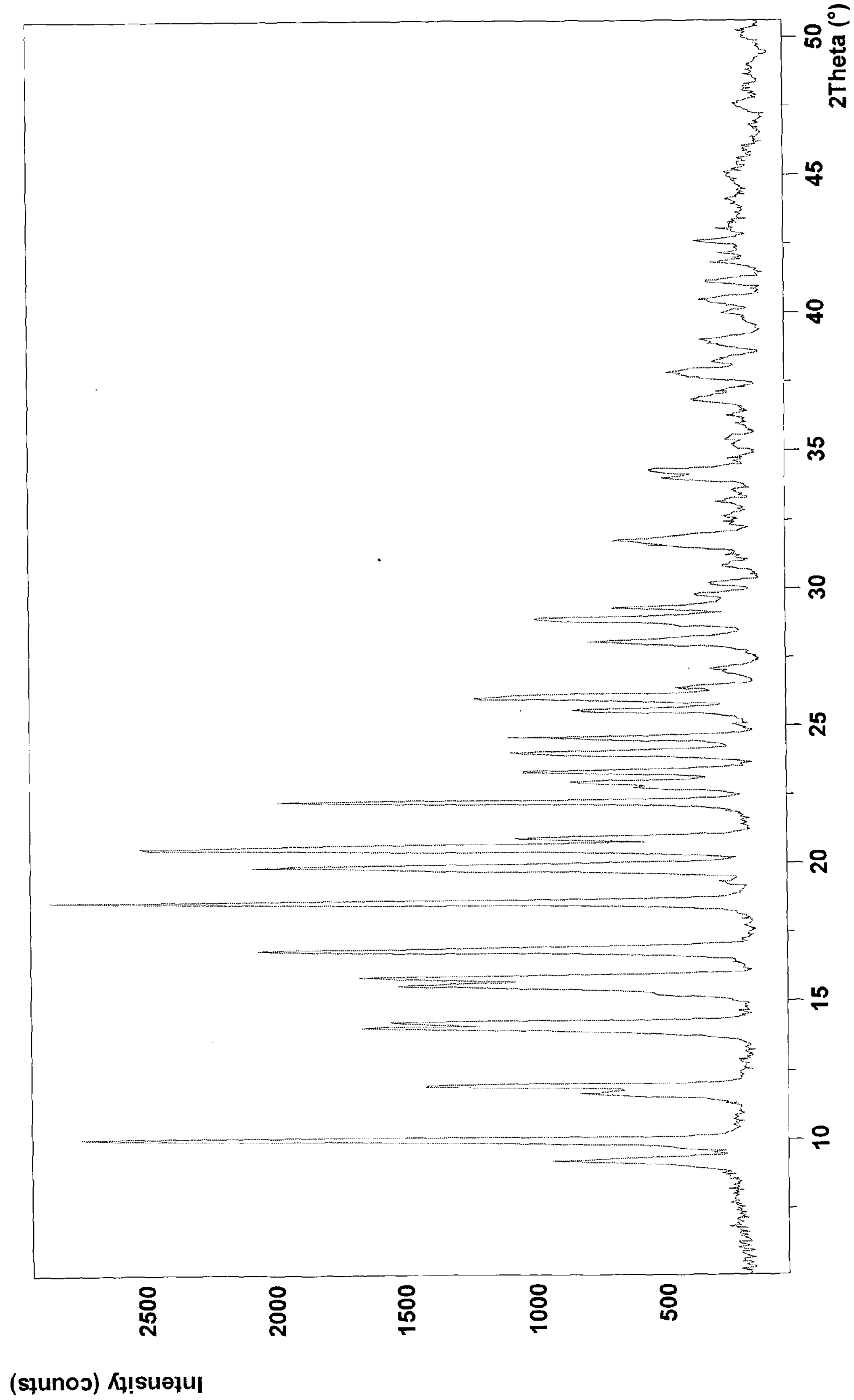


FIG. 21
IR spectrum Polymorph B (Example 17)

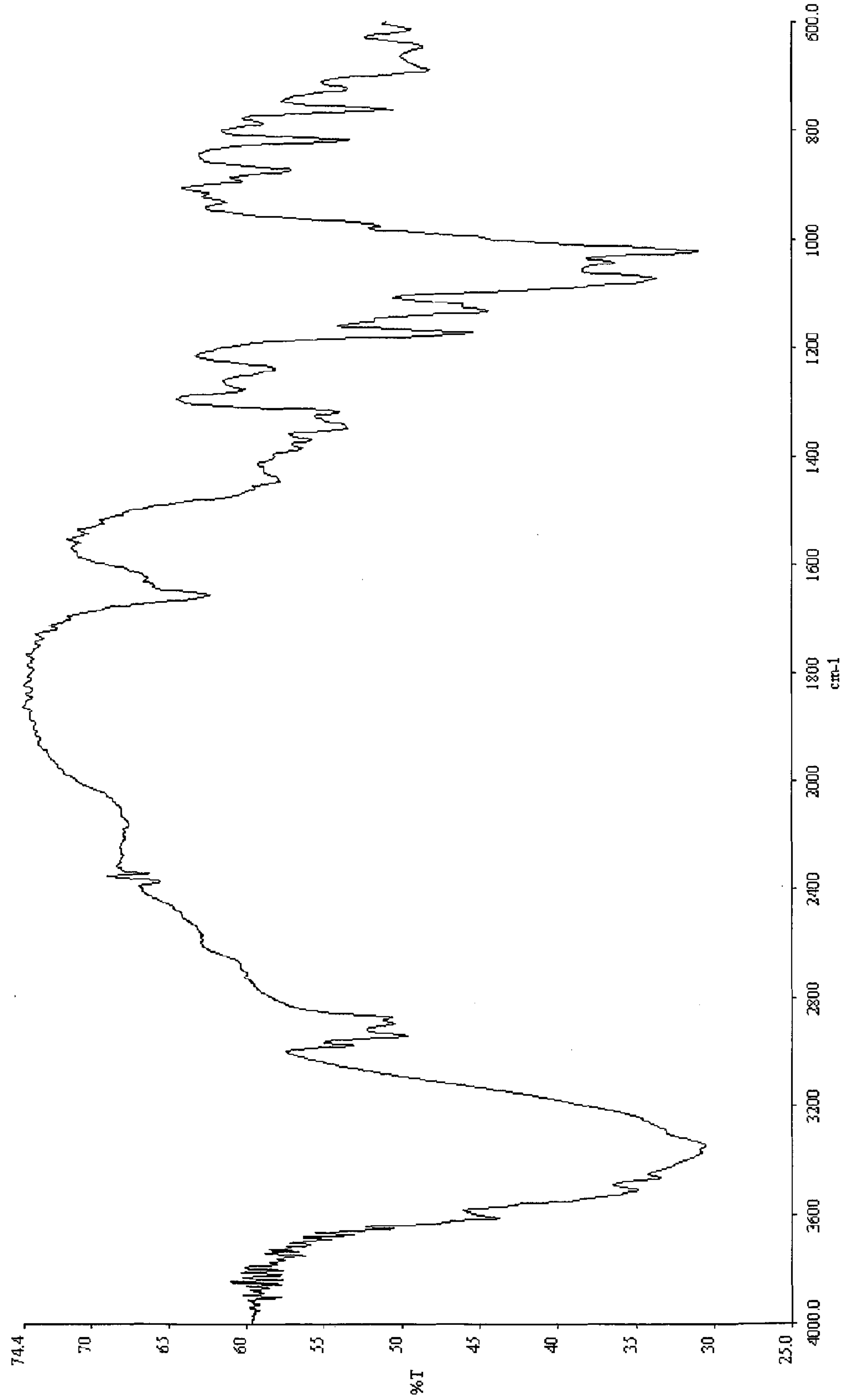


FIG. 22
DSC Polymorph B (Example 17)

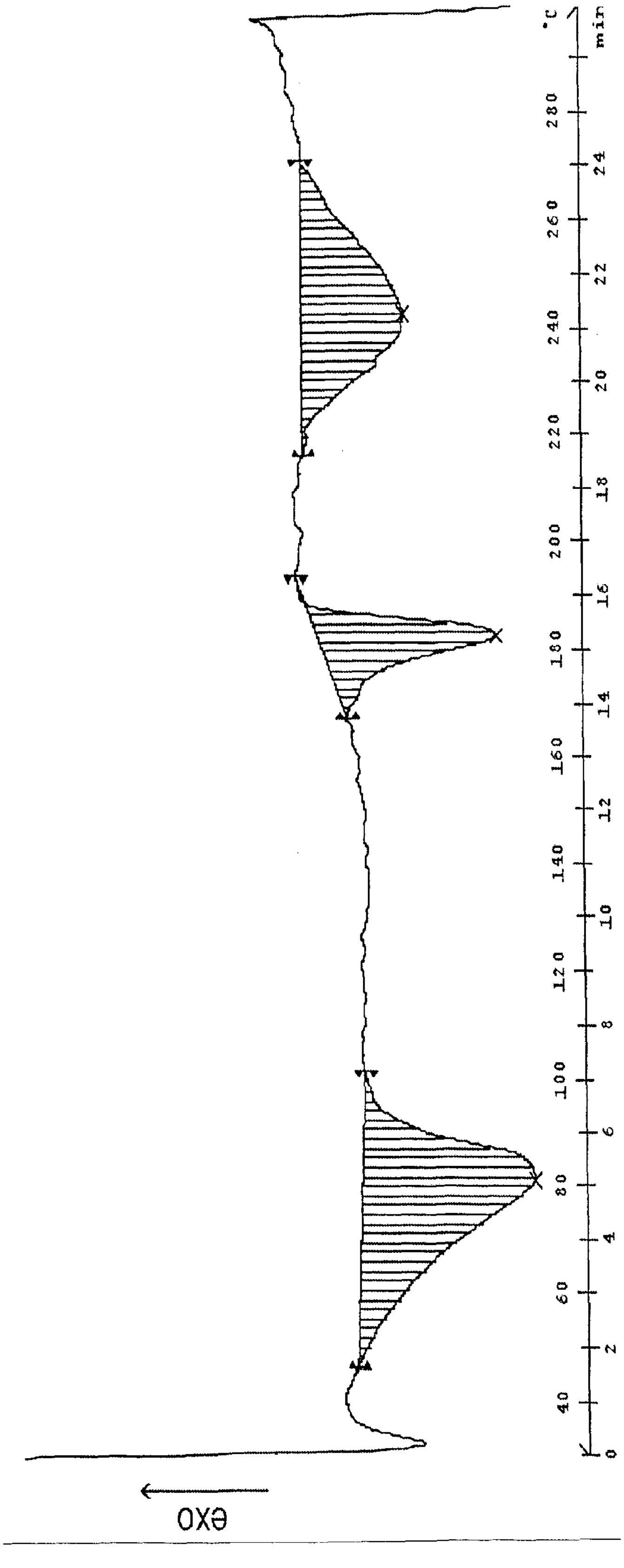


FIG. 23

TGA and DTA Polymorph B (Example 17)

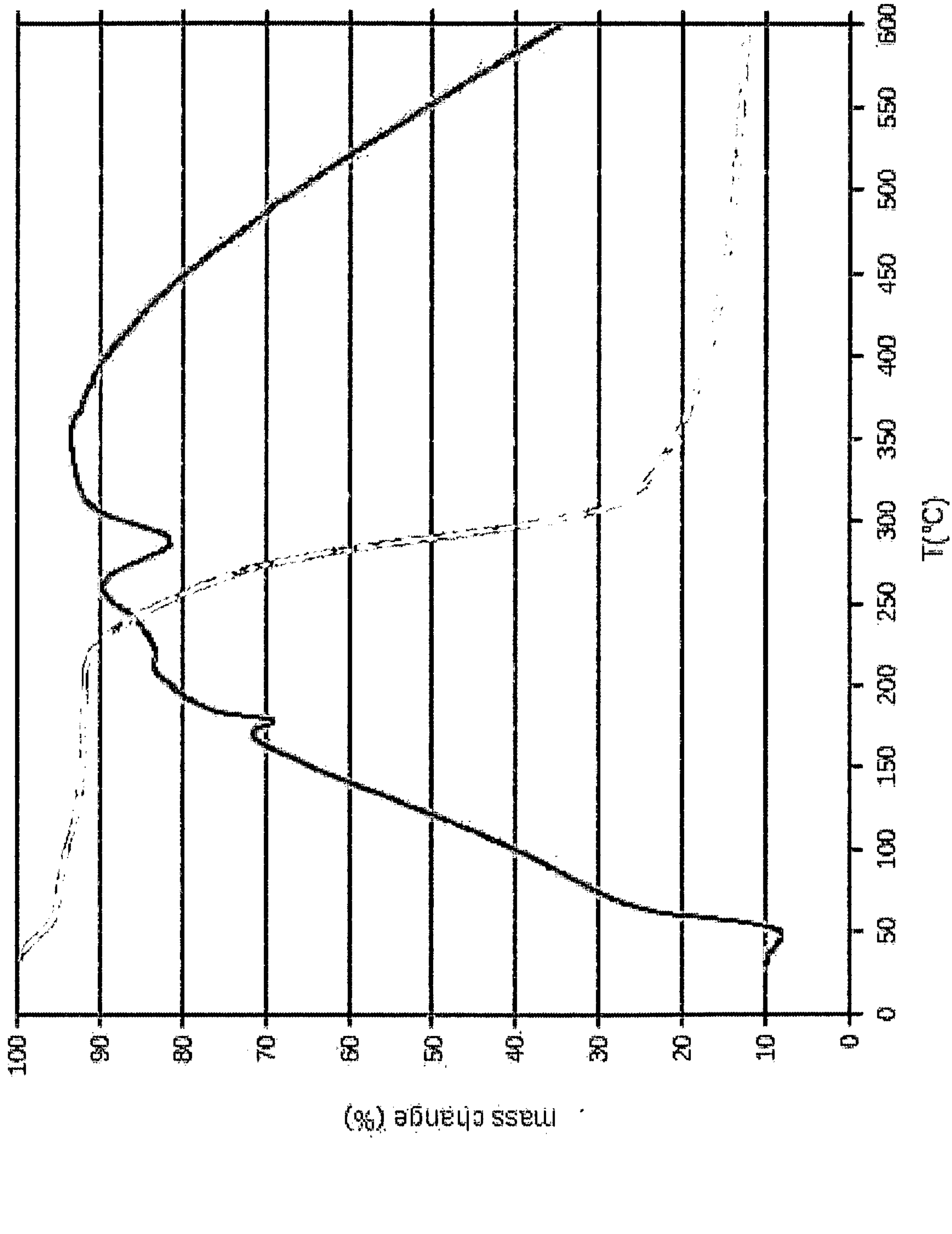


FIG. 24

XRPD Polymorph B (Example 17)

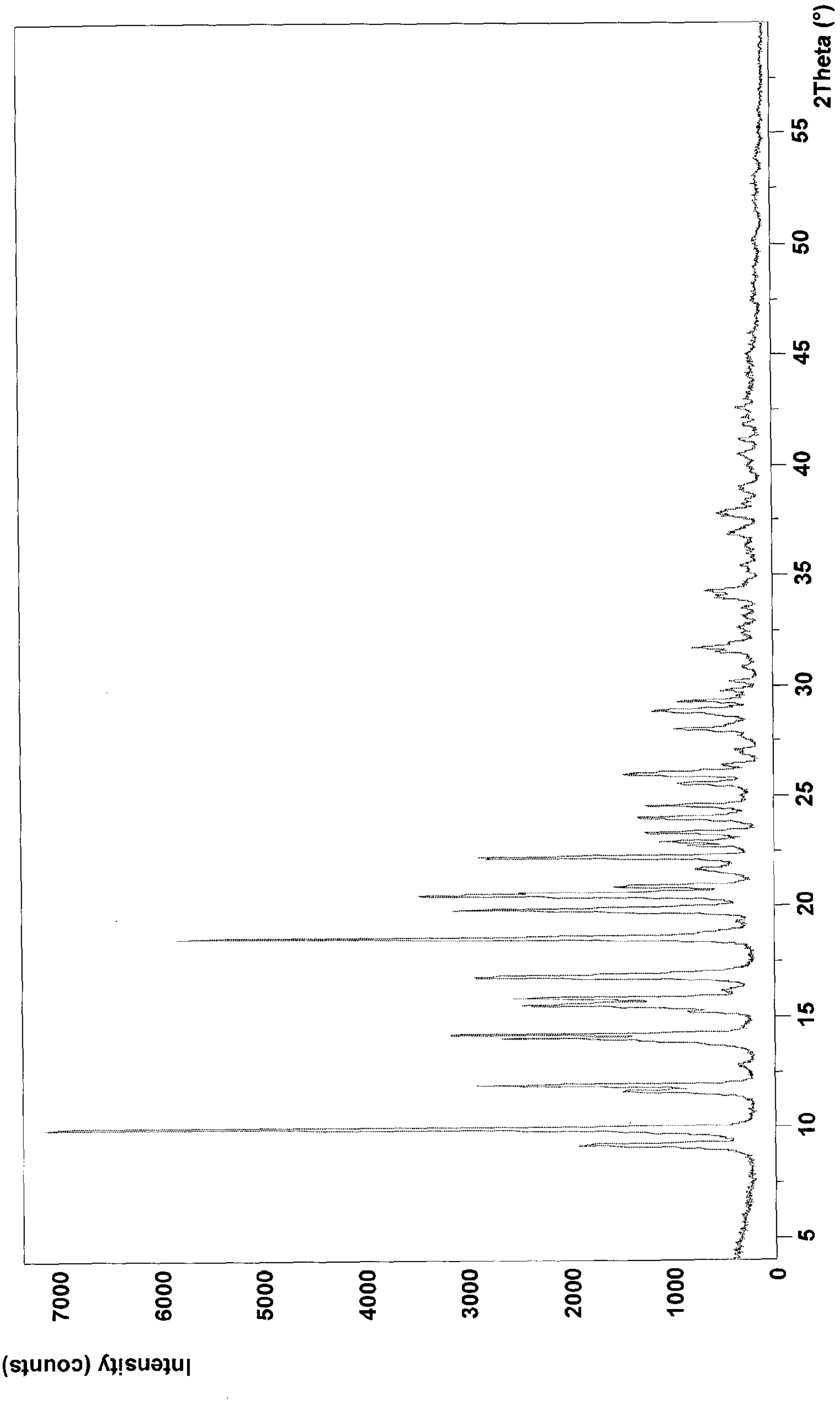
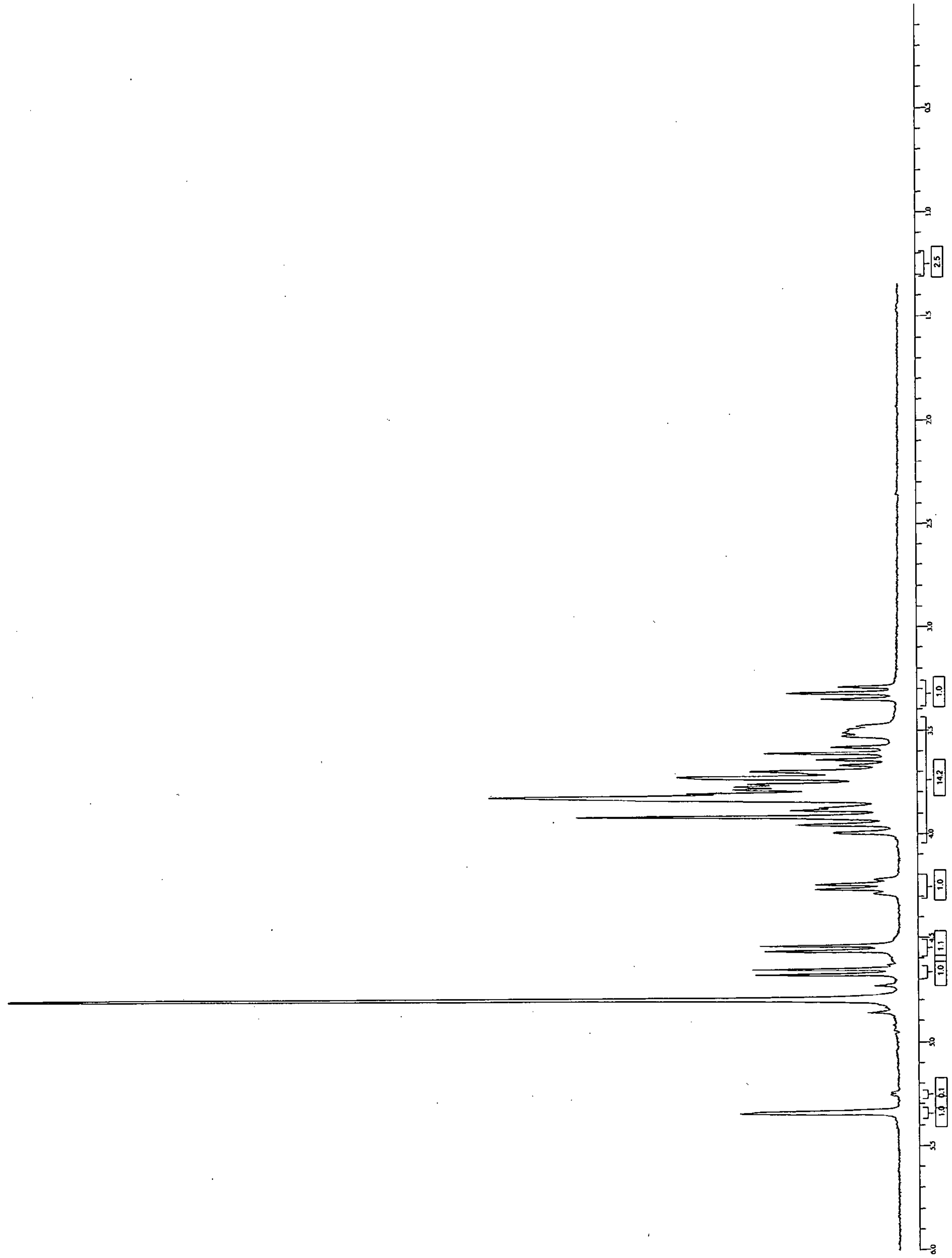


FIG. 25

^{1}HNMR Polymorph B (Example 18)



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- WO 2010115935 A **[0018]**

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- **KUHN et al.** *Chem. Ber.,* 1955, vol. 88, 1135 **[0006]**
- *Chem. Ber.,* 1956, vol. 89, 2513 **[0006]**