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(71) Applicant *(for all designated States except US)*: **LUPIN LIMITED** [IN/IN]; 159, CST Road, Kalina, Santacruz (East), Mumbai 400 098, Maharashtra (IN).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **ROY, Bhairab, Nath** [IN/IN]; Lupin Limited (Research Park), 46A/47A, Village Nande, Taluka Mulshi, Pune 411 042, Maharashtra (IN). **SINGH, Girij, Pal** [IN/IN]; Lupin Limited (Research Park), 46A/47A, Village Nande, Taluka Mulshi, Pune 411 042, Maharashtra (IN). **SRIVASTAVA, Dhananjai** [IN/IN]; Lupin Limited (Research Park), 46A/47A, Village Nande, Taluka Mulshi, Pune 411 042, Maharashtra (IN). **JADHAV, Harishchandra, Sambhaji** [IN/IN]; Lupin Limited (Research Park), 46A/47A, Village Nande, Taluka Mulshi, Pune 411 042, Maharashtra (IN). **DESIRAJU, Gautam R.** [IN/IN]; School of Chemistry, University of Hyderabad, Hyderabad 500 046 (IN). **BHATT, Prashant, M.** [IN/IN]; School of Chemistry, University of Hyderabad, Hyderabad 500 046 (IN).

(74) Agents: **MAJUMDAR, Subhatosh** et al.; S. Majumdar & Co., 5, Harish Mukherjee Road, Kolkata 700 025 (IN).

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(54) **Title**: LAMIVUDINE : ZIDOVUDINE : WATER 1 : 1 : 1 COCRYSTAL

(57) **Abstract**: Co-crystal of lamivudine with zidovudine comprising one molecule each of lamivudine, zidovudine and water; having distinct X-ray diffraction peaks at 2θ of 9.57, 11.45, 13.68, 15.57, 16.49, 18.35, 18.82, 20.45, 22.18, 22.78, 24.52, 24.99, 26.36, 26.55, 26.58 ± 0.2°. Process for preparation of co-crystal of lamivudine with zidovudine comprising of trituration of equimolar quantities of Lamivudine and zidovudine in presence of water at ambient temperature, optionally adding organic solvent during trituration, and drying the product. Process for preparation of co-crystal of lamivudine with zidovudine comprising stirring equimolar quantities of lamivudine and zidovudine in an organic solvent in presence of water at ambient temperature, evaporating solvents of the solution, allowing crystallization of product with optional seeding, isolating co-crystals and drying. Pharmaceutical composition comprising therapeutically effective amount of co-crystals of lamivudine, zidovudine. Use of therapeutically effective amount of co-crystal Zidovudine, Lamivudine for manufacture of medicament for treatment of HIV infections of humans.

LAMIVUDINE : ZIDOVUDINE : WATER 1 : 1 : 1 COCRYSTAL

Field of Invention:

The invention relates to pharmaceutical co-crystal of Lamivudine with Zidovudine and the process of preparation thereof.

Back Ground:

To be a drug product not only the active pharmaceutical ingredient (API) should have desirable pharmacological and pharmacokinetic properties but also must be amenable for formulation into a suitable dosage form so that on ingestion it also gives desirable pharmacological, pharmacokinetic and pharmacodynamic effects.

The physicochemical characteristics of API more often than not determines formulation development work.

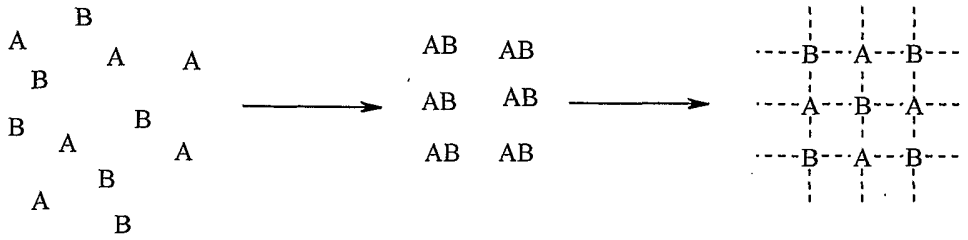
The important characteristics of API amongst other include (a) optimum solubility and dissolution rate (b) stability, should not be degraded and converted to other substance both by physical factors like heat, light as well as chemical interaction (c) must have desirable flow properties, bulk density and should not stick to the walls of the vessel, and most importantly (d) should be bioavailable / bioequivalent. All of these characters of an API are essentially dependant on its crystal structure.

As mentioned earlier crystal structure is a center point of solid stage manipulation and formulation development of API. Hence attempts are being made to obtain a crystal structure that is a desired polymorph. Therefore attempts have been made, though pain taking, to obtain crystal structure, which would give desirable dosage forms having all the properties mentioned hereinbefore.

When crystallization of a single substance takes place, identical molecules are incorporated in the crystal lattice. In short, a crystal lattice contains the same

molecules arranged in periodic order packed with the help of non-covalent bonds like hydrogen bonds, co-ordinate bonds, van der Waals forces, etc.

However, when two or more molecules are crystallized together, the crystallization could be homo or hetero. When it is hetero, the formation of a crystal lattice (A.B) is called as co-crystal.



Such incorporation of a chemically distinct molecule could drastically change the physicochemical characteristics like solubility, dissolution rate, stability, etc. and hence can alter the bioavailability of the active pharmaceutical ingredient. For example US 2007/0059356 describes how effectively co-crystallization enabled to convert the injectable API to conventional oral solid dosage form. In short effect of co-crystallization can be so dramatic to reach a far reaching of consequences and ramification.

It would not be out of place to review the advancement in the research in co-crystals in view of active pharmaceutical ingredients.

Co-crystals are crystals that contains two or more non-identical molecules. Various co-crystals were reported in Etter MC, and Adsmond DA "The use of co-crystallization as a method of studying hydrogen bond preferences of 2-aminopyridine" J. Chem. Soc, Chem. Commun. (1990) 589-591, Etter MC, et al., "Graph-set analysis of hydrogen-bond patterns in organic crystals" Acta Crystallogr. Sect. B, Struct. Sci. (1990), B46 256-262; Etter MC, et al., "Hydrogen bond directed

co-crystallization and molecular recognition properties of diarylureas" J. Am. Chem. Soc. (1990) 112 8415-8426, which are incorporated herein by reference in their entireties. The following articles are also incorporated herein by reference in their entireties: Carl HG and Hans PH,"On the inclusion of solvent molecules in the crystal structures of organic compounds"; Acta Cryst. (2000), B56, 625-534; SenthilKumar VS, Nangia A, et al., "Molecular Complexes of Some Mono- and Dicarboxylic Acids with *trans*-l,4,-Dithiane-l,4-dioxide" Crystal Growth & Design (2002), 2(4), 325 - 328; and Desiraju G. R., "Crystal and co-crystal" Cryst..Eng. Comm. (2003), 5, 466-467.

By co-crystallizing an active agent with a guest, one creates a new solid form which has unique properties compared to the existing solid forms of that active agent. For example, a co-crystal may have different dissolution and solubility properties than the active agent itself or as a salt. The guest molecule is often a pharmaceutically acceptable compound (which could also be an API). Co-crystals containing APIs can be used to deliver APIs therapeutically. New drug formulations comprising co-crystals of APIs with pharmaceutically acceptable guests may have superior properties over existing drug formulations (US 2007/0059356 and WO 2007/067727). Active agents and guests may also include nutraceuticals, agricultural chemicals, pigments, dyes, explosives, polymer additives, lubricant additives, photographic chemicals, and structural and electronic materials.

It has been speculated (Chem. Commun. 2005, 4601-4603) that the formation of co-crystal might considerably reduce the possibility of polymorphism in API. The authors found that among the approximately 1450 structures of hydrogen-bonded co-crystals in the Cambridge Structure Database only 11 entries were polymorphic. This behavior of the co-crystals can be attributed to the stronger intermolecular interactions between the active molecule and the host molecule that impart physical stability to the solid form. Reduction in chances of unanticipated polymorphism in

solid co-crystals of the active ingredient would minimize the problems faced by the formulation chemists during manufacture of dosage forms of metastable solid forms.

Combivir® is a pharmaceutical fixed dose combination containing Lamivudine and Zidovudine for treatment of HIV infection. Fixed dose combination of Lamivudine and Zidovudine is disclosed in US patent number 5,627,186 and 5,859,021. The ratio of Lamivudine as to Zidovudine varied from 1:2 to 1:1 by weight. During formulation both the drugs were physically mixed along with the excipients and compressed to form a tablet.

US 5905082 describes two polymorphic modifications of Lamivudine *viz* form I and II. and WO2007119248 describes a novel polymorph III. Polymorph III and I are metastable hydrated forms and polymorph II is an anhydrous form. Though polymorph II is thought to be the thermodynamically most stable form, form I and form III can also be formulated into a solid dosage form. As described in WO2007119248 all these polymorphic forms of Lamivudine get converted to other polymorphs under certain conditions. Moreover, form I is needle shaped and therefore poses problems in mixing and has poor flow property and bulk density. Therefore, it is desirable to make a stable crystal form of Lamivudine having better flow property and bulk density, thereby possessing better manufacturability.

During the crystallization study of Lamivudine, the present inventors have found that interestingly Lamivudine forms a 1:1:1 mole co-crystal with Zidovudine and water, and surprisingly, the co-crystal formed is stable when stirred with either water or any other solvents like isopropanol and the DSC thermogram shows single endothermic peak at about 100°, which shows that it is a stable crystal form, *i.e.* does not undergo modification on heating.

Objects of the invention

Thus an object of the present invention is to provide a novel co-crystal monohydrate of Lamivudine with Zidovudine.

Another object of the present invention is to provide a physically stable crystal form of Lamivudine co-crystallized with Zidovudine.

A further object of the present invention is to provide a process for preparation of a novel co-crystal of Lamivudine with Zidovudine using the eco-friendly solvent "water" and using minimum quantity of solvent thereby reducing the generation of effluents.

Summary of invention

Thus in the present invention there is provided a co-crystal of Lamivudine with 1 molecule of Zidovudine and one molecule of water having characteristic powder X-ray difractogram as shown in figure 1 with characteristic 2θ values as given in Table -I.

According to another aspect there is provided a process of preparation of co-crystal of 1 molecule each of Lamivudine, Zidovudine and water by wet grinding Lamivudine and Zidovudine in presence of water.

Brief description of the Accompanying drawings

Figure 1: powder X-ray diffractogram of co-crystal.

Figure 2: DSC of co-crystal

Figure 3: Powder X-ray diffractogram of equimolar physical mixture of Lamivudine and Zidovudine

Figure 4: FTIR spectra of co-crystal of Lamivudine.

Figure 5: FTIR spectra of equimolar physical mixture of lamivudine and zidovudine.

Figure 6: TGA of co-crystal

Figure 7: ORTEP diagram of co-crystal

Figure 8. Different intermolecular interactions of zidovudine molecule in the crystal structure of co-crystal.

Figure 9. Different intermolecular interactions of lamivudine molecule in the crystal structure of co-crystal.

Figure 10. 1 D ribbon type arrangement of lamivudine and ziodvudine molecules in the crystal structure of co-crystal.

Figure 11. 1 D ribbons from perpendicular view and interconnectivity between them in the crystal structure of co-crystal.

Figure 12. Simulated PXRD of co-crystal

Figure 13: DSC thermogram of crystalline form I of Lamivudine

Figure 14 TGA thermogram of crystalline form I of Lamivudine.

Figure 15: DSC thermogram of crystalline form II of Lamivudine

Figure 16: TGA thermogram of crystalline form II of Lamivudine

Figure 17: DSC thermogram of crystalline form III of Lamivudine

Figure 18: TGA thermogram of crystalline form III of Lamivudine.

Figure 19: DSC thermogram of Zidovudine

Figure 20: TGA thermogram of Zidovudine.

Figure 21: DSC thermogram of equimolar physical mixture of Lamivudine and Zidovudine

Figure 22: TGA thermogram of equimolar physical mixture of Lamivudine and Zidovudine

Description of the invention:

Three polymorphic modifications of Lamivudine *viz* form I, II. and III get interconverted under certain conditions.. Polymorphic form I and III are the metastable forms according to the DSC thermogram, both of them are converted to polymorphic form II (as described in page 1 to 5 of WO2007/119248). Form I and II when stirred with water get converted to polymorphic form III (see page 5 and

examples 11 and 12 of WO2007/119248). Hence, attempts to formulate form I or II using water as a solvent would result in form III. These interconversions may cause instability to the pharmaceutical formulations.

The co-crystal of Lamivudine with Zidovudine is stable when stirred in water and other organic solvents as revealed by powder X ray diffraction and other solid state properties like FTIR, DSC and TGA. The DSC thermogram (Figure 2) of the co-crystal shows a single peak of the endothermic deformation corresponding to its melting point. There is no other peak in DSC, which shows that the co-crystal is stable on heating.

When the experiments were carried out to study the stability of the co-crystal in various solvents like water and isopropanol, no change in the crystal structure of the co-crystal was observed. Hence, there would be no restriction in choosing a solvent for the formulation of lamivudine.

The solid-state properties like powder X-ray diffractogram and FTIR of the co-crystal are distinct over the equimolar physical mixture of Lamivudine and Zidovudine. The comparative data for the physical mixture and the co-crystal of Lamivudine are provided in Table I.

The co-crystal of Lamivudine has better flow property and bulk density, which are important parameters for formulation (Table II).

Table I:

Powder X-ray diffraction		FTIR	
Co-crystal (2θ values) (Fig. 1)	**Physical mixture** (2θ values) (Figure 3)	**Co-crystal** (cm^{-1}) (Figure 4)	**Physical mixture** (cm^{-1}) (Figure 5)
9.57	8.92	3532, 3414,	3461, 3329,
11.45	10.67	3310, 3219,	3184, 3029,
13.68	12.14	3087, 3014,	2825, 2117,
15.57	13.39	2956, 2920,	2083, 1672,
16.49	14.74	2832, 2160,	1613, 1487,
18.35	14.27	2093, 1713,	1399, 1360,
18.82	15.59	1642, 1524,	1336, 1316,
20.45	15.81	1498, 1473,	1282, 1259,
22.18	15.96	1436, 1269,	1181, 1160,
22.78	20.61	1104, 1053,	1142, 1089,
24.52	21.42	827, 782,	1059, 1031,
24.99	21.48	758, 741,	918, 897,
26.36	22.33	619, 601,	851, 806,
26.55	24.42	563, 496.	788, 763,
26.58	24.92		734, 632,
			593, 562,
			538, 495.

Table II

Property	Lamivudine	Zidovudine	Physical Mixture	Co-crystal
Bulk Density (gm /cc)	0.48	0.30	0.42	0.70
Tap Density (gm /cc)	0.77	0.46	0.60	0.92
Flow Property (Angle of Repose[$])	39.80°	37.14°	31.37	28.52°

Thermogravimetric analysis (as shown in fig. 6) of the co-crystal of Lamivudine shows 1.2 to 1.9 % 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 equimolar quantities of Lamivudine, Zidovudine and water in the co-crystal.

The co-crystal monohydrate is obtained by pulverization of stoichiometric quantities of Lamivudine and Zidovudine in presence of water. The co-crystal hydrate can also

[$] 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)

be obtained by dissolving Lamivudine and zidovudine in an organic solvent containing water, concentrating the solution and allowing the concentrated solution to stand, which can optionally be seeded with the co-crystal.

For the study by single crystal X-ray crystallography methods the single crystal of the co-crystal was developed as follows: 22.9 mg of lamivudine and 26.7 mg of zidovudine were taken in a sample vial. 1 ml ethanol was added and the mixture was warmed for 2-3 minutes until a clear solution was obtained. The solution was allowed to evaporate slowly for 2 days. Diffraction quality single crystals were obtained from the crystalline mass obtained after 2 days.

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 1.033. A total of 11952 reflections were measured on the diffractometer with monochromatised Mo-Kα radiation. The data was collected at θ ranging from 1.10 to 26.06°. The structure was solved by direct methods and the non-hydrogen atoms were refined anisotropically. All H atoms were refined isotropically. Refinement converged to give R1 = 0.0377, wR2 = 0.0975. Minimum residual electron density was -0.232 e. $Å^{-3}$ and maximum residual electron density was 0.336 $Å^{-3}$. The data is as shown in Table III and figures 7 to 11.

Crystal structure obtained by single crystal X-ray diffraction analysis (Fig. 7) reveals the association of one molecule each of Lamivudine, Zidovudine and water in the co-crystal with the help of hydrogen bonds as follows:

The crystal structure of the co-crystal has a large number of intermolecular interactions present. Each zidovudine molecule forms five different interactions with three different lamivudine molecules (N–H...O, O–H...O) and two different water molecules (N–H...O, O–H...O) as shown in figure 8. Each lamivudine molecule

forms four different interactions with three different zidovudine molecules (N–H...O, O–H...O) and one water molecule (O–H...N) as shown in figure 9. Lamivudine, zidovudine and water make an infinite 1 D ribbon-type arrangement as shown in figure 10. These ribbons are further connected by O–H...O interactions between zidovudine and lamivudine as shown in figure 11.

Powder X-ray diffractogram generated from computer simulation of single crystal X-ray data is shown in figure 12.

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

Table III

Unit Cell	Monoclinic
Space group	$P2_1$
Unit cell dimensions	a = 8.6899 (10) Å, b = 7.2501 (9) Å, c = 18.504 (2) Å; $\alpha = 90°$, $\beta = 92.506°$, $\gamma = 90°$
Z, calculated density	2, 1.493 Mg/m^3.
Cell volume	1164.7 (2) $Å^3$
Cell Measurement temperature	298 (2) K
R factor	0.0385
Flack Parameter	0.03 (8)
Goodness of fit	1.016
Completeness	99.3%

Attempts to obtain an anhydrous co-crystal were unsuccessful since it is evident from the single crystal X-ray diffraction data of co-crystal that water molecule plays an important role in formation of hydrogen bonds between lamivudine and zidovudine.

Comparative thermal analysis data is tabulated in Table IV

Table IV

Product	Melting Point	DSC	TGA
Lamivudine form I	135 - 145°C 124 – 127°C (a) 135°C (b)	Two endothermic peaks at 123° and 177° (fig. 13)	One step weight loss between temp 80°C to 140°C = 1.52% (Fig. 14)
Lamivudine form II	177 – 178°C 177 – 178 (a & b)	Single endothermic peak at 177°C (Fig. 15)	No weight loss due to crystal bound water. (Fig. 16)
Lamivudine form III	176 - 177°C (c)	Two endothermic peaks at 100°C and 177°C. (Fig. 17)	One step weight loss between temp 80°C to 140°C = 4.14% (Fig. 18)
Zidovudine	106 – 112°C (d) 120 – 122°C (e)	Single endothermic peak at 125.62°C (figure 19)	
Physical Mixture	112 – 115°C	Single endothermic peak at 119.53°C (figure 21)	No weight loss due to crystal bound water. (Fig. 22)
Co-crystal	94 – 95°	Single endothermic peak at 101.85°C (figure 2)	One step weight loss between temp 80°C to 100°C = 1.2 to 1.9% (Fig. 6)

(a) US5905082; **(b)** Journal of Pharm. Sci Vol 85 (2), page 195 (1996); **(c)** WO2007119248; **(d)** *J. Org. Chem.* 29, 2076 (1964); **(e)** *J. Org. Chem.* **38,** 4299 (1973)

The solubilities of the co-crystal and equimolar physical mixture in methanol are comparable. There is a slight decrease in the aqueous solubility of the co-crystal as compared to that of the physical mixture. Comparative solubility data of the co-crystal and the physical mixture at a temperature of 25 ± 0.5 °C are provided in table V.

Table V

Product		***Solubility in water (gm/ml)***	***Solubility in methanol (gm/ml)***
Physical Mixture	Lamivudine	0.018	0.048
	Zidovudine	0.018	0.089
Co-crystal	Lamivudine	0.013	0.048
	Zidovudine	0.017	0.091

Pharmaceutical formulations suitable for administration may conveniently be presented as discrete units such as capsules, cachets or tablets each containing a

predetermined amount of the co-crystal or combinations of the co-crystal with other antiviral compounds or co-crystals thereof.

Lamivudine polymorphs I, II and III were prepared for comparison by referring to the examples provided in US 5905082 and WO2007/119248. 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. The term 'Lamivudine' in the examples refers to Lamivudine form II unless specified.

Example 1:

458 mg of lamivudine and 534 mg of zidovudine were taken in a mortar and pestle and the mixture was triturated for 5 minutes to make a homogeneous mixture. 5-6 drops of ethanol and 5-6 drops of water were added to mixture at that stage and the mixture was then vigorously ground for a subsequent 15 minutes. The product obtained was further dried at 40°C in vacuum drier for four hours. Yield: 0.992 gm

The melting point is 94 - 95 °C.

FTIR [KBr] (cm^{-1}): 3532, 3414, 3310, 3219, 3087, 3014, 2956, 2920, 2832, 2160, 2093, 1713, 1642, 1524, 1498, 1473, 1436, 1269, 1104, 1053, 827, 782, 758, 741, 619, 601, 563, 496. (Figure 4)

The powder X-ray diffraction analysis shows peaks at about 9.57, 10.15, 11.45, 13.11, 14.36, 15.52, 15.92, 16.49, 16.75, 18.00, 18.36, 18.83, 20.45, 20.80, 21.36, 22.18, 22.24, 22.79, 23.87, 24.52, 24.99, 25.41, 25.72, 26.37, 26.59, 27.02, 27.12, 27.54, 28.17, 28.55, 28.79, 30.20, 30.86, 31.36, 32.13, 32.67, 33.30, 33.58, 33.86, 34.96, 35.85, 36.15, 36.45, 36.99, 38.17, 38.85 ±0.2 degrees two theta. (Figure 1). The data/difractogram is superimposable with the one generated from single crystal data via computer simulation (Figure 12)

The differential scanning calorimetric analysis shows a single peak of the endotherm at 101.85°C (Fig. 2).

The thermogravimetric analysis exhibits a one-step weight loss of 1.45 % between 80°C to 100°C (Fig. 6) and Karl Fischer titration results in a moisture content of 3.60%.

Example 2

1.15 gm of lamivudine and 1.34 gm of zidovudine were taken in a mortar and pestle and the mixture was triturated for 5 minutes to make a homogeneous mixture. 5-6 drops of methanol and 5-6 drops of water were added to mixture at that stage and the mixture was then vigorously ground for a subsequent 20 minutes. The product obtained was further dried at 40°C in vacuum drier for four hours. Yield: 2.49 gm

The melting point of resultant material is 94 - 95°C.

Powder X-ray diffraction pattern and FTIR spectra is superimposable with that of the co-crystal as obtained in Example 1.

Example 3

1.15 gm of lamivudine and 1.34 gm of zidovudine were taken in a mortar and pestle and the mixture was triturated for 5 minutes to make a homogeneous mixture. 5-6 drops of ethanol and 5-6 drops of water were added to mixture at that stage and the mixture was then vigorously ground for a subsequent 15 minutes. The resulting material was then transferred to a 100 ml round bottom flask. 20 ml of distilled ethanol and 5 ml of distilled water was added and the mixture was stirred at 60 °C for 1hour. The resulting solution was transferred to a 250 ml conical flask and allowed to evaporate slowly. Seed crystals of the co-crystal were added to the

solution after 2 days. The crystalline mass obtained after one more day was filtered. The product obtained was further dried at 40°C in a vacuum drier for four hours. Yield 2.3 gm

The melting point of resultant material is 94 - 95°C.

Powder X-ray diffraction pattern and FTIR spectra were superimposable with that of the co-crystal as obtained in Example 1.

Example 4

1.15 gm of lamivudine and 1.34 gm of zidovudine were taken in a 100 ml round bottom flask. 15 ml of distilled methanol and 5 ml of distilled water were added. The solution was concentrated in a rotavapor at around 80 °C. The resulting solution (≈ 10 ml) was allowed to stand. The crystalline mass obtained after a few hours was filtered. The product obtained was further dried at 40°C in vacuum drier for four hours. Yield: 2.25 gm

The melting point of the resultant material is 94 - 95°C.

Powder X-ray diffraction pattern and FTIR spectra are superimposable with that of the co-crystal as obtained in Example 1.

Example 5

1.15 gm of lamivudine and 1.34 gm of zidovudine were taken in a 100 ml round bottom flask. 15 ml of distilled methanol and 5 ml of distilled water was added. The solution was concentrated in rotavapor at around 80 °C. Resulting solution (≈ 10 ml) was allowed to stand and seed crystals of co-crystal were added into it. Crystalline mass obtained after around 1 hour was filtered. The product obtained was further dried at 40°C in vacuum drier for four hours. Yield: 2.3 gm

The melting point of the resultant material is 94 - 95°C.

Powder X-ray diffraction pattern and FTIR spectra are superimposable with that of the co-crystal as obtained in Example 1.

Example 6

1.15 gm of lamivudine and 1.34 gm of zidovudine was taken in a 100 ml round bottom flask. 15 ml of distilled ethanol and 1.5 ml of distilled water was added in to it. The solution was concentrated in rotavapor at around 95 °C. The resulting solution (≈ 7 ml) was allowed to stand. The crystalline mass obtained after few hours was filtered. The product obtained was further dried at 40°C in vacuum drier for four hours. Yield: 2.3 gm

The melting point of the resultant material is 94 - 95°C.

Powder X-ray diffraction pattern and FTIR spectra are superimposable with that of the co-crystal as obtained in Example 1.

Example 7

4.58 gm of lamivudine and 5.34 gm of zidovudine were taken in a 100 ml round bottom flask. 50 ml of distilled ethanol and 5 ml of distilled water were added. The solution was concentrated in rotavapor at around 95 °C. The resulting solution (≈ 15 ml) was allowed to stand and seed crystals of co-crystal were added. The crystalline mass obtained after one hour was filtered. The product obtained was further dried at 40°C in a vacuum drier for four hours. Yield: 9.8 gms

The melting point of the resultant material is 94 - 95°C.

Powder X-ray diffraction pattern and FTIR spectra are superimposable with that of the co-crystal as obtained in Example 1.

Example 8

1.15 gm of lamivudine and 1.345 gm of zidovudine were taken in a 100 ml round bottom flask. 11 ml of acetone and 4 ml of distilled water were added. The mixture was stirred for 10 minutes to make a homogeneous clear solution. The solution was allowed to evaporate for 2 days. A crystalline mass was obtained. The product obtained was further dried at 40°C in a vacuum drier for four hours. Yield: 2.49 gm

The melting point of the resultant material is 94 - 95°C.

Powder X-ray diffraction pattern and FTIR spectra are superimposable with that of the co-crystal as obtained in Example 1.

Example 9

1.15 gm of lamivudine and 1.345 gm of zidovudine were taken in a 100 ml round bottom flask. 11 ml of acetonitrile and 4 ml of distilled water were added. The mixture was stirred for 10 minutes to make a homogeneous clear solution. The solution was allowed to evaporate for 2 days. The product obtained was further dried at 40°C in a vacuum drier for four hours. Yield: 2.60 gm

The melting point of the resultant material is 94 - 95°C.

Powder X-ray diffraction pattern and FTIR spectra are superimposable with that of the co-crystal as obtained in Example 1.

Example 10

1.15 gm of lamivudine and 1.34 gm of zidovudine were taken in a mortar and pestle and the mixture was triturated for 5 minutes to make a homogeneous mixture. 11 drops of acetone and 4 drops of water were added to the mixture at this stage and the mixture was then vigorously ground for a subsequent 20 minutes. The product obtained was further dried at 40°C in a vacuum drier for four hours. Yield: 2.49 gm.

The melting point of the resultant material is 94 - 95°C.

Powder X-ray diffraction pattern and FTIR spectra are superimposable with that of the co-crystal as obtained in Example 1.

Example 11

1.15 gm of lamivudine and 1.34 gm of zidovudine were taken in a mortar and pestle and the mixture was triturated for 5 minutes to make a homogeneous mixture. 11 drops of acetonitrile and 4 drops of water were added to the mixture at this stage and the mixture was then vigorously ground for a subsequent 20 minutes. The product obtained was further dried at 40°C in vacuum drier for four hours. Yield: 2.49 gm

The melting point of the resultant material is 94 - 95°C.

Powder X-ray diffraction pattern and FTIR spectra are superimposable with that of the co-crystal as obtained in Example 1.

Example 12:

1.15 gm of lamivudine and 1.34 gm of zidovudine were taken in a mortar and pestle and the mixture was triturated for 2 minutes to make a homogenous mixture. 2 ml of ethanol and 0.5 ml of water were added to the mixture in 4 portions and ground for 1

hour. The product obtained was further dried at 40°C in a vacuum drier for four hours. Yield: 2.49 gm

The melting point of the resultant material is 94 - 95°C.

Powder X-ray diffraction pattern and FTIR spectra are superimposable with that of the co-crystal as obtained in Example 1.

Example 13:

1.15 gm of lamivudine and 1.34 gm of zidovudine were taken in a mortar and pestle and the mixture was triturated for 5 minutes to make a homogeneous mixture. 5-6 drops of water were added to the mixture and vigorously ground for a subsequent 20-30 minutes. The sample was then dried under vacuum at 40 °C for 4 hours. Yield: 2.48 gm.

The melting point of the resultant material is 94 - 95°C.

Powder X-ray diffraction pattern and FTIR spectra are superimposable with that of the co-crystal as obtained in Example 1.

Example 14:

1.15 gm of lamivudine and 1.34 gm of zidovudine were taken in a mortar and pestle. The solid material was triturated and blended homogeneously for around 20 minutes to obtain 2.49 gm of a homogeneous physical mixture of equimolar quantities of lamivudine and zidovudine.

The melting point is 112 - 115 °C.

FTIR [KBr] (cm^{-1}): 3461, 3329, 3184, 3029, 2825, 2117, 2083, 1672, 1613, 1487, 1399, 1360, 1336, 1316, 1282, 1259, 1181, 1160, 1142, 1089, 1059, 1031, 918, 897, 851, 806, 788, 763, 734, 632, 593, 562, 538, 495. **(Figure 5)**

X-ray powder diffraction analysis shows peaks at about 8.92, 10.04, 10.68, 12.15, 13.39, 14.28, 14.75, 15.60, 15.82, 15.97, 16.81, 17.20, 17.53, 17.88, 18.24, 19.37, 19.66, 20.15, 20.61, 20.68, 20.88, 21.15, 21.43, 21.49, 21.81, 22.33, 22.76, 23.00, 23.26, 23.74, 24.23, 24.42, 24.92, 25.00, 25.69, 26.48, 27.21, 27.47, 27.89, 28.40, 28.73, 29.46, 29.72, 30.19, 31.45, 31.53, 31.81, 32.39, 32.59, 33.12, 33.99, 34.80, 35.19, 35.52, 36.19, 36.95, 37.25, 37.75, 38.43 ±0.2 degrees two theta. (Figure 3)

The differential scanning calorimetric analysis shows a single peak of endotherm at 119.53°C (Fig. 21).

The thermogravimetric analysis reveals that it is an anhydrous product. (Fig. 22) and Karl Fischer titration results moisture content of 0.2 %.

Example 15:

1.15 gm of lamivudine and 1.34 gm of zidovudine were taken in mortar and pestle. The mixture was triturated for 5 minutes to make a homogeneous mixture. 10 – 12 drops of methanol added to the homogeneous mixture and the trituration was further continued for 20 minutes. The product obtained was further dried at 40°C in a vacuum drier for four hours. Yield: 2.49 gm

The analysis of the product (FTIR, powder X-ray diffraction, TGA, DSC, melting point) revealed that it is exactly the same as that of physical mixture obtained in example 14.

Example 16:

5 gm of co-crystal product as obtained by practicing the process of example 1 to 13 was added to 20 ml of isopropanol and the slurry so obtained was further stirred at ambient temperature for an hour. The slurry was further filtered and the product was dried at 40°C in vacuum drier for four hours. Yield: 4.8 gm

The melting point of the resultant material is 94 - 95°C.

Powder X-ray diffraction pattern and FTIR spectra are superimposable with that of the co-crystal as obtained in Example 1.

Example 17:

5 gm of co-crystal product as obtained by practicing the process of example 1 to 13 was added to 20 ml of water and the slurry so obtained was further stirred at ambient temperature for an hour. The slurry was further filtered and the product was dried at 40°C in a vacuum drier for four hours. Yield 4.5 gm

The melting point of resultant material is 94 - 95°C.

Powder X-ray diffraction pattern and FTIR spectra are superimposable with that of the co-crystal as obtained in Example 1.

Example 18: Preparation of tablets containing Lamivudine co-crystals and comparison with the tablets made out of physical mixture of Lamivudine and Zidovudine:

** Qty. varies based on Assay OAB and water content of Lamivuidne and Zidovudine*
*** Qty. to be adjusted based on Assay OAB and water content of Lamivuidne and*

S. No.	Ingredients	Mg/Tablet TRIAL I	TRIAL II
INTRAGRANULAR			
1.	Lamivudine and Zidovudine Co-Crystal*	336.38	-
2.	Lamivudine (Form I)*	-	150.00
3.	Zidovudine USP*	-	174.89
4.	Lactose monohydrate NF** (Pharmatose DCL 11)	231.82	243.61
5.	Dibasic Calcium phosphate USP (Di-Tab)	29.625	29.625
6.	Sodium Starch Glycolate NF (Primojel Type A)	56.25	56.25
7.	Povidone USP (PVP K-30)	15.00	15.00
8.	Isopropyl Alcohol USP***	Qs	Qs
EXTRAGRANULAR			
9.	Sodium Starch Glycolate NF (Primojel Type A)	75.00	75.00
10.	Magnesium stearate NF	5.625	5.625
	Total Weight	**750.00**	**750.00**

Zidovudine to keep tablet weight constant
***** Used as solvent for granulation. Evaporates during processing.

Dry mix was prepared by sifting Lactose monohydrate, Dibasic calcium Phosphate dihydrate, Sodium starch Glycolate through 30# ASTM S.S. Sieve and mixing together with Lamivudine and Zidovudine Co-crystal (for trial I) or Lamivudine and Zidovudine (For trial II) sifted through 30# ASTM S.S. Sieve. The dry mix was further granulated using Povidine solution in isopropyl alcohol as binder. Dried the granulated mass in dryer at 45±5°C and dried granules were sifted through 20# ASTM S.S. Sieve. Oversize granules were milled and sifted through 20# ASTM S.S. Sieve. The granules were blended with extragranular sodium starch glycolate and lubricated with Mg stearate. Lubricated blend was compressed using 17.5 X 7.18 mm modified-capsule-shaped punches.

Analysis of Tablets:

Tablet Parameters	TRIAL I		TRIAL II	
Tablet weight	750± 10 mg		750± 10 mg	
Thickness	6.40 – 6.60 mm		6.60 – 6.80 mm	
Hardness	100 to 130 N		100 to 130 N	
% Friability	0.05%		0.08%	
Assay (% of Label Claim) Lamivudine Zidovudine	 101.2 103.7		 99.2 101.2	
DISSOLUTION (0.1 N HCl, 900 mL, at 37°C, Paddle, 50 RPM)				
Time in Minutes	% Drug Release		% Drug Release	
	Lamivudine	Zidovudine	Lamivudine	Zidovudine
5	83.4	77.1	92.3	80.4
10	99.1	89.8	101.2	87.6
20	98.6	93.4	101.6	89.8
30	98.8	93.2	102.0	92.2
40	100.1	94.9	102.6	89.8

5

CLAIMS

1. Co-crystal of lamivudine with zidovudine comprising one molecule each of lamivudine, zidovudine and water; having distinct X-ray diffraction peaks at 2θ of 9.57, 11.45, 13.68, 15.57, 16.49, 18.35, 18.82, 20.45, 22.18, 22.78, 24.52, 24.99, 26.36, 26.55, 26.58 ± 0.2°.
2. The co-crystal as claimed in claim 1 having peak of endotherm at about 100°C in its differential scanning calorimetry profile.
3. The co-crystal as claimed in claim 1 showing sharp weight loss of 1.2 to 1.9% between the temperatures 80° and 100° in its thermogravimetric analysis.
4. The co-crystal as claimed in claim 1 having unit cell dimensions of

a = 8.6899 (10) Å	α = 90°
b = 7.2501 (9) Å	β = 92.506°
c = 18.504 (2) Å	γ = 90°

5. A process for preparation of co-crystal of lamivudine with zidovudine comprising of trituration of equimolar quantities of Lamivudine and zidovudine in the presence of water at ambient temperature, with optional addition of organic solvent during trituration, and drying of the product.
6. The process as claimed in claim 5 wherein the organic solvent is selected from the group of ethanol, methanol, acetone, acetonitrile or mixtures thereof.
7. The process as claimed in claim 5 wherein the solid mixture obtained after trituration is added to the mixture of organic solvent and water, stirred at 55 – 65°C, allowing the solvents to evaporate slowly and seeded to obtain co-crystal.
8. The process as claimed in claim 7 wherein the organic solvent employed is selected from C1 to C4 aliphatic alcohols
9. The process as claimed in claim 8 wherein the organic solvent employed is ethanol.

10. A process for preparation of the co-crystal of lamivudine with zidovudine comprising stirring equimolar quantities of lamivudine and zidovudine in an organic solvent in the presence of water at ambient temperature, evaporation of solvents of the solution, allowing the product to get crystallized with optional seeding, isolation of the co-crystals and drying.

11. The process as claimed in claim 10 wherein the organic solvent employed is selected from methanol, ethanol, acetone, acetonitrile or mixtures thereof.

12. A pharmaceutical composition in solid dosage unit form comprising of a therapeutically effective amount of co-crystals of equimolar proportion of lamivudine, zidovudine and water in combination with a pharmaceutically acceptable carrier therefor.

13. A pharmaceutical composition according to claim 12 in oral administration form.

14. A pharmaceutical composition according to claim 13 in tablet form.

15. Use of therapeutically effective amount of co-crystal of equimolar proportions of Zidovudine, Lamivudine and water for the manufacture of medicament for treatment of HIV infections in humans.

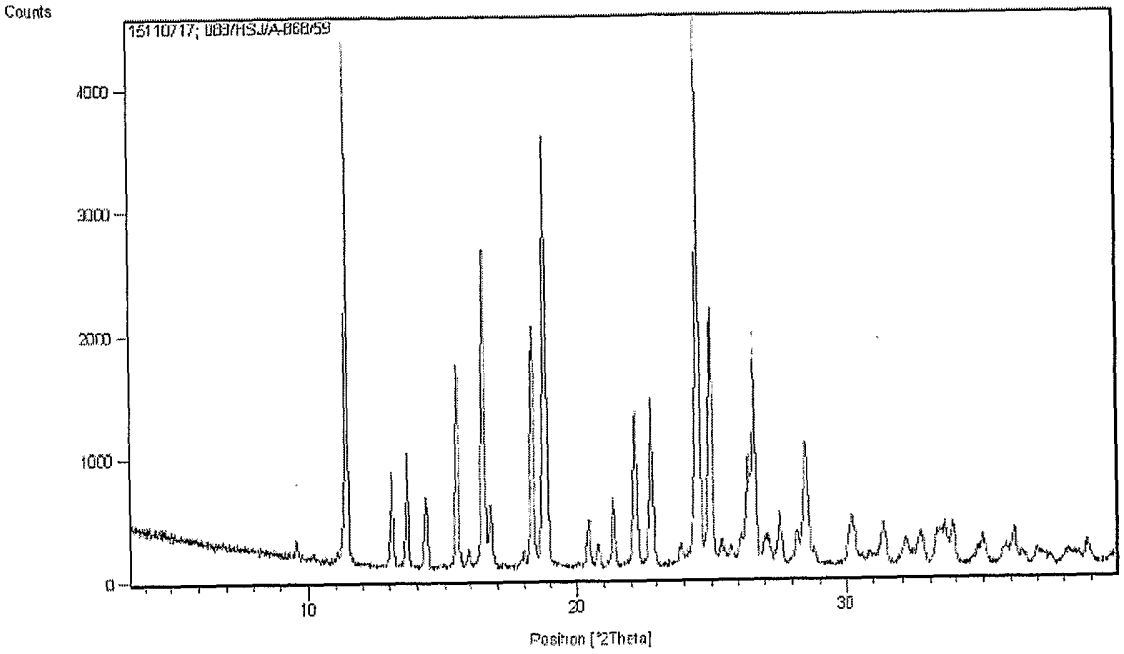


FIGURE 1

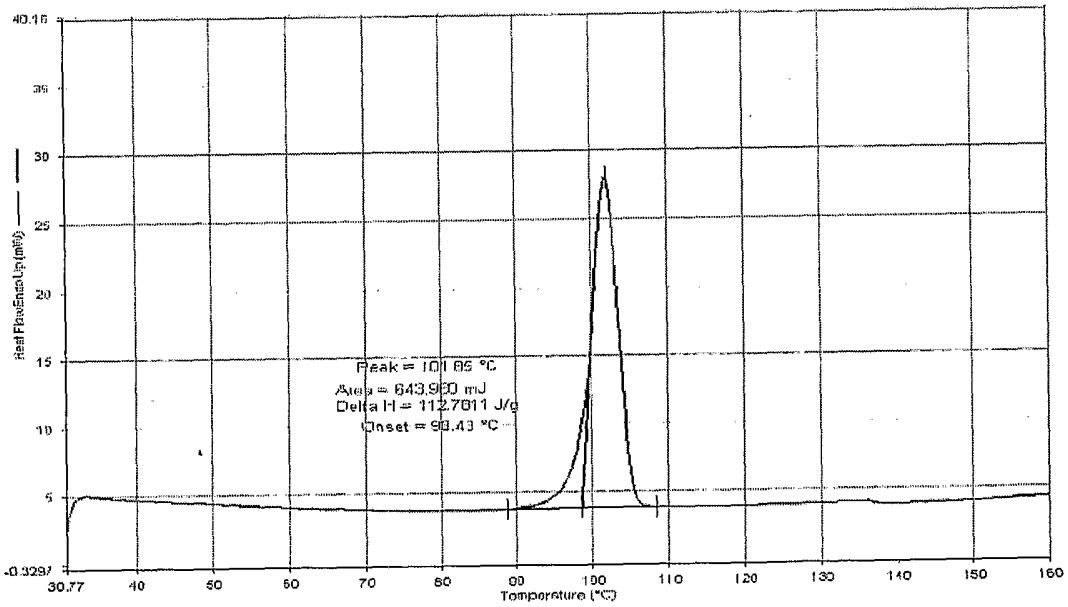


FIGURE 2

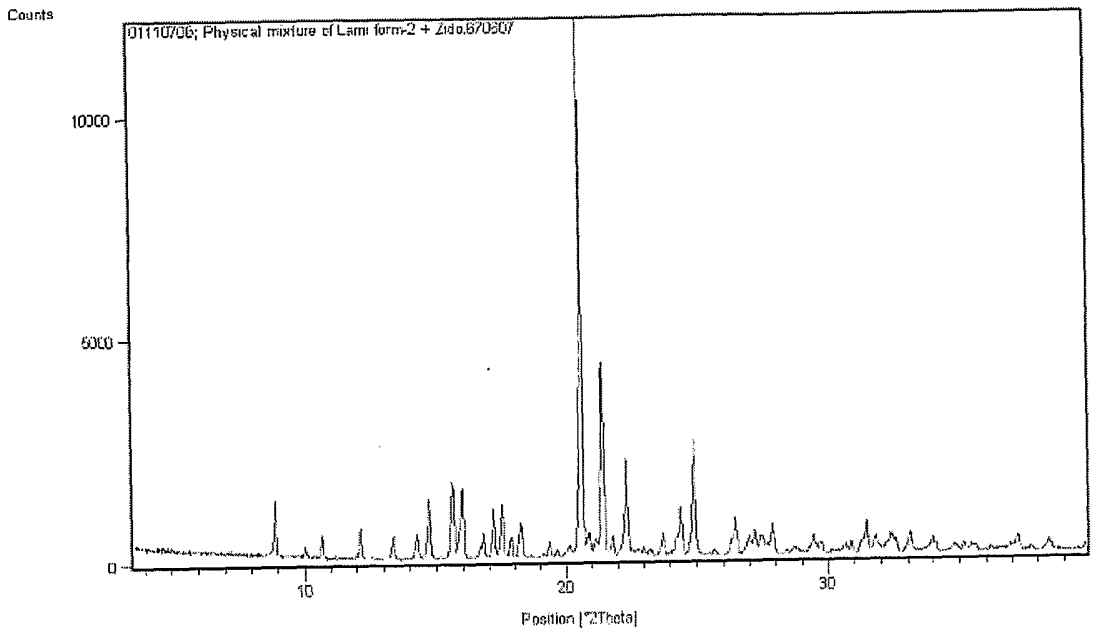


FIGURE 3

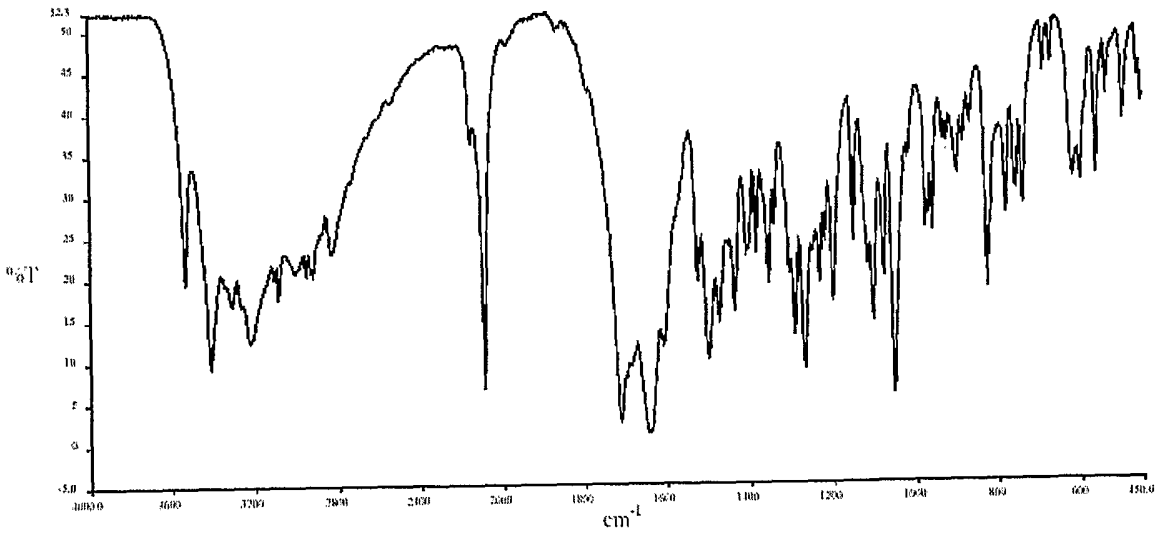


FIGURE 4

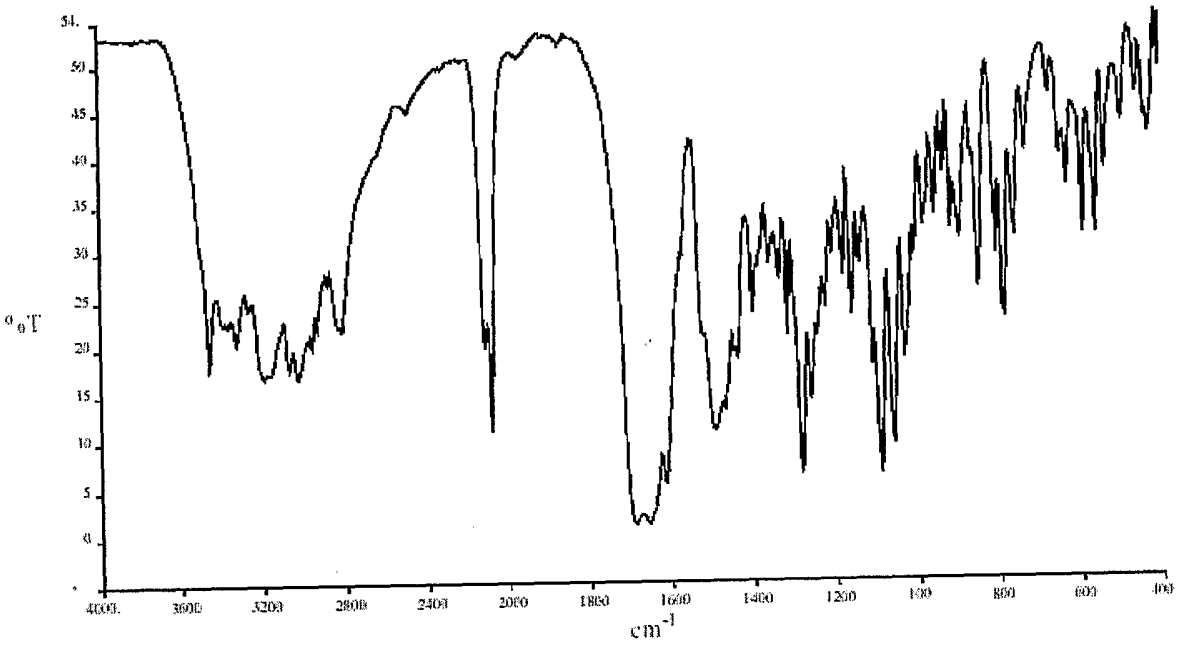


FIGURE 5

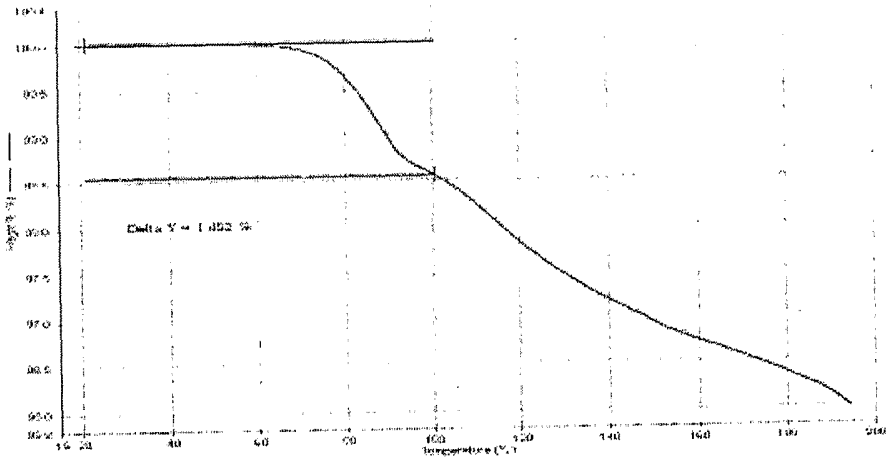


FIGURE 6

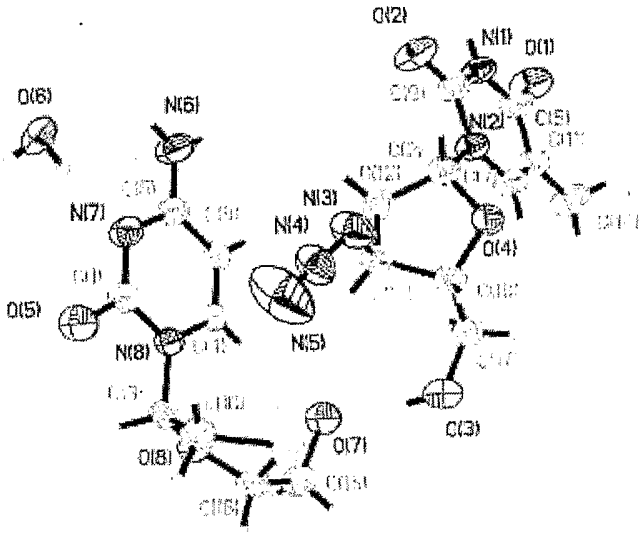


FIGURE 7

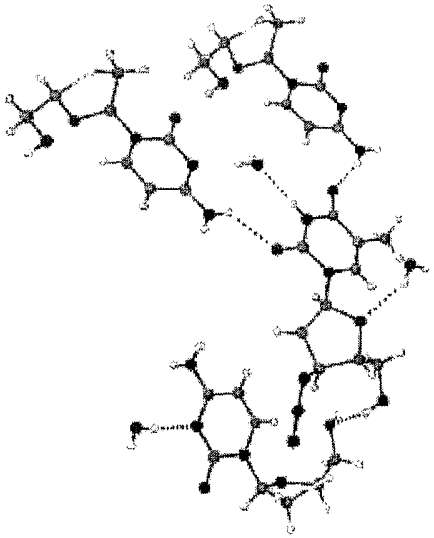


FIGURE 8

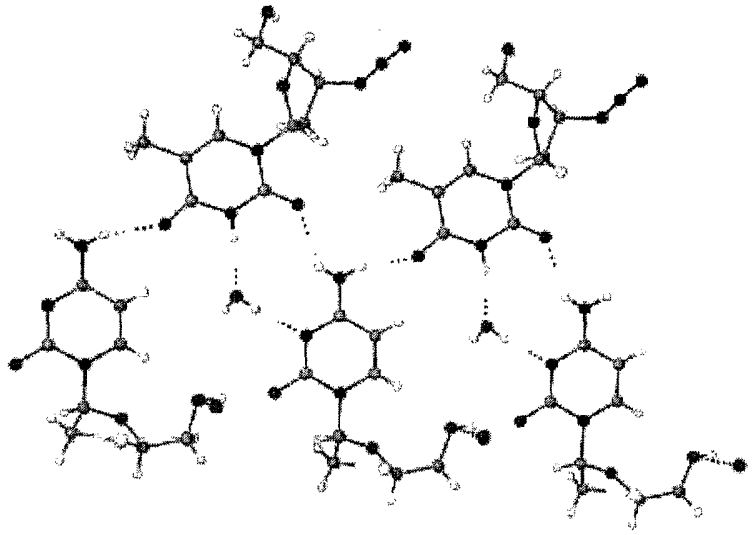


FIGURE 9

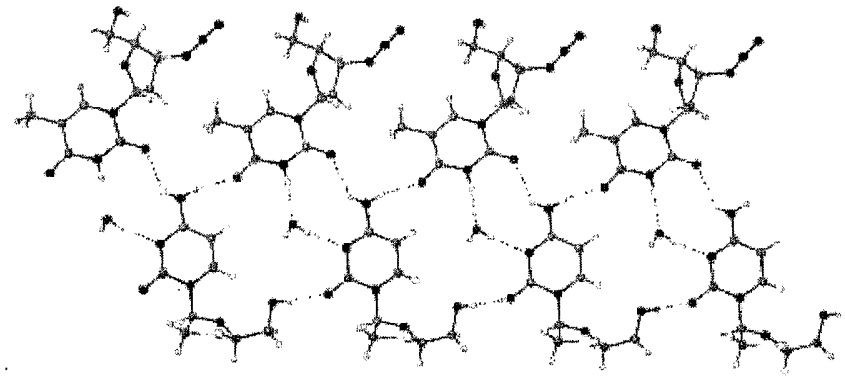


FIGURE 10

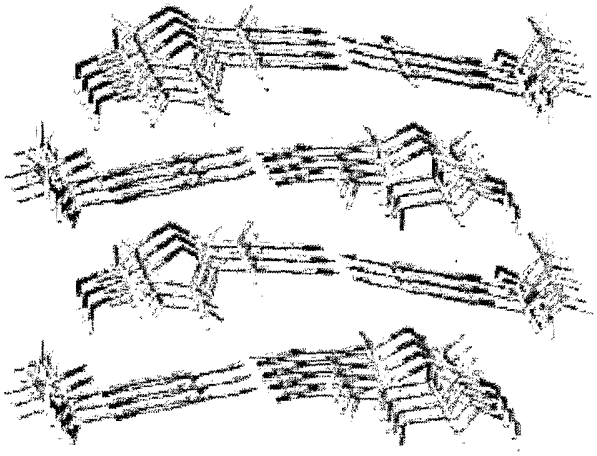


FIGURE 11

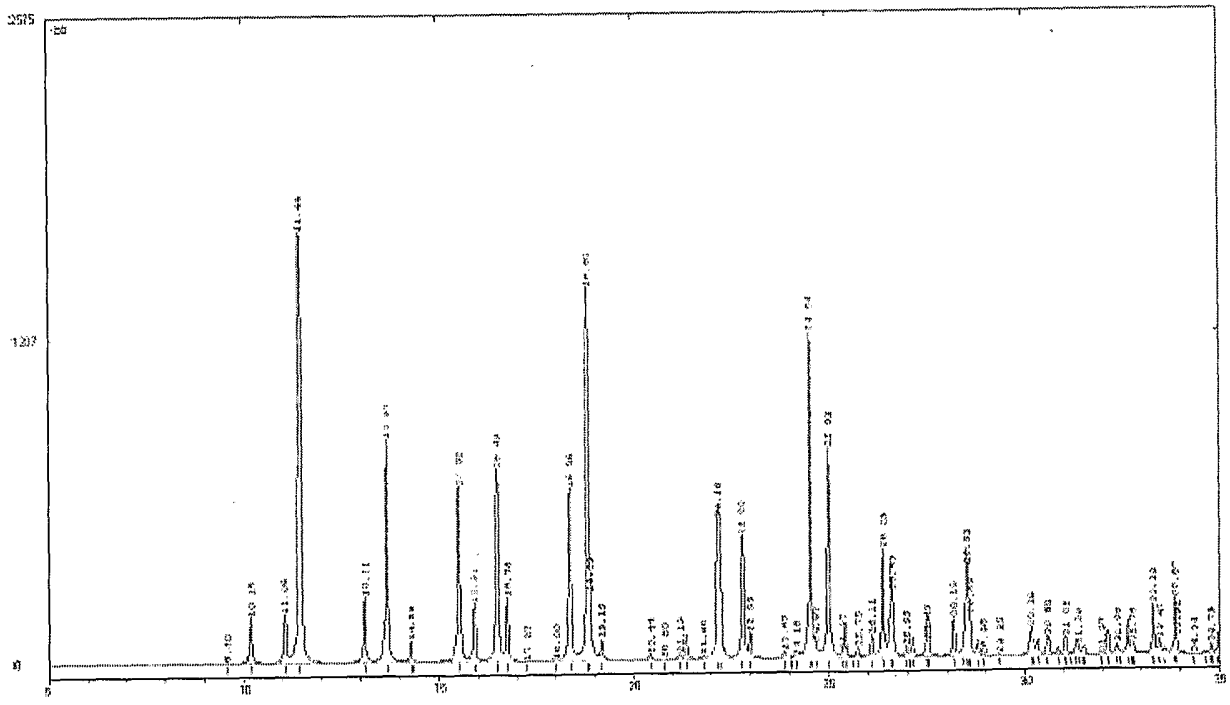


FIGURE 12

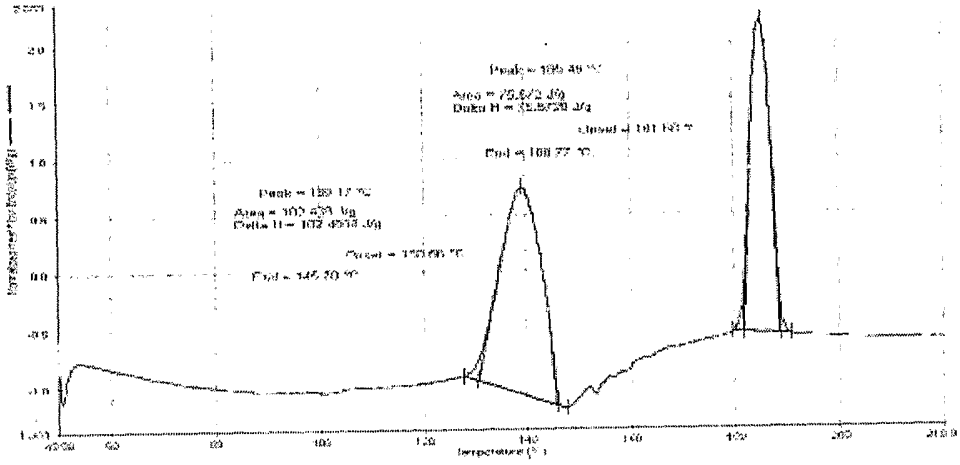


FIGURE 13

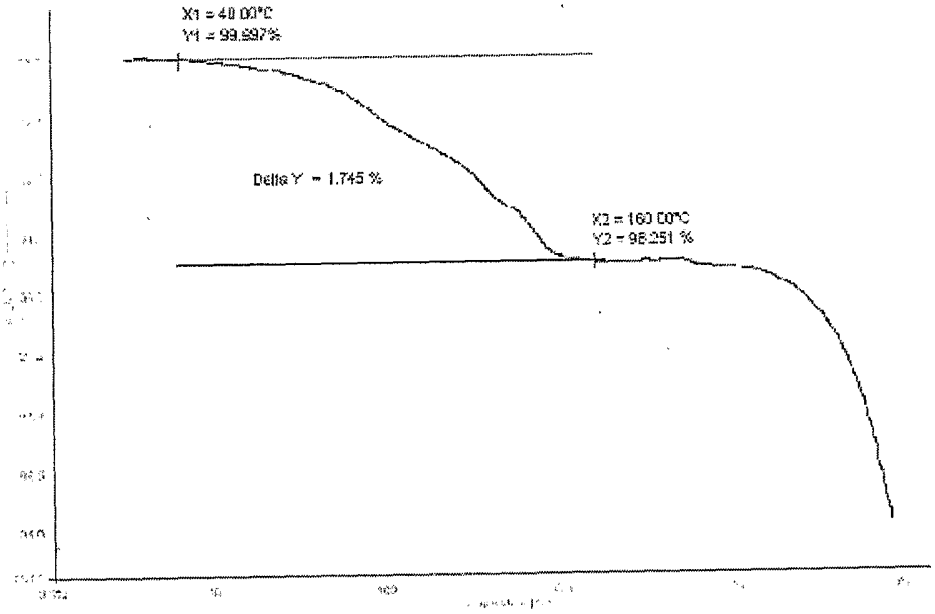


FIGURE 14

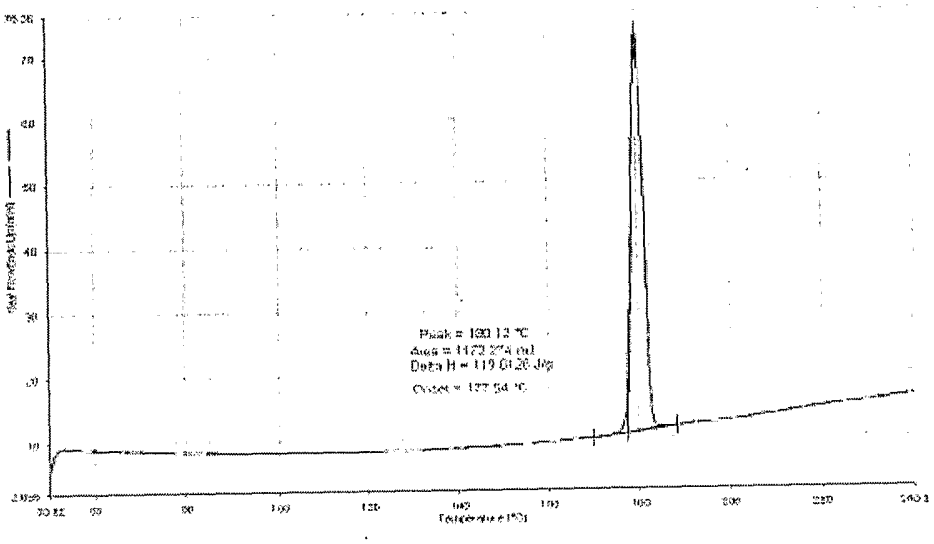


FIGURE 15

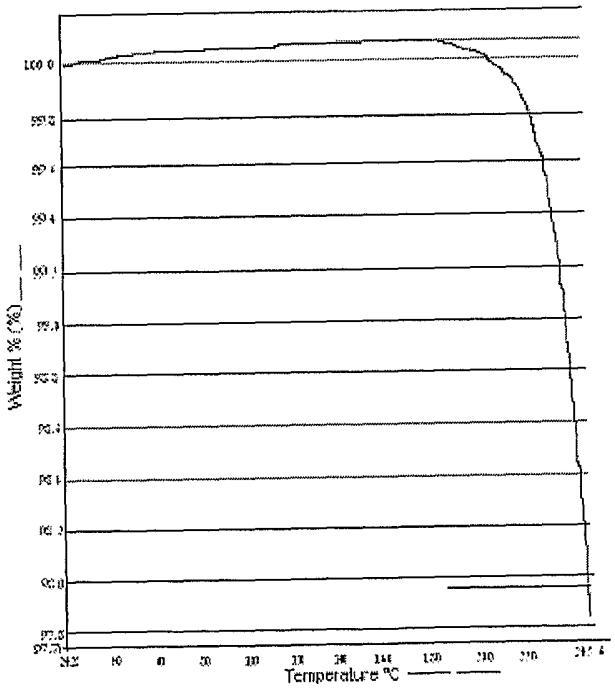


FIGURE 16

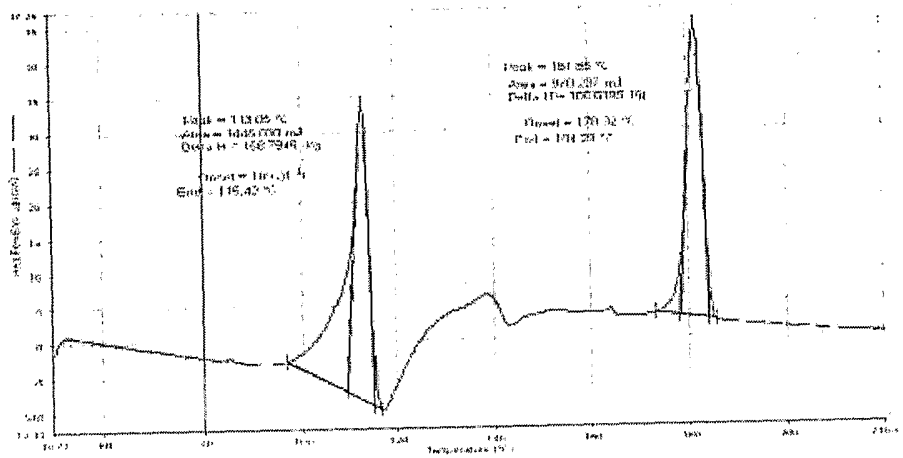


FIGURE 17

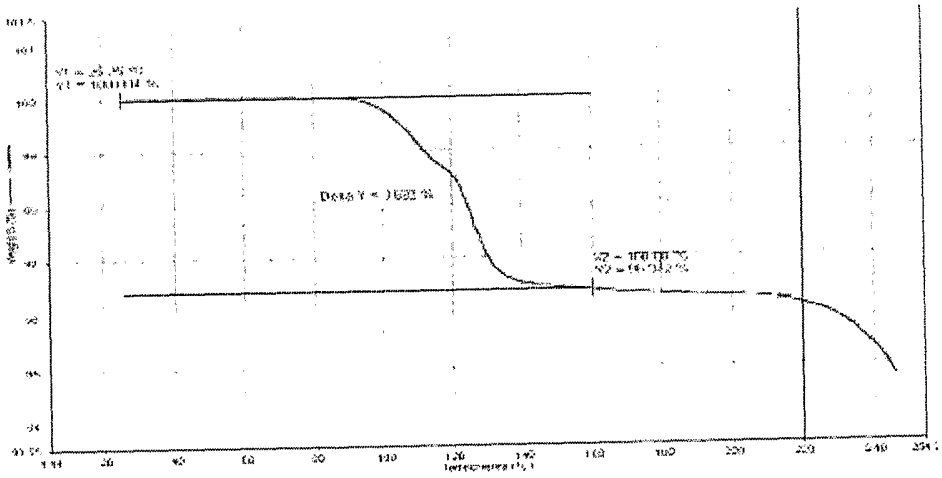


FIGURE 18

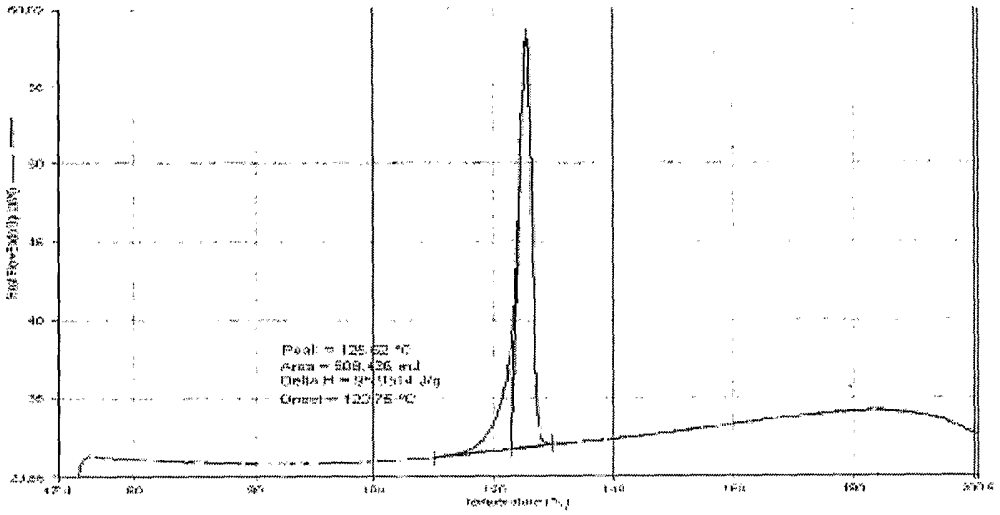


FIGURE 19

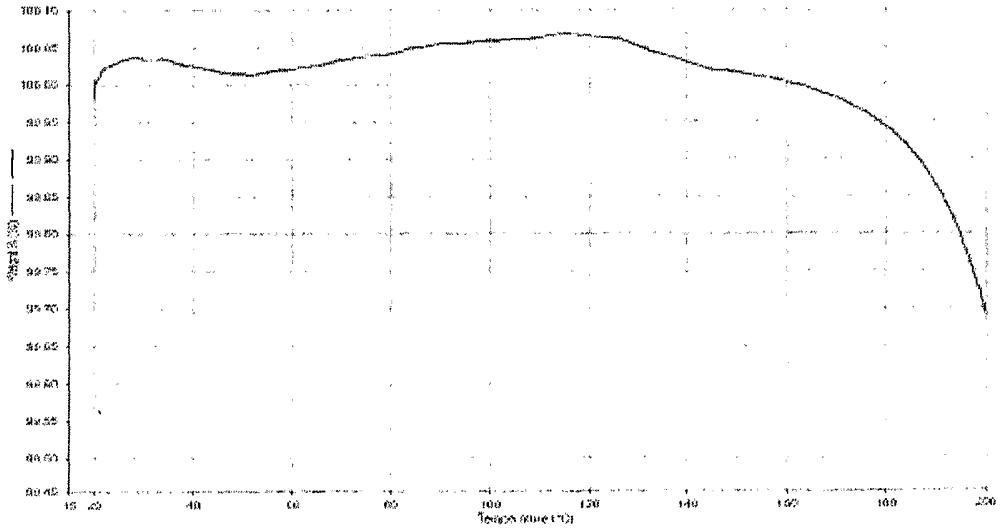


FIGURE 20

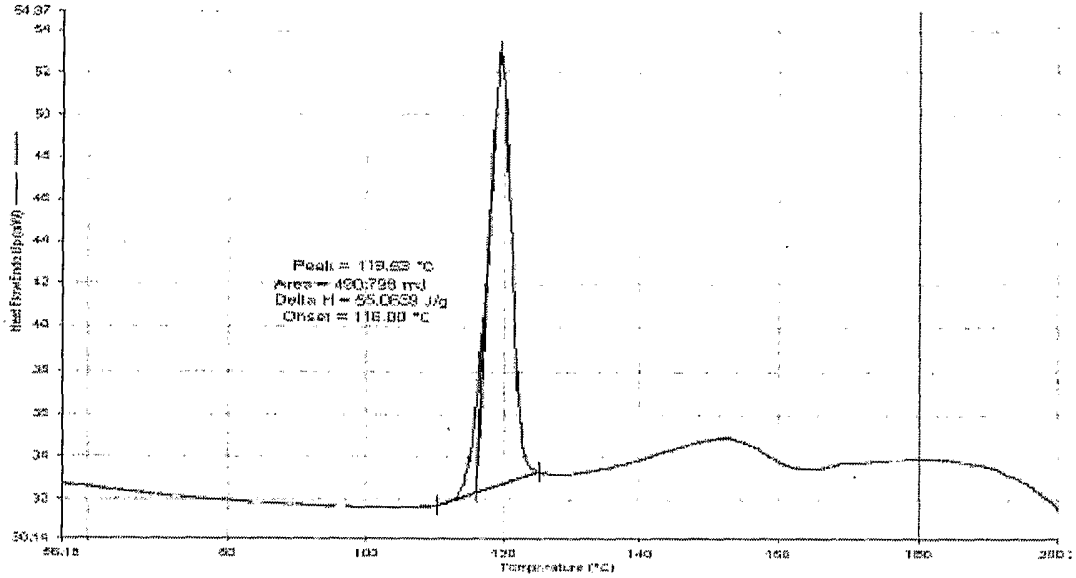


FIGURE 21

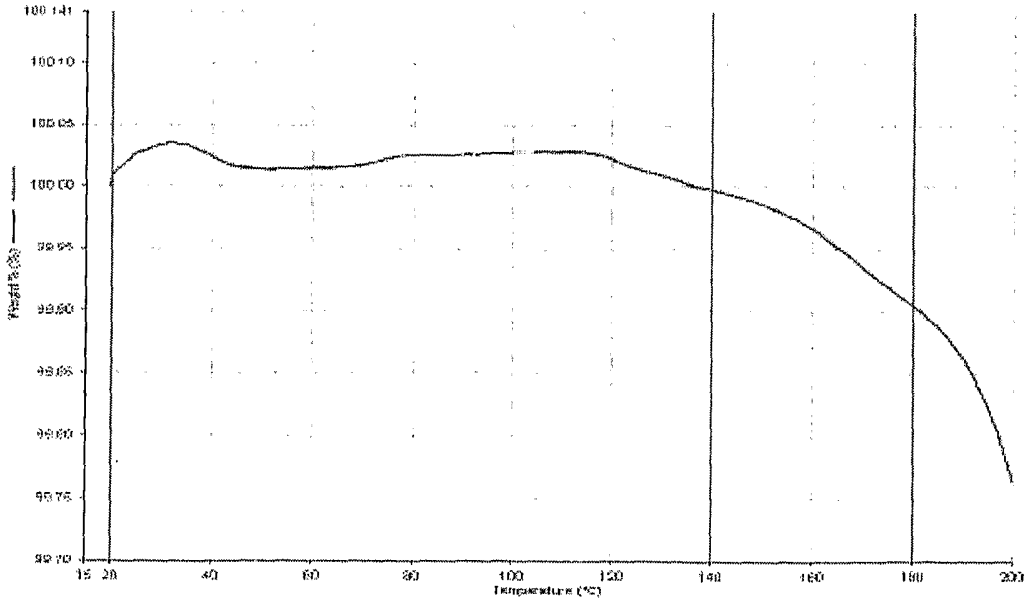


FIGURE 22

INTERNATIONAL SEARCH REPORT

International application No
PCT/IN2008/000276

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D405/04 C07D411/04 A61K31/513 A61P31/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07D A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2007/119248 A (LUPIN LTD [IN]; SINGH GIRIJ PAL [IN]; SRIVASTAVA DHANANJAI [IN]; SAINI) 25 October 2007 (2007-10-25) pages 15-16; example 12b	1-9, 12-15

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 October 2008	23/10/2008

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Fax: (+31–70) 340–3016	Lange, Tim

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IN2008/000276

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2007119248 A	25-10-2007	NONE	