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(54) **Title:** A NOVEL CRYSTALLINE FORM OF LAMIVUDINE

(57) **Abstract:** A new Lamivudine polymorphic form, pharmaceutical formulations thereof. This (-) cis-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one in the form of monoclinic crystals has characteristic powder X-ray difractogram as shown in figure 1. A process for preparation of monoclinic crystals of (-) cis-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one having characteristic powder X-ray difractogram as shown in figure 1, called crystalline form III comprising of dissolution of Lamivudine in water at 45°C, then slowly cooling the solution under stirring, separation of the crystalline form from mother liquor, optional washing with organic solvent and drying of the product. A pharmaceutical composition in solid dosage unit form comprising a therapeutically effective amount (-)-cis-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one in monoclinic crystalline form having characteristic powder X-ray difractogram as shown in figure 1,in combination with a pharmaceutically acceptable carrier therefore. A pharmaceutical composition useful for treating HIV infections in humans which comprises a therapeutically effective amount of a combination of 3'-azido-3'-deoxythymidine (AZT) and (-)-cis-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-IH-pyrimidin-2-one in monoclinic crystalline form having characteristic powder X-ray difractogram as shown in figure 1, in combination with a pharmaceutically acceptable carrier.

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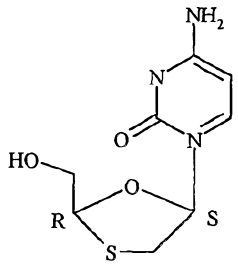
A NOVEL CRYSTALLINE FORM OF LAMIVUDINE

Field of invention

The present invention relates to a new Lamivudine polymorphic form, pharmaceutical formulations thereof.

Background of the invention

Lamivudine (I) (CAS No. 134678-17-4) is chemically known as (2R-cis)-4-amino-1-[2—(hydroxymethyl)-1,3-oxathiolan-5-yl]-2(1H)-pyrimidinone, also known as (-) cis-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one



(I)

Lamivudine is a reverse transcriptase inhibitor used in the treatment of HIV infection alone or in combination with other class of Anti HIV drugs.

Lamivudine is commercially available in a pharmaceutical composition under the brand name EPIVIR® marketed by GlaxoSmithKine and is covered under US 5047407.

US 5047407 claims 1,3-oxathiolane derivatives, their geometric and optical isomers and mixtures thereof. The patent also discloses the preparation of cis and trans isomers of 2,5 substituted 1,3-oxathiolane derivatives.

US 5905082 describes two polymorphic modifications of Lamivudine *viz* form I and II. Form 1 crystals are short rods or long thin needles with orthorhombic crystal system. Form 1 is a hydrate of Lamivudine consisting of one molecule of water per five molecules of Lamivudine. This form melts at 146°C (Journal of Chem. Soc., Perkin Trans. 2, page 2655 (1997)). The DSC thermogram (the rate of heating: 2°C/min) of this form shows first an endotherm at 123.6°C followed by an exotherm at 128°C, finally another

endotherm at 179.6°C. This second endotherm is due to conversion of crystal form I to form II, hence form 1 is a metastable crystalline form.

However with rate of heating of 100°C/min form I shows a single endotherm at 146°C, which is it's melting point. The TGA shows a single step sharp weight loss of 2 %.

Form I as per US 5905082 is prepared by heating a suspension of 64.8 gm Lamivudine in 200 ml water at 45°C to give a solution and cooling the solution to 30°C. The product crystallizes out as an unstirrable mass. Further breaking this mass and cooling it to 10°C with stirring and thereafter filtering and drying at 45°C for 24 hours gives form I crystals.

Form II crystals as disclosed in US 5905082 are bipyramidal in shape with tetragonal crystal system. It is an anhydrous form of Lamivudine. This form melts at 177°C (Journal of Chem. Soc., Perkin Trans. 2, page 2655 (1997)). The DSC thermogram of this form at all scan speeds shows a single peak of endotherm at 177°C. Form II is a stable crystalline form of Lamivudine and is claimed in US 5905082.

Form II as per US 5905082 is prepared by following procedure: Heat a suspension of 10 gm Lamivudine in 200 ml of industrial methylated spirit to reflux to obtain a clear solution. Filter the solution while hot; distil half the amount of the solvent from the filtrate then stop heating and seed the concentrated solution with authentic form II crystals. The seeded solution is then cooled from 80°C to 25°C during one hour. Crystal formation starts at 79°C. Further cooling the suspension to 15°C and stirring for an hour, filtration, washing with IMS and drying gives Form II crystals.

Crystalline form I have inferior flow property and also lower bulk density, which create problem in handling the product during formulation. In view of the literature cited hereinbefore Lamivudine form I also suffers from stability issues. Therefore, it is desirable to develop a crystalline form of Lamivudine having improved stability and also comparable if not better bioavailability.

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When slurried in water both crystal form I and II get converted to another polymorphic form not yet reported in the literature, which is really not a desirable feature for manufacturing practices. Form I converts to form II during milling and formulation operation and because of this the invention embodied in US 5905082 for getting form II, a thermodynamically stable polymorph, used for formulation.

The present inventors have surprisingly i found that Lamivudine can also be obtained in a third crystalline form (hereinafter form III), which not only have distinct powder X-ray diffractogram but also have entirely different single crystal X-ray diffraction when compared to form I and II.

Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present disclosure as it existed before the priority date of each claim of this application.

Preferred aims of the invention

Thus a preferred aim of the present invention is to provide a novel crystalline hemihydrate form of Lamivudine with better flow property and bulk density, which enables to have a formulation without any difficulty.

Another preferred aim of the present invention is to provide a novel crystalline hemihydrate form of Lamivudine with comparable dissolution rate with the reported polymorphic forms of lamivudine.

Yet another preferred aim of the present invention is to provide a novel crystalline form of Lamivudine that is stable during wet granulation using water as a granulating solvent, thereby ensuring the physical stability of the finished solid dosage form.

A further preferred aim of the present invention is to provide a process for preparation of novel crystalline hemihydrate of Lamivudine using eco-friendly solvent "water".

Another preferred aim of the present invention is to provide suitable pharmaceutical dosage forms of novel crystalline hemihydrate of Lamivudine alone or in combination with other anti HIV agents.

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Summary of invention

Herein, there is disclosed a crystalline hemihydrate (form III) of Lamivudine having characteristic powder and single crystal X-ray diffraction as shown in figure 1 and 16 with characteristic 2θ values as given in Table III.

Also herein, there is disclosed a method for formation of Form III by dissolving Lamivudine in water at 45°C, then cooling the clear solution to 30°C, optionally seeding with form III crystals and further cooling to 10°C at the rate ranging from 0.5°C /min to 3.5°C/min, isolating the crystals by filtration optionally washing with alcohol and drying at 45 - 55°C.

In a first aspect, the invention provides a novel crystalline form of (-)-cis-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one in the form of monoclinic crystals having characteristic powder X-ray diffractometric peaks at 2θ value 5.50, 7.60, 9.00, 9.62, 10.98, 11.97, 12.52, 12.81, 13.52, 15.19, 15.71, 15.94, 16.57, 16.72, 17.11, 17.57, 17.98, 18.30, 19.26, 19.68, 20.37, 21.04, 22.00, 22.86, 23.40, 23.70, 24.04, 24.68, 25.15, 26.97, 27.70, 28.74, 30.35, 30.60, 31.94, 33.25 ±0.2; and having endothermic peaks at 100 °C and 179.6 °C in its differential scanning calorimetry profile at the heating rate of 2 °C per minute.

In a second aspect, the invention provides a process for preparation of monoclinic crystals of (-)-cis-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one according to the first aspect, comprising the dissolution of Lamivudine in water at 45 °C, then slowly cooling the solution under stirring, optionally seeding with pure crystals of (-)-cis-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one having characteristic powder X-ray diffractometric peaks at 2θ value 5.50, 7.60, 9.00, 9.62, 10.98, 11.97, 12.52, 12.81, 13.52, 15.19, 15.71, 15.94, 16.57, 16.72, 17.11, 17.57, 17.98, 18.30, 19.26, 19.68, 20.37, 21.04, 22.00, 22.86, 23.40, 23.70, 24.04, 24.68, 25.15, 26.97, 27.70, 28.74, 30.35, 30.60, 31.94, 33.25 ±0.2; and having endothermic peaks at 100 °C and 179.6 °C in its differential scanning calorimetry profile at the heating rate of 2 °C per

minute at 30 °C, separation of the crystalline form from mother liquor, optionally washing with organic solvent and drying of the product.

In a third aspect, the invention provides a process for preparation of monoclinic crystals of (-)-cis-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one according to the first aspect, comprising stirring Lamivudine crystal form I or crystal form II in water at a temperature between 20 to 45 °C, then slowly cooling the mixture under stirring, separation of the crystalline form from mother liquor, optional washing with organic solvent and drying of the product.

In a fourth aspect, the invention provides a pharmaceutical composition in solid dosage unit form comprising a therapeutically effective amount of (-)-cis-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one in monoclinic crystalline form according to the first aspect, in combination with a pharmaceutically acceptable carrier.

In a fifth aspect, the invention provides a pharmaceutical composition for treating HIV infections in humans which comprises a therapeutically effective amount of a combination of 3'-azido-3'-deoxythymidine (AZT) and (-)-cis-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one in monoclinic crystalline form according to the first aspect, in combination with a pharmaceutically acceptable carrier.

In a sixth aspect, the invention provides a method of treating HIV infections in humans which comprises administering to a human in need thereof a therapeutically effective amount of 3'-azido-3'-deoxythymidine (AZT) and (-)-cis-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5yl)-(1H)-pyrimidin-2-one in monoclinic crystalline form according to the first aspect, in combination with a pharmaceutically acceptable carrier.

In a seventh aspect, the invention provides a use of a composition comprising a therapeutically effective amount of a combination of 3'-azido-3'-deoxythymidine (AZT) and (-)-cis-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one in monoclinic crystalline form according to the first aspect in the preparation of a medicament for the treatment of HIV infections in humans.

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In an eighth aspect, the invention provides a use of a novel crystalline form of (-)-cis-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one in the form of monoclinic crystals according to the first aspect in the preparation of a medicament for the treatment of HIV infections in humans.

Description of the invention

As mentioned earlier both form I and form II polymorphs when slurried in water get converted to polymorphic form III, which happens to be thermodynamically stable and does not undergo any change in crystal structure during milling.

This crystal form has been found to have better flow property and higher bulk density in comparison with literature reported forms.

Further study on single crystal X-ray diffraction reveals that it is a hemihydrate form (four molecules of Lamivudine with two molecules of water) of Lamivudine.. This product melts at 176 - 177°C. The DSC thermogram (at the rate of heating = 2°C/min) shows first peak of endotherm (Δ H = 16.61 J/g) at 100°C and the second peak of endotherm (Δ H = 101.68 J/g) at 179.6°. This crystal form is found to be stable and has better flow property than form 1, and is found to posses comparable bioavailability.

The crystal form III of Lamivudine is obtained by subjecting the hot (45°C) supersaturated solution of Lamivudine for controlled cooling. Whereas if such solution is cooled suddenly it gives form 1 crystals of Lamivudine.

Thermogravimetric analysis (as shown in fig. 6) of form III crystals of Lamivudine shows 3.5 to 4 % single step loss of weight. Moisture content of this crystal form by Karl Fischer titration is in the range of 3.5 to 4.0%, which confirms presence of approximately one mole of water per every two moles of Lamivudine.

Single crystal structure X-ray data (Fig. 16) reveals two molecules of water are associated with four molecules of lamivudine presumably through hydrogen bonds in polymorphic form III. In other words the material of present invention is a hemihydrate having four molecules of lamivudine and two molecules of water. Form III thus obtained has a melting point of 176 to 177°C.

The novel crystalline hemihydrate form (form III) of Lamivudine has better flow property and bulk density, which are important parameters for formulation (Table I).

Table I

Property	**Form I**	**Form II**	**Form III**
Bulk Density (gm /cc)	0.46	0.38	0.64
Tap Density (gm /cc)	0.60	0.55	0.83
Flow Property (Angle of Repose[$])	33.66°	32.00°	32.00°

Lamivudine Form I and Form II when slurried in water at ambient temperature for 24 to 48 hours get converted to Form III, which is not at all desirable since during formulation especially in wet granulation such conversion would lead to physical instability of the finished formulation. Hence, use of Lamivudine Form III crystals would certainly have an added advantage over other polymorphic forms mentioned in the literature.

The crystalline form III of Lamivudine as disclosed herein was found to be stable for more than three months when stored at 40±2°C RH 75±5%.

[$] measured as per the procedure provided on page 317 of 'The Theory and Practice of Industrial Pharmacy' by Leon Lachman et al., Third Ed. Varghese Publishing House, Bombay; (1987)

Comparative thermal analysis data is tabulated in Table II

Table II

Crystal Form	Melting Point	DSC	TGA
I	135 - 145°C 124 – 127°C* 135°C#	@ 2°C/min: exotherm at 123° then at 177° (fig. 7) @ 100°C/min: 146°C (Fig. 8)	One step weight loss between temp 80°C to 140°C = 1.52% (Fig. 4)
II	177 – 178°C 177 – 178°C * #	@ 2°C/min and 100°C/min: 177°C (Fig. 9 & 10)	No weight loss due to crystal bound water. (Fig. 5)
III	176 - 177°C	@ 2°C / min first peak at 100°C and second at 177°C. (Fig. 11) @ 100°C/min: 120°C (Fig. 12)	One step weight loss between temp 80°C to 140°C = 4.14% (Fig. 6)

The powder X-ray diffraction analysis of form III also shows characteristic 2θ values. Comparative data of 2θ values form III and other literature reported polymorphic forms is provided in Table III

Table III:

Form I (Fig. 1) (2θ values)	**Form II** (Fig. 2) (2θ values)	**Form III** (Fig. 3) (2θ values)
5.20	10.70	5.50
6.66	12.17	7.60
8.53	13.42	9.00
8.81	14.30	9.62
9.65	14.76	10.98
9.85	15.86	11.97
10.15	16.83	12.52
10.41	17.55	12.81
11.27	18.63	13.52
11.38	19.68	15.19
11.63	20.63	15.71
12.34	21.44	15.94
12.60	22.13	16.57
12.93	22.60	16.72
13.22	23.03	17.11
14.60	24.44	17.57
15.01	24.94	17.98
15.17	25.70	18.30
15.67	26.51	19.26
15.81	27.68	19.68
16.51	28.41	20.37
17.59	28.93	21.04
17.98	29.72	22.00
18.13	30.67	22.86
18.72	30.90	23.40
19.10	31.30	23.70
19.30	31.47	24.04
19.76	31.99	24.68
21.788	32.40	25.15
23.487	32.59	26.97
23.706	33.14	27.70
25.44	34.01	28.74
25.90	35.20	30.35
27.34	35.49	30.60
29.46	37.27	31.94
31.00	38.46	33.25

The single crystal X-ray diffraction data obtained for form III crystalline form of Lamivudine is tabulated in Table IV

Suitable pharmaceutical formulations may conveniently be presented containing predetermined amount of lamivudine in crystalline form III

Description of accompanying figures:

Figure 1: Powder X-ray diffractogram of crystalline form I of Lamivudine.

Figure 2: Powder X-ray diffractogram of crystalline form II of Lamivudine.

Figure 3: Powder X-ray diffractogram of crystalline form III of Lamivudine.

Figure 4: TGA thermogram of crystalline form I of Lamivudine.

Figure 5: TGA thermogram of crystalline form II of Lamivudine.

Figure 6: TGA thermogram of crystalline form III of Lamivudine.

Figure 7: DSC thermogram of crystalline form I of Lamivudine at heating rate 2°C/min.

Figure 8: DSC thermogram of crystalline form I of Lamivudine at heating rate 100°C/min.

Figure 9: DSC thermogram of crystalline form II of Lamivudine at heating rate 2°C/min.

Figure 10: DSC thermogram of crystalline form II of Lamivudine at heating rate 100°C/min.

Figure 11: DSC thermogram of crystalline form III of Lamivudine at heating rate 2°C/min.

Figure 12: DSC thermogram of crystalline form III of Lamivudine at heating rate 100°C/min.

Figure 13: FTIR spectra of crystalline form I of Lamivudine.

Figure 14: FTIR spectra of crystalline form II of Lamivudine.

Figure 15: FTIR spectra of crystalline form III of Lamivudine.

Figure 16: crystal structure and packing diagram of crystalline form III of Lamivudine obtained by Single crystal X-ray diffraction analysis

The present invention is illustrated in more detail by referring to the following Examples, which are not to be construed as limiting the scope of the invention.

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

Example 1: Preparation of Lamivudine form III

A suspension of the Lamivudine form –II (25.0) g in water (75.0 ml) was heated to 45^{0}C in 20 min to give a clear solution. The solution was cooled to 30^{0}C during a period of 30 min. The crystallization started at 30°C. The mass was further cooled to 10^{0}C during a period of 20 min and stirred for 1 hour. The product was filtered and washed with ethanol (2 x10ml) then dried in vacuum at 45^{0}C for 24 hours. Yield = 23.0 gms.

IR Spectra [Nujol Mull] (cm^{-1}): 3330, 3160, 2923, 2854, 1640, 1600, 1522, 1460, 1376, 1296, 1226, 1193, 1155, 1135, 1106, 1044, 976, 927, 844, 788, 722 (Figure 15)

X-ray powder diffraction analysis shows peaks at about 5.50, 7.60, 9.00, 9.62, 10.98, 11.97, 12.52, 12.81, 13.52, 15.19, 15.71, 15.94, 16.57, 16.72, 17.11, 17.57, 17.98, 18.30, 19.26, 19.68, 20.37, 21.04, 22.00, 22.86, 23.40, 23.70, 24.04, 24.68, 25.15, 26.97, 27.70, 28.74, 30.35, 30.60, 31.94, 33.25 ±0.2 °2θ.

The single crystal X-ray analysis is carried out using SMART APEX CCD diffractometer by full-matrix least-squares refinement on F^2; goodness of fit on F^2 was 1.050. A total of 20474 reflections were measured on diffractometer with monochromatised Cu-Kα radiation. The data was collected at θ ranging from 1.26 to 25°. The structure was solved by direct method and the non-hydrogen atoms refined anisotropically. All H atoms were refined isotropically. Refinement converged to give R1 = 0.0538, wR2 = 0.1428. Minimum residual electron density was -.403 e. Å^{-3} and maximum residual electron density was 0.887 Å^{-3}. The data is as shown below in Table IV:

Table IV:

Empirical Formula	$2(C_8H_{11}N_3O_3S).(H_2O)$
Formula weight	476.53
Crystal System	Monoclinic
Space group	$P2_1$
Unit cell dimensions	a = 11.714 (9) Å α = 90° b = 11.214 (9) Å β = 94.68° c = 16.197 (12) Å γ = 90°
Z, calculated density	2, 1.493 Mg/m^3.
Cell volume	2120.4 (3) Å^3
Crystal size	0.18 X 0.11 X 0.09

Powder pattern generated from single crystal data using MERCURY software was found to be identical to the experimental powder X-ray diffraction pattern of the material of invention (as provided for Form III in Table III and in Figure 3).

The differential scanning calorimetric analysis at the rate of heating 2°C / min shows first peak of endotherm at 100°C and second at 177°C (Fig. 11), and at the rate of heating 100°C/min shows single peak of endotherm at 120°C (Fig. 12).

The thermogravimetric analysis exhibits one-step weight loss of 4.14% between temp 80°C to 140°C (Fig. 6).

Example 2: Preparation of Lamivudine form III

A suspension of the Lamivudine form –II (20.0) g in water (60.0 ml) was heated to 45^0C in 25 min to give a solution. The solution was cooled to 30^0C in 15 min. The mass was then cooled to 10^0C in 20 min and stirred for 1 h. The product was filtered and washed with IMS (2x10ml) then dried in vacuum at 45^0C for 24 h. Yield = 17 gms.

Powder X-ray diffraction pattern superimposable with that of form III as obtained in Example 1.

Example 3: Preparation of Lamivudine form III

A suspension of the Lamivudine form –II (20.0) g in water (60.0 ml) was heated to 45^0C in 25 min to give a solution. The solution was cooled to 30^0C in 30 min. The mass was then cooled to 10^0C in 20 min and stirred for 1 h. The product was filtered and washed with ethanol (2 x10ml), then dried in vacuum at 45^0C for 24h. Yield = 17 gms.

Powder X-ray diffraction pattern superimposable with that of form III as obtained in Example 1.

Example 4: Preparation of Lamivudine form III

A suspension of the Lamivudine form –II (10.0) g in water (30.0 ml) was heated to 45^0C in 20 min to give a clear solution. The solution was cooled to 30^0C in 15 min. The reaction mass was then cooled to 10^0C in 20 min and stirred for 1 h. The product was filtered and dried in vacuum at 45^0C for 24h. Yield = 8.5 gms.

Powder X-ray diffraction pattern superimposable with that of form III as obtained in Example 1.

Example 5: Preparation of Lamivudine form III

A suspension of the Lamivudine form –1 (10.0) g in water (30.0 ml) was heated to 45^{0}C in 20 min to give a clear solution. The solution was then cooled to 10^{0}C in 10 min and stirred for 1 h. The product was filtered and dried in vacuum at 45^{0}C for 24h. Yield = 7 gms

Powder X-ray diffraction pattern superimposable with that of form III as obtained in Example 1.

Example 6: Preparation of Lamivudine form III

A suspension of the Lamivudine form –II (10.0) g in water (30.0 ml) was heated to 45^{0}C in 20 min to give a clear solution. The solution was then cooled to 10^{0}C in 10 min and stirred for 1 hr. The product was filtered and dried in vacuum at 45^{0}C for 24hr. Yield = 8 gm.

Powder X-ray diffraction pattern superimposable with that of form III as obtained in Example 1.

Example 7: Preparation of Lamivudine form III

A suspension of the Lamivudine form –II (50.0) g in water (150.0 ml) was heated to 45^{0}C in 17 min. to give a clear solution. The solution was cooled slowly to 30^{0}C in 1.0 hr 40 min. The product was then cooled to 10^{0}C in 10 min and stirred for 1 h. The product was filtered and dried in vacuum 1.0 mm at 45^{0}C for 24h. Yield = 44 gm

Powder X-ray diffraction pattern superimposable with that of form III as obtained in Example 1.

Example 8: Preparation of Lamivudine form III

A suspension of the Lamivudine form –II (20.0) g in water (80.0 ml) was heated to 45°C in 25 min to give a clear solution. The solution was cooled slowly to 30°C in 55 min.The product was then cooled to 10°C in 5 min and stirred for 1 h at the same temperature. The product was filtered and dried in vacuum for 24 hr at 50-55°C. Yield: 18 gm.

Powder X-ray diffraction pattern superimposable with that of form III as obtained in Example 1.

Example 9: Preparation of Lamivudine form III

A suspension of the Lamivudine form –II (20.0) g in water (100.00) was heated to 45°C in 25 min to give a clear solution. The solution was cooled slowly to 30°C in 55 min. The product was then cooled to 10°C in 5 min and stirred for 1 h at the same temperature. The product was filtered and dried in vacuum for 24 hr at 50-55°C. Yield 18.7 gm.

Powder X-ray diffraction pattern superimposable with that of form III as obtained in Example 1.

Example 10: Preparation of Lamivudine form III

A suspension of lamivudine (Form I or Form II or mixture thereof) (35 gm) in water (105 ml) was heated to 45°C in 17 minutes to give a clear solution. The solution was cooled slowly to 37°C in 50 minutes. The solution was seeded with lamivudine form III. The mixture was then cooled to 10°C in 10 minutes and stirred for one hour. The product was filtered and dried in vacuum at 45°C for 24 hours. Yield 32 gm.

Powder X-ray diffraction pattern superimposable with that of form III as obtained in Example 1.

Example 11: Preparation of Lamivudine form III

A suspension of the Lamivudine form – II (5.0 gm) in water (5.0 ml) was stirred at 25°C for 48 hours. The suspension was cooled and stirred at 10°C for one hour. The product was filtered and then dried under vacuum at 45°C for 24 hours. Yield = 4.5 gms

Powder X-ray diffraction pattern superimposable with that of form III as obtained in Example 1.

Example 12: Preparation of Lamivudine form III

A suspension of the Lamivudine form – I (2.0 gm) in water (2.0 ml) was stirred at 25°C for 24 hours. The suspension was cooled and stirred at 10°C for one hour. The product was filtered and then dried under vacuum at 45°C for 24 hours. Yield = 1.6 gms

Powder X-ray diffraction pattern superimposable with that of form III as obtained in Example 1.

Example 13: Preparation of Lamivudine Form I

A suspension of the Lamivudine (10.0) g in water (30.0 ml) was heated to 45^0C in 30 min to give a solution. The solution was cooled to 30^0C in 0.5 min. The product was crystallized as an unstirrable mass. This was broken up and suspension stirred at 10.0° C for 1 hr. The product was filtered and washed with IMS (2x5ml) then dried in vacuum at 45^0C for 24hr. Yield = 6.0 gm

IR Spectra [Nujol Mull] (cm^{-1}): 3356, 3199, 2923, 2854, 1639, 1611, 1461, 1402, 1376, 1309, 1288, 1252, 1196, 1166, 1145, 1107, 1052, 970, 932, 839, 786, 720 (Figure 13).

X-ray powder diffraction analysis shows peaks at about 5.20, 6.66, 8.53, 8.81, 9.65, 9.85, 10.15, 10.41, 11.27, 11.38, 11.63, 12.34, 12.60, 12.93, 13.22, 14.60, 15.01, 15.17, 15.67, 15.81, 16.51, 17.59, 17.98, 18.13, 18.72, 19.10, 19.30, 19.76, 21.79, 23.49, 23.71, 25.44, 25.90, 27.34, 29.46, 31.00 ±0.2 °2θ.

The differential scanning calorimetric analysis at the rate of heating 2°C / min shows first peak of endotherm at 123°C and second at 177°C (Fig. 7), and at the rate of heating 100°C/min shows single peak of endotherm at 146°C (Fig. 8).

The thermogravimetric analysis exhibits one-step weight loss of 1.52 % between temp 80°C to 140°C (Fig. 4).

Example 14: Preparation of Lamivudine Form I

A suspension of the Lamivudine (250.0 g) in the mixture of water (750.0 ml) and DNS (250.0 ml) was heated to 45^{0}C in 12 min to give a solution. The solution was cooled to 30^{0}C in 15 min and seeded with form I crystals. The product was then cooled to 10^{0}C in 30 min and stirred for 1 h. The product was filtered washed wished with 100 ml water DNS mixture (3:1) and dried in vacuum at 45^{0}C for 24h. Yield: 220.0 gm.

Powder X-ray diffraction pattern superimposable with that of form I as obtained in Example 13.

Example 15: Preparation of Lamivudine Form II

A suspension of the Lamivudine (10.0) g in ethanol (200.0 ml) was heated to refluxed to give a clear solution. The solution thus formed was subjected to distillation and about 100 ml of ethanol was distilled out at atmospheric pressure. The remaining solution was then cooled to 15^{0}C in 35 min. The suspension stirred at 15°C for 1.0 hr .The product was filtered and washed with ethanol (10.0ml) then dried in vacuum at 50^{0}C for 12hr to get 8.2 gm.

IR Spectra [Nujol Mull] (cm^{-1}): 3322, 3194, 2950, 2870, 1651, 1611, 1496, 1456, 1396, 1376, 1337, 1316, 1285, 1222, 1180, 1158, 1087, 1058, 1030, 918, 851, 806, 786, 723 (Figure 14).

X-ray powder diffraction analysis shows peaks at about 10.70, 12.17, 13.42, 14.30, 14.76, 15.86, 16.83, 17.55, 18.63, 19.68, 20.63, 21.44, 22.13, 22.60, 23.03, 24.44, 24.94, 25.70, 26.51, 27.68, 28.41, 28.93, 29.72, 30.67, 30.90, 31.30, 31.47, 31.99, 32.40, 32.59, 33.14, 34.01, 35.20, 35.49, 37.27, 38.46 ± 0.2 °2θ.

The differential scanning calorimetric analysis at the rate of heating 2°C / min and 100°C/min shows single peak of endotherm at 177°C (Fig. 9 and Fig. 10).

The thermogravimetric analysis reveals that it is an anhydrous product. (Fig. 5).

Example 12: Pharmaceutical Formulations

(a) 150 mg Lamivudine Tablet

Ingredients per Tablets	Weight (mg.)
Lamivudine (Form III)	150
Microcrystalline cellulose NF	269.62
Sodium starch glyclolate NF	22.50
Colloidal silicon dioxide NF	2.25
Magnessium Stearate NF	5.63
Total Weight	**450.00**

Lamivudine (form III), microcrystalline cellulose, sodium starch glycolate and colloidal silicon dioxide were sieved and blended in octagonal for about 15 minutes. Sieved magnesium stearate was then added and blending continued for a further 2 minutes

The blend was compressed in standard tabletting equipment.

Analysis:

Tablet weight: 450 mg ±5%

Thickness: 5.0 – 5.2 mm

Hardness: 150 to 200 N

Disintigration Time: 25 seconds.

% friability: 0.1 %.

(b) Lamivudine form III /Zidovudine combination tablets:

Ingredients per Tablets	Weight (mg.)
Intra-granular	
Lamivudine (Form III)	150.00
Zidovudine	300.00
Dicalcium phosphate dihydrate NF	181.87
Sodium starch glyclolate NF	56.25
Purified water	Qs
Extra-granular	
Sodium starch glycolate NF	18.75
Dicalcium phosphate dihydrate NF	37.50
Magnessium stearate NF	5.63
Coating	
Opadry YS-1 7706G White	15
Total Weight	**765.00**

Lamivudine (form III), Zidovudine, sodium starch glycolate and dicalcium phosphate dihydrate were sieved and mixed in rapid mixer granulator for about 15 minutes. The drymixture obtained was granulated using purified water as granulating agent. The granules were then dried and sifted. Previously sifted sodium starch glycolate and dicalcium phosphate dihydrate blended with the dry granules in octagonal blened for 10 minutes. Previously sifted magnesium stearate was added to this blend and blending continued for further two minutes.The blend was compressed in standard tabletting equipment and then film coated with an aqueous suspension of Opadry YS-1 7706 G White to produce aesthetically acceptable tablets.

Analysis:

Tablet weight: 750 mg ±10 mg

Thickness: 5.5 – 5.6 mm

Hardness: 120 to 130 N

Disintigration Time: 35 seconds (coats), 50 seconds.

% friability: 0.2 %.

Dissolution in 0.1 N HCl, 50 rpm, paddle, 900 ml:

Time (minutes)	Lamivudine (%)	Zidovudine (%)
5	80.9	81.1
10	86.2	87.8
20	92.0	95.2
30	96.0	100.4
40	96.7	101.5

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THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:-

1. A novel crystalline form of (-)-cis-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one in the form of monoclinic crystals having characteristic powder X-ray diffractometric peaks at 2θ value 5.50, 7.60, 9.00, 9.62, 10.98, 11.97, 12.52, 12.81, 13.52, 15.19, 15.71, 15.94, 16.57, 16.72, 17.11, 17.57, 17.98, 18.30, 19.26, 19.68, 20.37, 21.04, 22.00, 22.86, 23.40, 23.70, 24.04, 24.68, 25.15, 26.97, 27.70, 28.74, 30.35, 30.60, 31.94, 33.25 ±0.2; and having endothermic peaks at 100 °C and 179.6 °C in its differential scanning calorimetry profile at the heating rate of 2 °C per minute.

2. The crystal form as claimed in claim 1 having an endothermic peak between 115 and 130 °C in its differential scanning calorimetry profile at the heating rate of 100 °C per minute.

3. The crystal form as claimed in claim 1 showing weight loss of 4 to 4.5% between the temperatures 80 °C and 140 °C in thermogravimetric analysis.

4. A process for preparation of monoclinic crystals of (-)-cis-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one as claimed in claim 1 comprising the dissolution of Lamivudine in water at 45 °C, then slowly cooling the solution under stirring, optionally seeding with pure crystals of (-)-cis-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one having characteristic powder X-ray diffractometric peaks at 2θ value 5.50, 7.60, 9.00, 9.62, 10.98, 11.97, 12.52, 12.81, 13.52, 15.19, 15.71, 15.94, 16.57, 16.72, 17.11, 17.57, 17.98, 18.30, 19.26, 19.68, 20.37, 21.04, 22.00, 22.86, 23.40, 23.70, 24.04, 24.68, 25.15, 26.97, 27.70, 28.74, 30.35, 30.60, 31.94, 33.25 ±0.2; and having endothermic peaks at 100 °C and 179.6 °C in its differential scanning calorimetry profile at the heating rate of 2 °C per minute at 30 °C, separation of the crystalline form from mother liquor, optionally washing with organic solvent and drying of the product.

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5. The process as claimed in claim 4 wherein the rate of cooling is in the range of 0.5 °C/min to 3.5 °C/min.

6. A process for preparation of monoclinic crystals of (-)-cis-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one as claimed in claim 1 comprising stirring Lamivudine crystal form I or crystal form II in water at a temperature between 20 to 45 °C, then slowly cooling the mixture under stirring, separation of the crystalline form from mother liquor, optional washing with organic solvent and drying of the product.

7. The process as claimed in claim 6 wherein the organic solvent employed for washing the crystals is selected from C1 to C4 aliphatic alcohols.

8. The process as claimed in claim 7, wherein the organic solvent employed for washing the crystals is ethanol.

9. A pharmaceutical composition in solid dosage unit form comprising a therapeutically effective amount of (-)-cis-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one in monoclinic crystalline form as claimed in claim 1, in combination with a pharmaceutically acceptable carrier.

10. A pharmaceutical composition which comprises a therapeutically effective amount of a combination of 3'-azido-3'-deoxythymidine (AZT) and (-)-cis-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one in monoclinic crystalline form as claimed in claim 1, in combination with a pharmaceutically acceptable carrier.

11. A pharmaceutical composition according to claim 10 in oral administration form.

12. A pharmaceutical composition according to claim 11 in tablet or capsule form.

13. A method of treating HIV infections in humans which comprises administering to a human in need thereof a therapeutically effective amount of 3'-azido-3'-deoxythymidine (AZT) and (-)-cis-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5yl)-(1H)-pyrimidin-2-one in monoclinic crystalline form as claimed in claim 1, in combination with a pharmaceutically acceptable carrier.

14. The method according to claim 13 wherein the administration is sequential.

15. The method according to claim 13 wherein the administration is simultaneous.

16. The method according to claim 14 or claim 15 wherein the administration is oral.

17. The method according to claim 16 wherein the oral administration is in tablet or capsule form.

18. Use of a composition comprising a therapeutically effective amount of a combination of 3'-azido-3'-deoxythymidine (AZT) and (-)-cis-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one in monoclinic crystalline form as claimed in claim 1 in the preparation of a medicament for the treatment of HIV infections in humans.

19. Use of a novel crystalline form of (-)-cis-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one in the form of monoclinic crystals as claimed in claim 1 in the preparation of a medicament for the treatment of HIV infections in humans.

20. A novel crystalline form of (-)-cis-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one in the form of monoclinic crystals as hereinbefore described with reference to any one of Figure 3, Figure 6, Figure 11, Figure 12, Figure 15, Figure 16 and Examples 1 to 12.

Figure 1: Powder X-ray diffractogram of crystalline form I of Lamivudine.

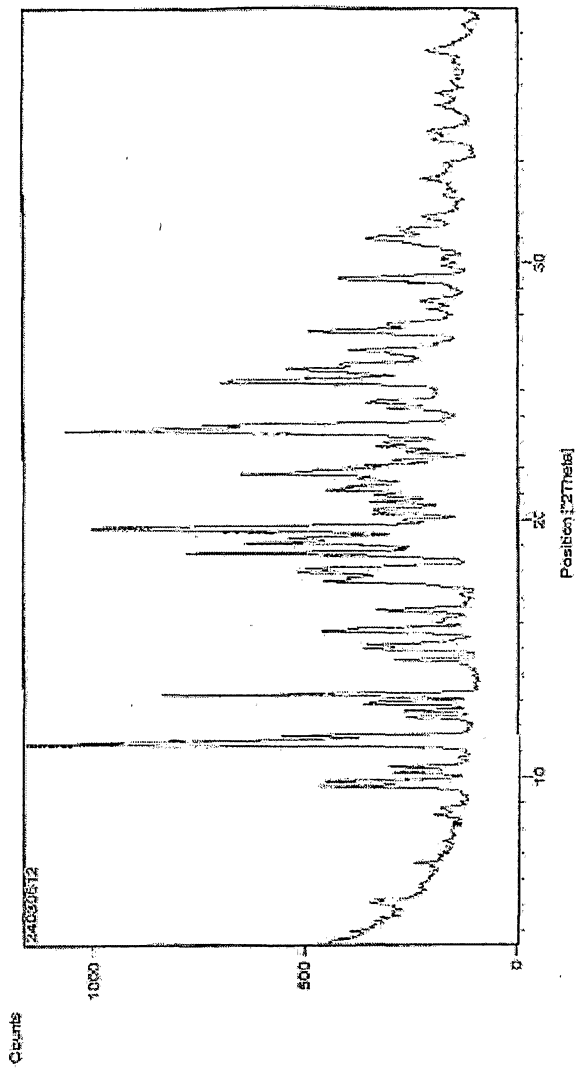


Figure 2: Powder X-ray diffractogram of crystalline form II of Lamivudine.

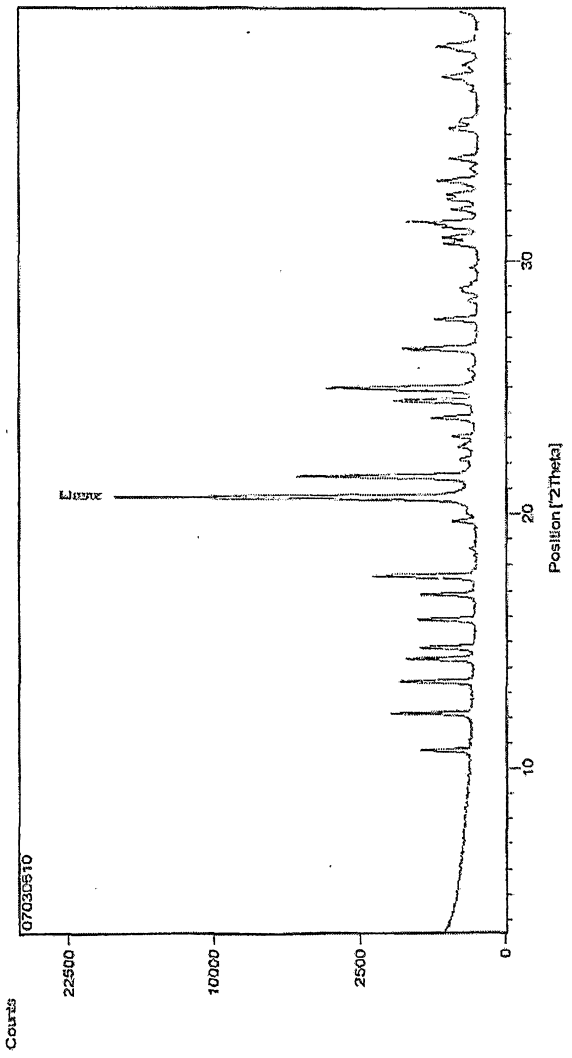


Figure 3: Powder X-ray diffractogram of crystalline form III of Lamivudine.

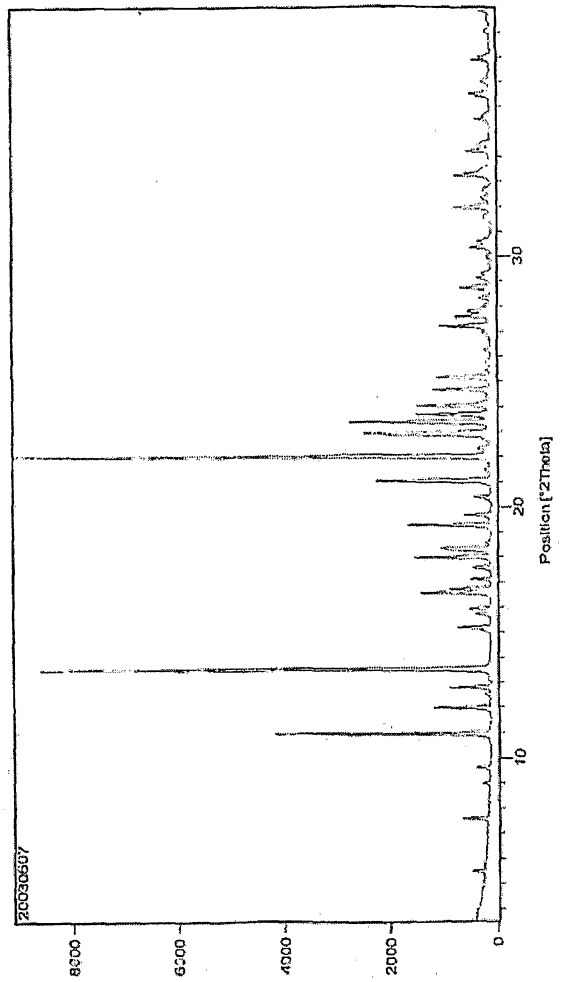


Figure 4: TGA thermogram of crystalline form I of Lamivudine.

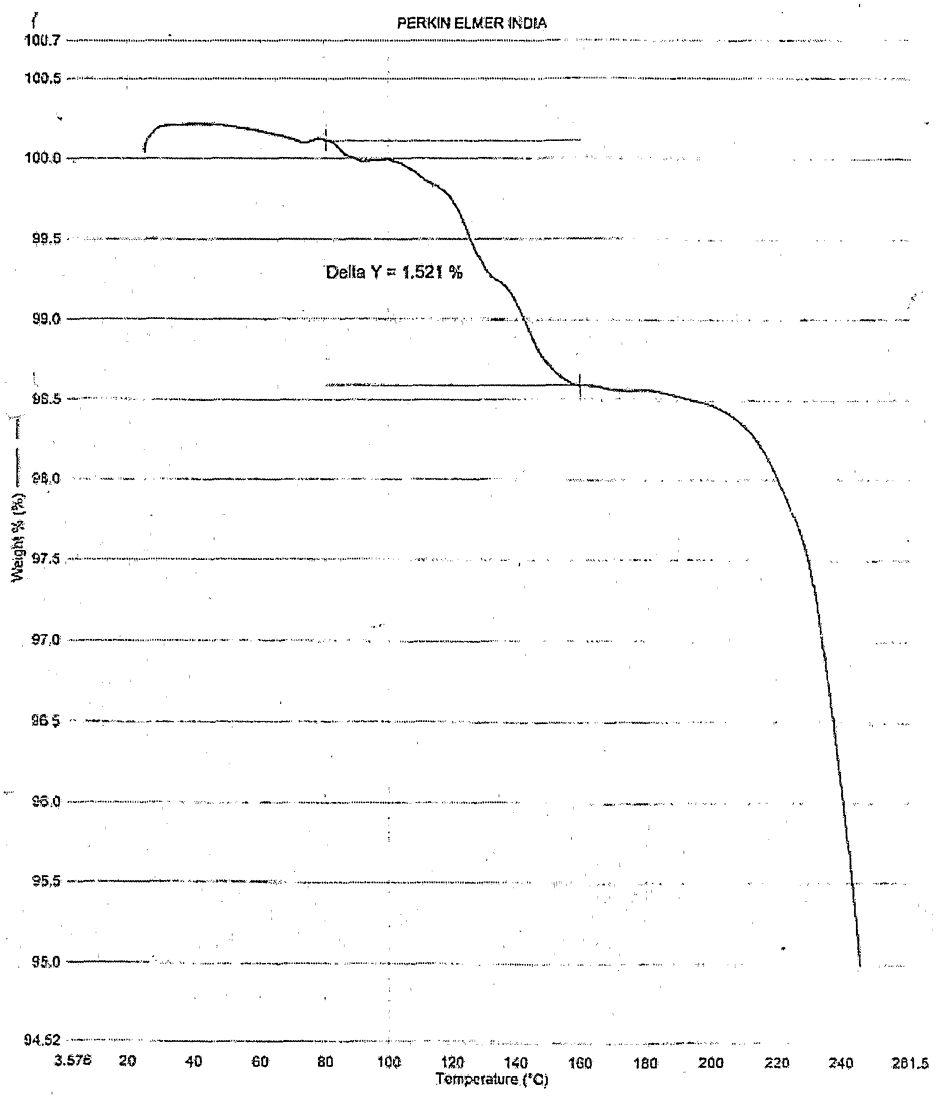


Figure 5: TGA thermogram of crystalline form II of Lamivudine.

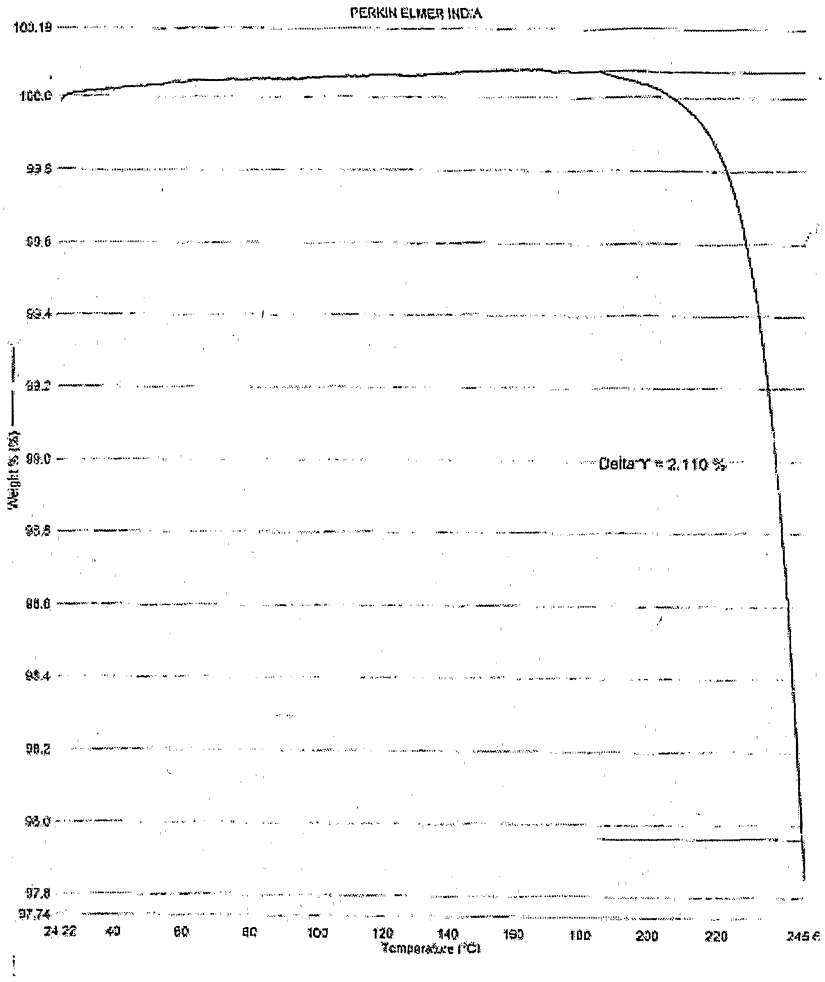


Figure 6: TGA thermogram of crystalline form III of Lamivudine.

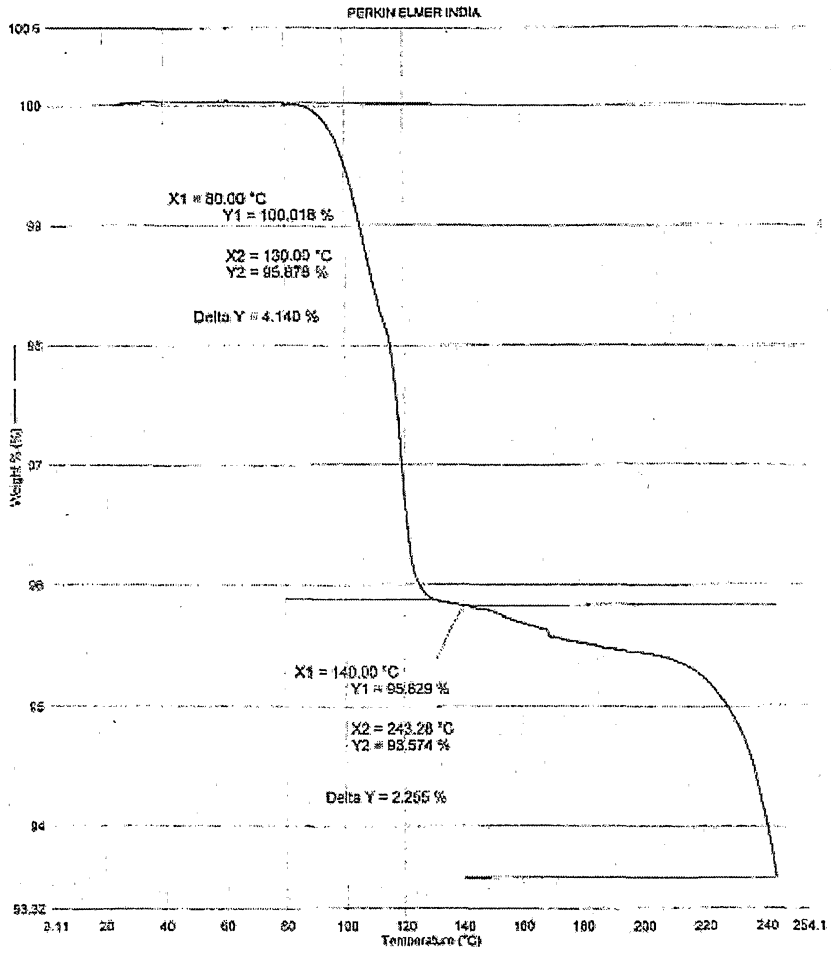


Figure 7: DSC thermogram of crystalline form I of Lamivudine at heating rate 2°C/min.

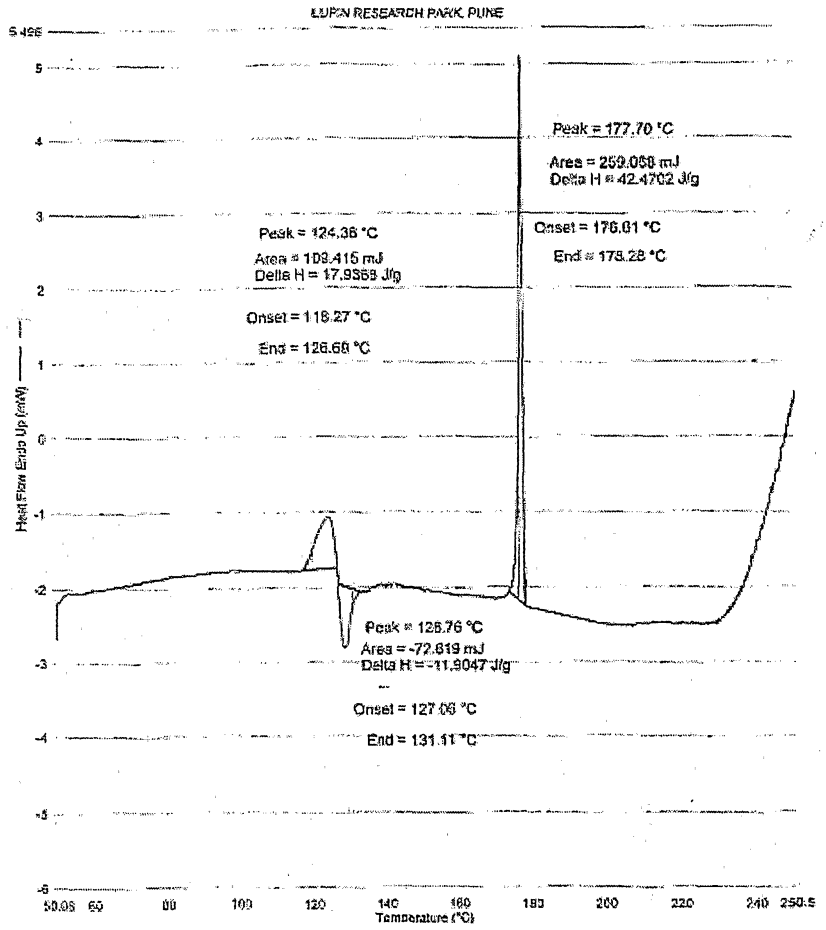


Figure 8: DSC thermogram of crystalline form I of Lamivudine at heating rate 100°C/min.

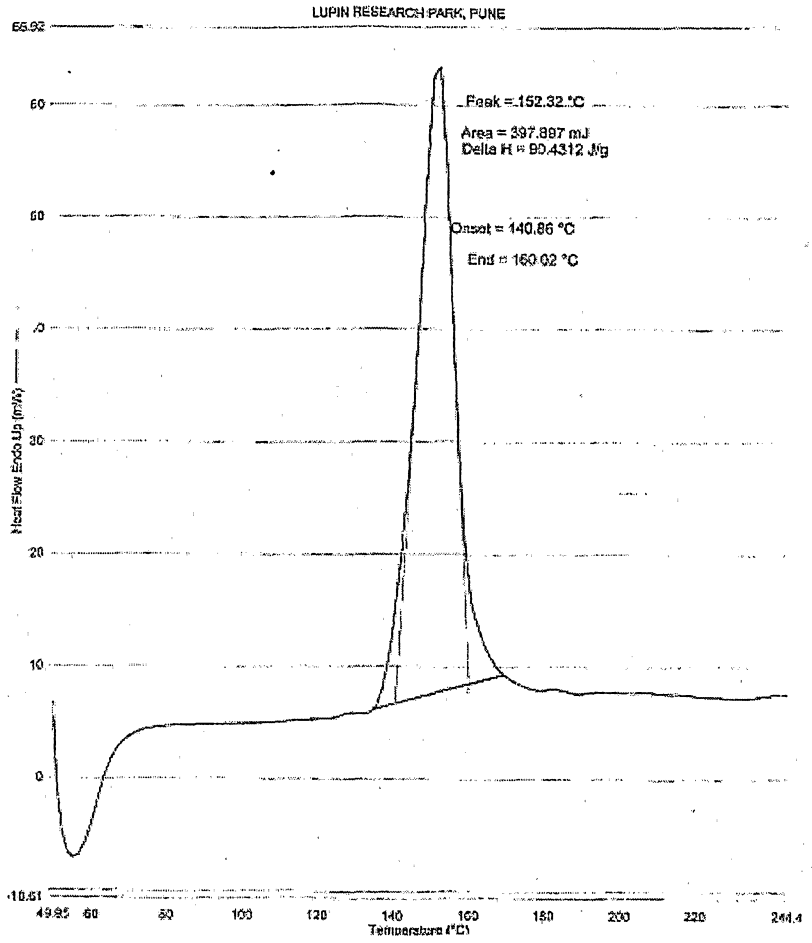


Figure 9: DSC thermogram of crystalline form II of Lamivudine at heating rate 2°C/min.

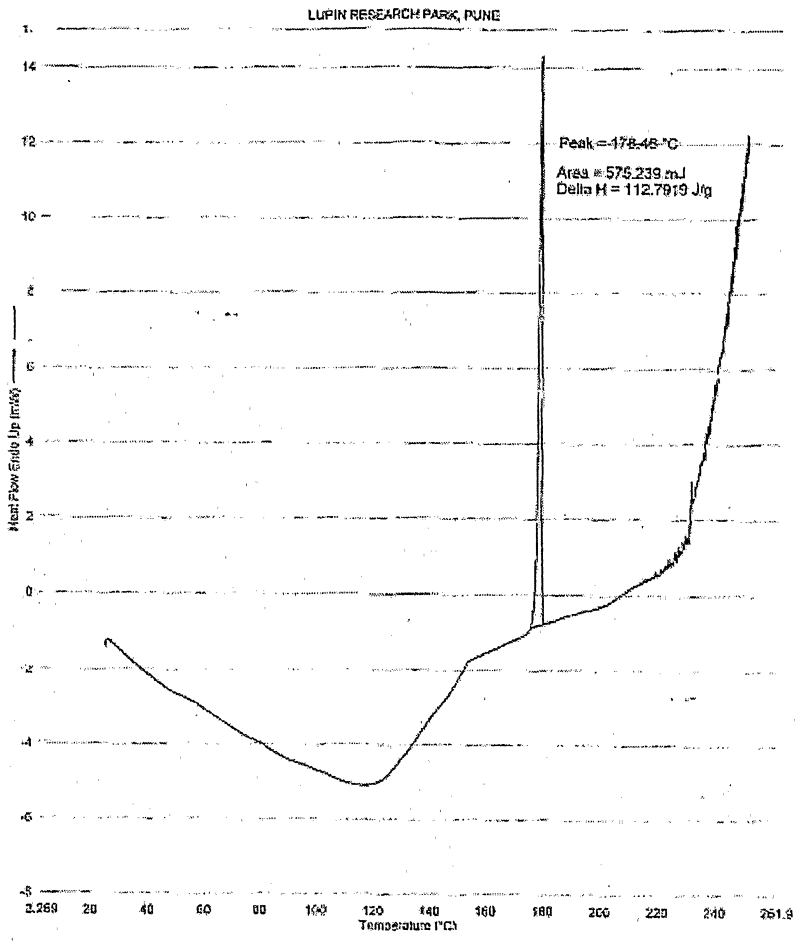


Figure 10: DSC thermogram of crystalline form II of Lamivudine at heating rate 100°C/min.

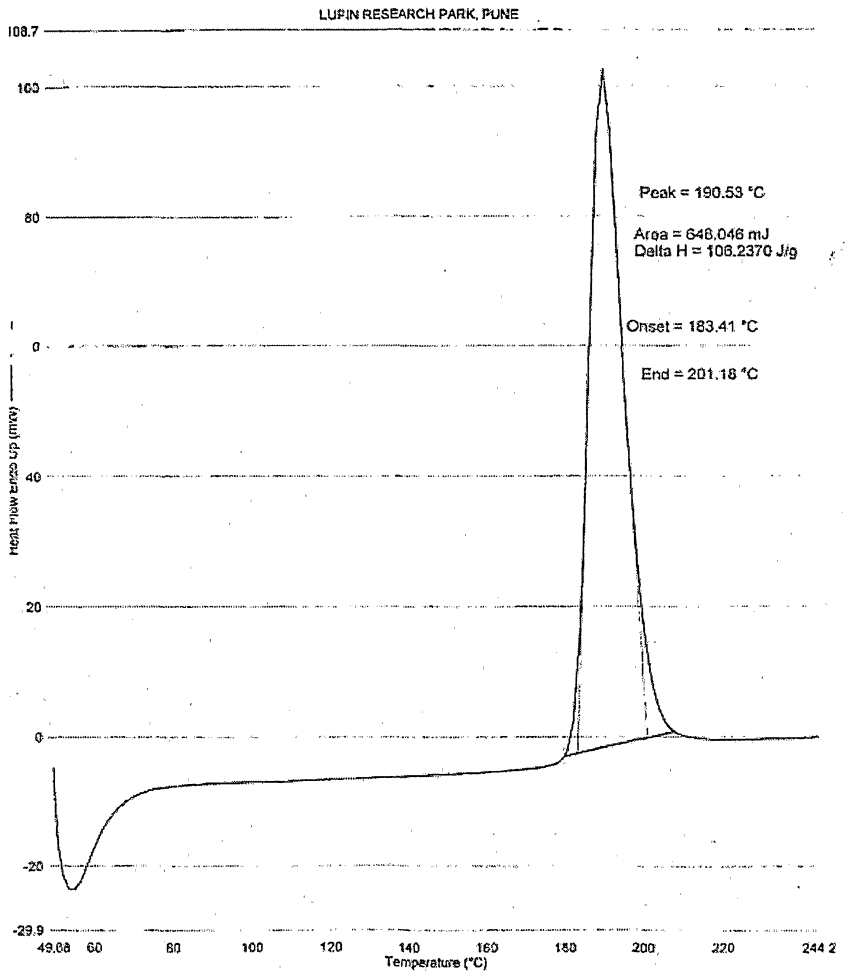


Figure 11: DSC thermogram of crystalline form III of Lamivudine at heating rate 2°C/min.

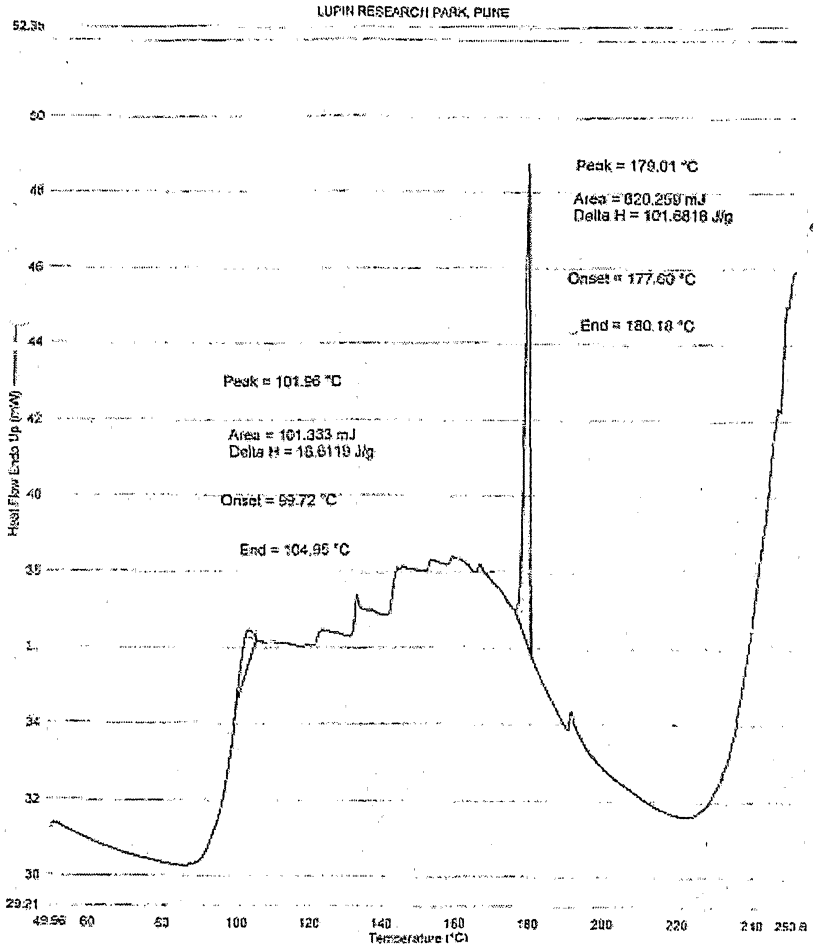


Figure 12: DSC thermogram of crystalline form III of Lamivudine at heating rate 100°C/min.

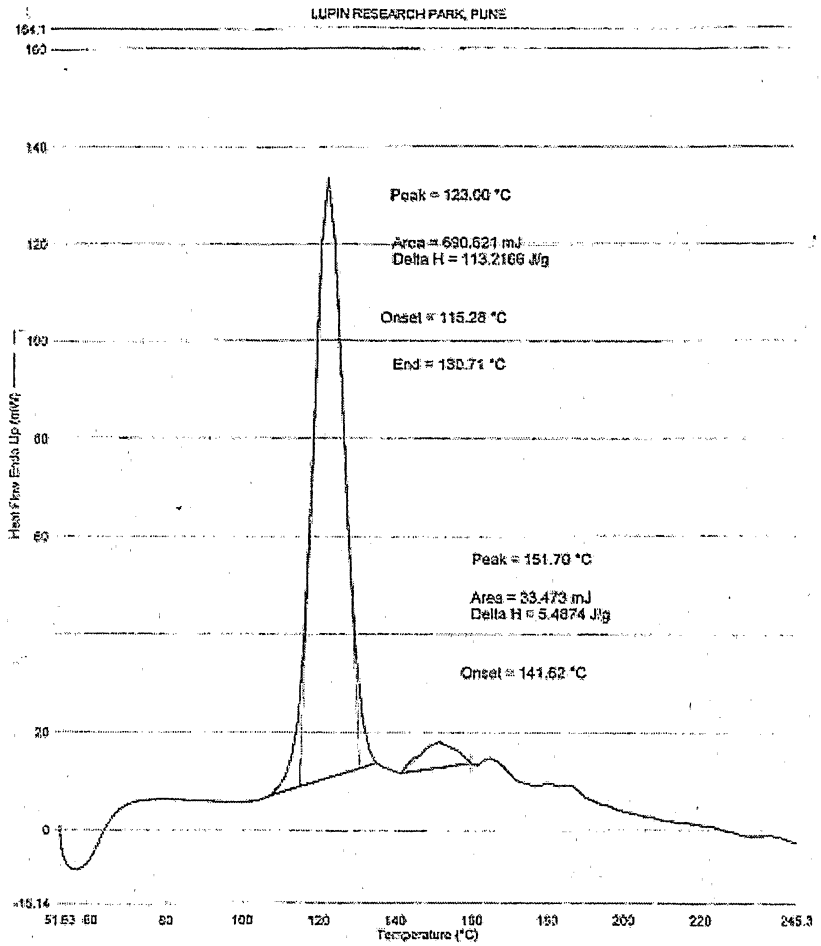


Figure 13: FTIR spectra of crystalline form I of Lamivudine.

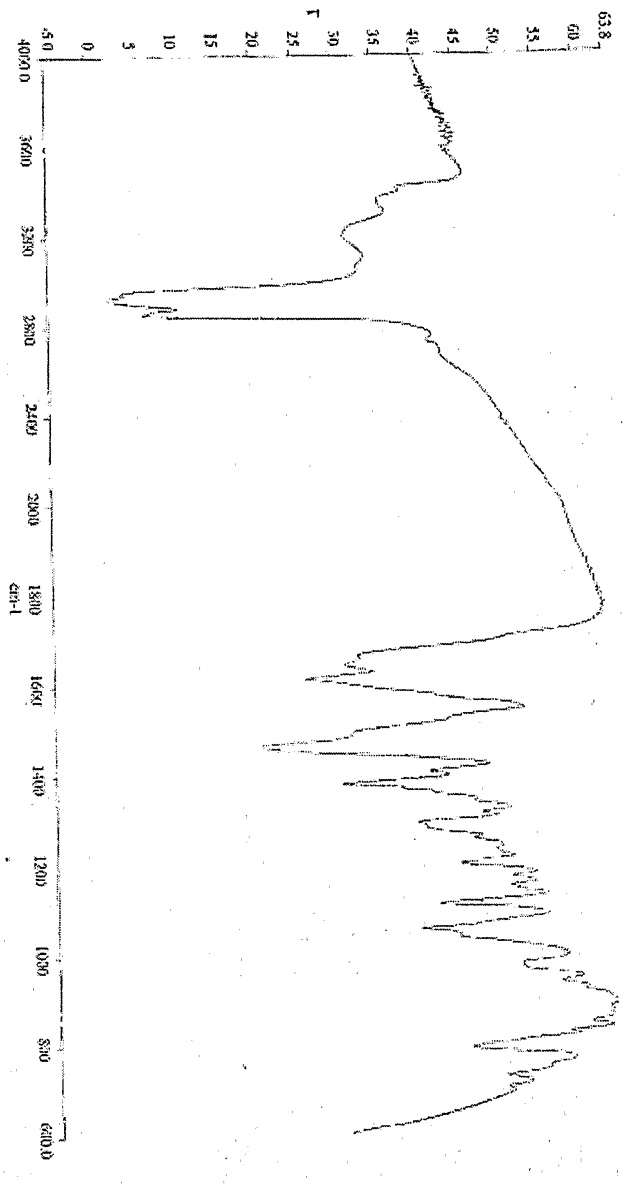


Figure 14: FTIR spectra of crystalline form II of Lamivudine.

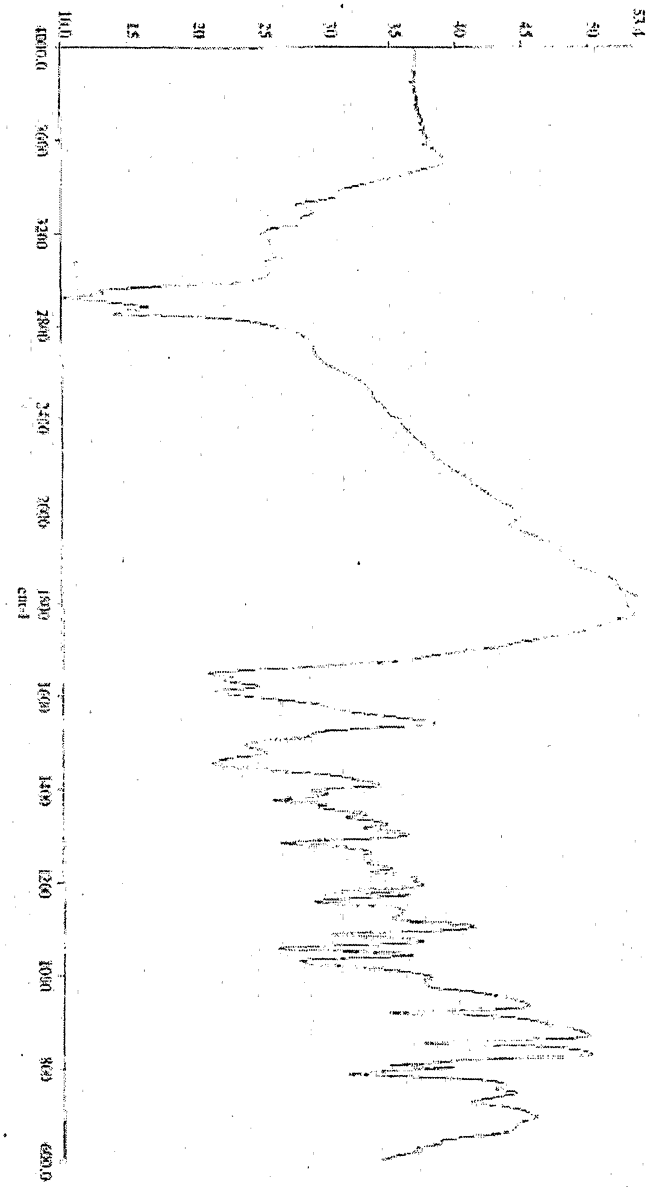


Figure 15: FTIR spectra of crystalline form III of Lamivudine.

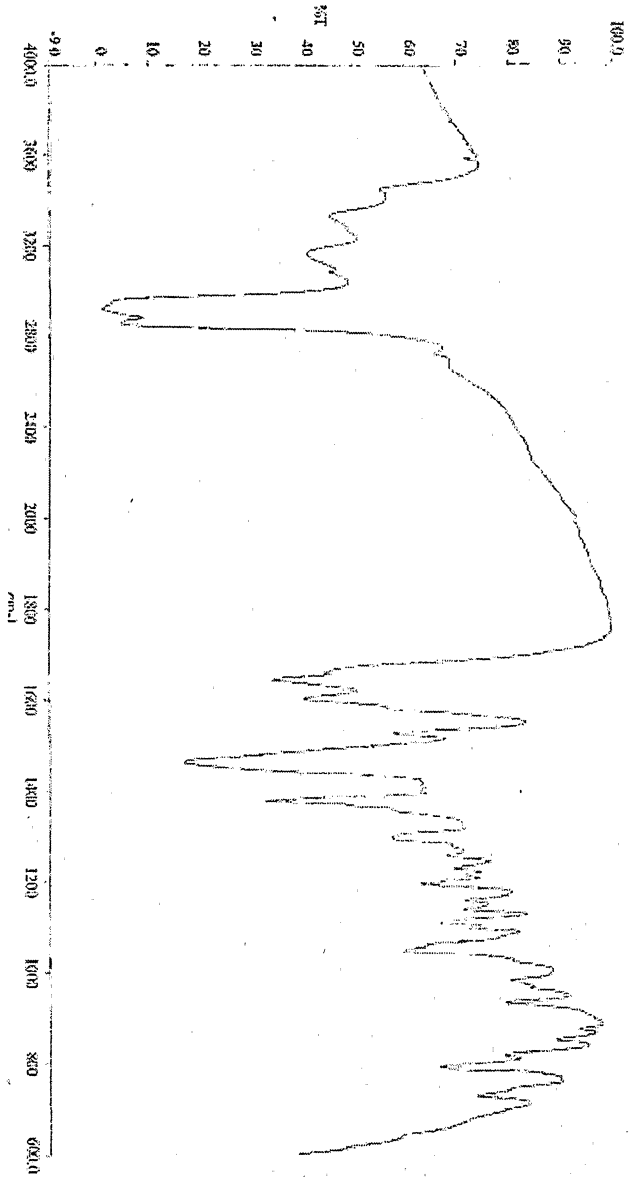
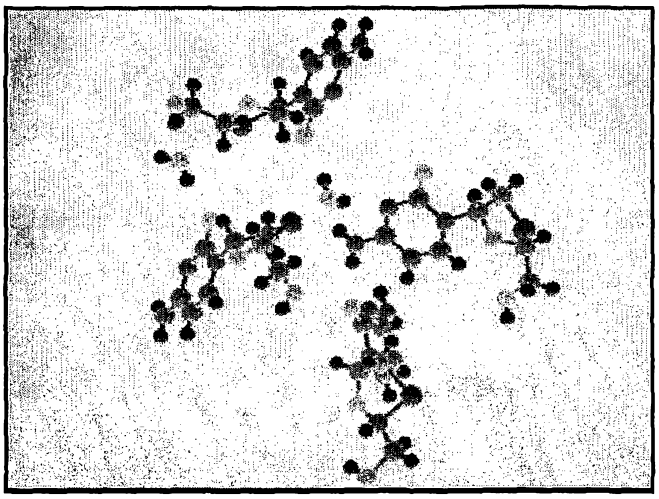
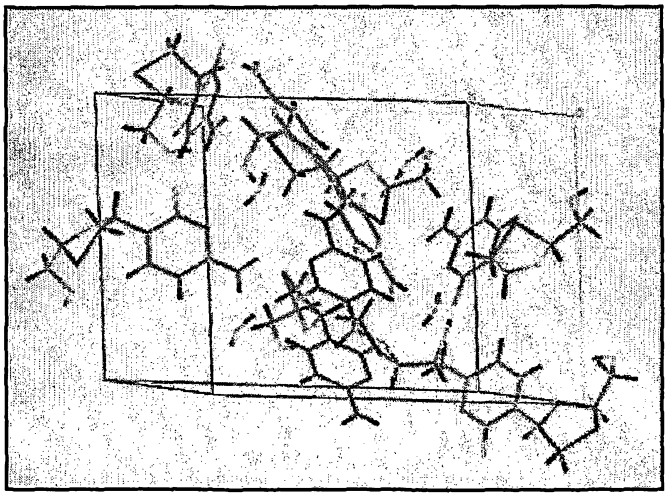


Figure 16: crystal structure and packing diagram of crystalline form III of Lamivudine obtained by Single crystal X-ray diffraction analysis



(a) Crystal Structure of Lamivudine form III. (Disordered atom of minor component S9D1 has been omitted for clarity.)



(b) Packing diagram of Lamivudine form III. (Disordered atom of minor component S9D1 has been omitted for clarity)