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(54) Title: THE SUCCINATE OF TENOFOVIR DISOPROXIL

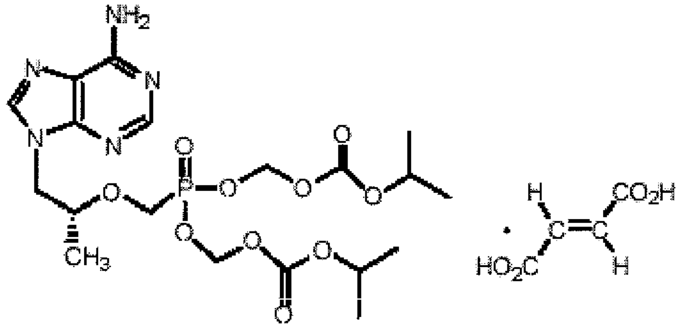
(57) Abstract: The present invention provides a succinate of Tenofovir disoproxil, methods for the preparation thereof and its use in pharmaceutical applications, in particular in anti-HIV medicaments. The succinate of Tenofovir disoproxil can be used in combination with other anti-HIV medicaments such as Efavirenz and Emtricitabine. The invention also provides methods for the treatment of HIV using the succinate of Tenofovir disoproxil medicaments such as Efavirenz and Emtricitabine.

Title: The succinate of Tenofovir disoproxil

The present invention relates to a novel solid form of Tenofovir disoproxil, in particular a succinate of Tenofovir disoproxil, methods for its preparation and its formulation and application in the field of medicine, in particular antiviral medicines

Tenofovir disoproxil fumarate (also known as Viread(R), Tenofovir DF, Tenofovir disoproxil, TDF, Bis-POC-PMPA, 9-[(R)-2-[[bis[[(isopropoxycarbonyl)oxy]methoxy] phosphinyl]methoxy]propyl] adenine (U.S. Pat. Nos. 5,935,946, 5,922,695, 5,977,089, 6,043,230, 6,069,249) is a prodrug of Tenofovir

The chemical name of Tenofovir disoproxil fumarate is 9-[(R)-2-[[bis[[(isopropoxycarbonyl)oxy]methoxy]phosphinyl]methoxy]propyl] adenine fumarate (1:1). The CAS Registry number is 202138-50-9. It has a molecular formula of C19H30N5O10P C4H4O4 and a molecular weight of 635.52. It has the following structural formula:



Tenofovir disoproxil fumarate (DF) is a nucleotide reverse transcriptase inhibitor approved *inter alia* in the United States for the treatment of HIV-I infection in combination with other antiretroviral agents Tenofovir disoproxil DF is available as Viread(R) (Gilead Sciences, Inc.).

Among the anti-HIV drugs which have been developed are those which target the HIV reverse transcriptase (RT) enzyme or protease enzyme, both of which enzymes are necessary for the replication of the virus. Examples of RT inhibitors include nucleoside/nucleotide RT inhibitors (NRTIs) and non-nucleoside RT inhibitors (NNRTIs). Currently, HIV-infected patients are routinely being treated with three-drug combinations. Regimens containing (at least) three NRTIs; two NRTIs in combination with one or two protease inhibitors (PI)(s); or two NRTIs in combination with a NNRTI, are widely used. When two or more PIs are used in these combinations, one of the PIs is often ritonavir, given at a low sub-therapeutic dose, which acts as an effective inhibitor of the elimination of the other

PI(s) in the regimen, resulting in maximal suppression of the virus and thereby reducing the emergence of resistance.

Clinical studies have shown that three-drug combinations of these anti-HIV drugs are much more effective than one drug used alone or two-drug combinations in preventing disease progression and death. Numerous studies of drug combinations with various combinations of such drugs have established that such combinations greatly reduce disease progression and deaths in people with HIV infections. The name now commonly given to combinations of anti-HIV drugs is HAART (Highly Active Anti-Retroviral Therapy).

Tenofovir DF is described *inter alia* in WO99/05150 and EP998480. This crystalline form is characterised as having XRPD peaks at about 4.9, 10.2, 10.5, 18.2, 20.0, 21.9, 24.0, 25.0, 25.5, 27.8, 30.1 and 30.4. Furthermore these crystals are described as opaque or off-white and exhibit a DSC absorption peak at about 118 °C with an onset at about 116 °C and an IR spectrum showing characteristic bands expressed in reciprocal centimetres at approximately 3224, 3107-3052, 2986-2939, 1759, 1678, 1620, 1269 and 1102. Bulk densities have been described of about 0.15-0.30 g/mL, usually about 0.2-0.25 g/mL. Hygroscopicity is well above industry limits of 4%, requiring a desiccant in the packaged product to ensure stability.

It has been found earlier that Tenofovir DF is highly polymorphic and that conversion from one form to other forms might occur under normal processing conditions such as wet granulation. Recently the present applicants have investigated the behaviour of Tenofovir disoproxil fumarate (WO2009064174, WO2008143500, WO2008140302) to overcome the problems associated with Tenofovir DF.

IN WO 2006135932, initial efforts to simply combine the three drugs (active pharmaceutical intermediates, or APIs) into a unitary, essentially homogeneous composition manufactured by wet granulation failed to produce a chemically stable tablet. The Tenofovir DF in this combination tablet was highly unstable and rapidly degraded in stability studies. The Efavirenz formulation was unexpectedly incompatible with Tenofovir DF, a result now attributed to the surfactant (sodium lauryl sulphate) found in the Efavirenz portion of the formulation.

In WO2006135932, another attempt was made to produce the triple combination, this time using a dry granulation of the three part combination and omitting the surfactant. This resulted in a tablet that failed to achieve bioequivalence with respect to Efavirenz in human clinical trials. The peak Efavirenz concentration in the blood stream and total drug exposure (Cmax and AUC) were both below the parameters determined for the commercial comparator, Sustiva (Efavirenz) tablets. The inventors of WO2006135932 concluded that at least the surfactant in the triple combination (Efavirenz /Emtricitabine/ Tenofovir disoproxil fumarate) tablets was essential to achieve bioequivalence to Sustiva

Further attempts to come to the triple formulation were based on wet granulating the Efavirenz component with the surfactant and other excipients, separately manufacturing the Truvada component using dry granulation, mixing the granulates together, compressing the mixture into tablets, and then film-coating the tablets. This approach also failed to produce the desired bioequivalence in between the commercial product, Sustiva (Efavirenz), and clinical trial material (i.e., proposed commercial triple combination product) Finally, the desired stability and bioequivalence objectives for the triple combination product were been achieved by providing a multicomponent dosage form, one component comprising tenofovir DF and, optionally, Emtricitabine, and the other comprising at least Efavirenz. Another embodiment of WO2006135932 is a dosage form comprising a tenofovir DF component and a surfactant component not in destabilizing contact with the tenofovir DF component.

As this is still a tedious and cumbersome formulation, the present inventors set out to improve on this topic.

It is a goal of the present inventors to overcome the problems associated with the current fumarate salt of Tenofovir disoproxil by looking for combinations of Tenofovir disoproxil and other weak organic acids.

Summary of the invention

The present invention therefore relates to a novel solid form of Tenofovir Disoproxil The present inventors have identified a novel solid form, herein depicted as a succinate of Tenofovir disoproxil. This solid form may be in the form of a salt or a co-crystal The present inventors have found that the particular succinate has an improved solubility paired with strongly reduced hygroscopicity, compared to the known TDF 1:1. It has also been found more stable compared to the known TDF 1:1. The succinate is also more compressible compared to the known succinates and/or fumarates. The succinate of the invention can be subjected to a wet granulation step and combination products can be made by wet granulating the mixture of API and tablets made by direct compression of the mixture

Description of the Drawings:

Figure 1 illustrates the X-Ray Powder Diffraction pattern of the succinate of Tenofovir Disoproxil

Figure 2 TGA thermogram of the succinate of Tenofovir Disoproxil.

Figure 3 illustrates the DVS isotherm plot of the succinate of Tenofovir Disoproxil.

Figure 4 illustrates the HPLC of the succinate of Tenofovir Disoproxil.

Figure 5 illustrates the FTIR of the succinate of Tenofovir Disoproxil

Figure 6 illustrates the ^{1}H NMR of the succinate of Tenofovir Disoproxil.

Figure 7 illustrates visual aspects of the succinate of Tenofovir Disoproxil.
Another solid form of a succinate (suc2) appears to consist predominantly of agglomerated particles; The succinate of the invention (suc8) and Tenofovir disoproxil hemifumarate particles show a more fluffy structure. The smaller particle size of the succinate of Tenofovir disoproxil results in an increase of the compressibility as compared to the Tenofovir disoproxil hemifumarate.

Figure 8 illustrates the X-Ray Powder Diffraction pattern of starting material Tenofovir Disoproxil Fumarate

Figure 9 illustrates the FTIR of Tenofovir Disoproxil Fumarate.

Figure 10 illustrates the HPLC of Tenofovir Disoproxil Fumarate

Figure 11 illustrates the X-Ray Powder Diffraction pattern of Tenofovir Disoproxil free base.

Figure 12 illustrates the FTIR of Tenofovir Disoproxil free base.

Figure 13 illustrates the HPLC of Tenofovir Disoproxil free base.

Figure 14 illustrates quantitative solubility of Tenofovir disoproxil succinate (SUC8) as compared to Suc2 and TDF.

Figure 15 illustrates the Raman spectrum of the succinate of Tenofovir Disoproxil.

Figure 16 illustrates the Particle size distribution of Tenofovir disoproxil fumarate.

Figure 17 illustrates the Particle size distribution of Tenofovir disoproxil succinate (SUC8).

Figure 18 illustrates the HPLC spectrogram of Emtricitabine (Starting material)

Figure 19 illustrates the HPLC spectrogram of Tenofovir disoproxil fumarate (Starting material)

Figure 20 illustrates the HPLC spectrogram of Efavirenz (Starting material)

Figure 21 illustrates the HPLC spectrogram of Tenofovir disoproxil succinate (Viread method)

Figure 22 illustrates the HPLC spectrogram of Emtricitabine/Tenofovir disoproxil succinate (30% w/w water)

Figure 23 illustrates the HPLC spectrogram of Emtricitabine/Tenofovir disoproxil succinate (40% w/w water)

Figure 24 illustrates the HPLC spectrogram of Emtricitabine/Tenofovir disoproxil succinate / Efavirenz (30% w/w water)

Figure 25 illustrates the HPLC spectrogram of Emtricitabine/Tenofovir disoproxil succinate/ Efavirenz (40% w/w water)

Detailed description of the invention:

The invention thus relates to a succinate salt of tenofovir disoproxil. The succinate salt of the invention can contain tenofovir disoproxil and succinate in a certain ratio, preferably 1:1 i.e. one molecule of tenofovir disoproxil for each molecule of succinic acid. In certain embodiments, it is possible that the succinate of the present invention contains tenofovir disoproxil and succinate in a ratio different from 1:1. Examples of such ratios are 2:1, 1:2, 1:3, 3:1, 1:4, 4:1, 2:3, 3:2.

Thus, in one aspect, the present invention provides a succinate of tenofovir disoproxil characterised by the selection of at least one, preferably at least two, more preferably at least three, even more preferably at least four, particularly preferred at least five and most preferred six X-ray powder diffraction peaks selected from the group consisting of 5.0, 9.9, 11.7, 12.7, 14.1, 15.7, 17.3, 18.2, 18.9, 19.9, 20.8, 25.0, 30.1 degrees two-theta +/- 0.3 degrees two-theta, preferably +/- 0.2 degrees two-theta, more preferably +/- 0.1 degrees two-theta, most preferably +/- 0.05 degrees two-theta. In a preferred embodiment, at least seven, more preferably at least eight, even more preferably at least nine, particularly preferred at least ten and most preferred eleven X-ray powder diffraction peaks are selected from the above group.

In another embodiment, the succinate of the invention can be characterised by the following set of XRPD peaks (Table 1) and, optionally, by the associated intensities:

Table 1	**Suc8**		**Preferred embodiment**	
Peak ID	**Angle (2θ)**	**Intensity***	**Angle (2θ)**	**Intensity***
1	4.96	L	5.0	L
2	9.93	H	9.9	H
3	10.58	L	10.6	L
4	10.81	L	10.8	L
5	11.42	L	11.4	L
6	11.73	L	11.7	L
7	12.67	L	12.7	L
8	13.45	L	13.5	L
9	14.13	L	14.1	L
10	15.72	M	15.7	M
11	16.14	L	16.1	L
12	16.57	L	16.6	L
13	17.27	M	17.3	M
14	17.79	L	17.8	L
15	18.17	L	18.2	L
16	18.85	L	18.8	L
17	19.31	L	19.3	L
18	19.93	M	19.9	M
19	20.24	L	20.2	L
20	20.77	H	20.8	H
21	24.01	L	24.0	L
22	24.99	H	25.0	H
23	27.88	L	27.9	L
24	30.09	L	30.1	L
normalised intensity values:		L	0	40

M	40	60
H	60	100

Thus, in one aspect, the present invention provides a succinate of tenofovir disoproxil characterised by the selection of at least one, preferably at least two, more preferably at least three, even more preferably at least four, particularly preferred at least five and most preferred six FTIR peaks selected from the group consisting of 634, 950, 1027, 1255, 1623, 1669, 1744, 1759 cm^{-1} +/- 0.3 cm^{-1}, preferably +/- 0.2 cm^{-1}, more preferably +/- 0.1 cm^{-1}, most preferably +/- 0.05 cm^{-1}. In another embodiment the succinate of Tenofovir Disoproxil can be characterised by the following set of FTIR peaks (Table 2A), optionally with their associated intensities.

Peak ID	Position (cm-1)	Intensity
13	634.5	M
22	950.1	H
25	1027	H
31	1255.4	H
38	1623.2	L
39	1669.3	M
40	1744.2	M
41	1758.9	M
normalised intensity values:		
Low	60	80
Medium	40	60
High	0	40

In an alternative embodiment the succinate of Tenofovir Disoproxil can be characterised by the following set of FTIR peaks (Table 3), optionally with their associated intensities

Table 3					
Peak ID	Position (cm-1)	Intensity	Peak ID	Position (cm-1)	Intensity
1	406	64	21	888.7	38
2	416.9	64	22	950.1	39
3	445.9	66	23	966.3	44
4	463.4	65	24	981.6	38
5	474.3	65	25	1027	35
6	501.5	60	26	1059.4	64
7	519	57	27	1102.3	49
8	531.1	56	28	1133	68
9	566.9	62	29	1156.1	60

10	583.3	57	30	1196.9	54
11	597.3	46	31	1255.4	31
12	621.1	49	32	1318.3	63
13	634.5	53	33	1336.3	64
14	691	67	34	1376.7	77
15	721.9	72	35	1388	78
16	755.1	63	36	1420.6	71
17	789.1	45	37	1468	80
18	804.8	53	38	1623.2	67
19	822.7	50	39	1669.3	53
20	833.8	52	40	1744.2	61

In another embodiment, the succinate of Tenofovir Disoproxil can be characterised by an XRPD substantially according to Fig 1.

In another embodiment, the succinate of Tenofovir Disoproxil can be characterised a TGA substantially according to Fig 2.

In another embodiment, the succinate of Tenofovir Disoproxil of the present invention can be characterised by a DVS isotherm substantially according to Fig 3.

In another embodiment, the succinate of Tenofovir Disoproxil of the present invention can be characterised by the HPLC substantially according to Fig 4.

In another embodiment, the succinate of Tenofovir Disoproxil of the present invention can be characterised by the FTIR substantially according to Fig 5.

In another embodiment, the succinate of Tenofovir Disoproxil of the present invention can be characterised by the ^{1}H NMR substantially according to Fig 6.

The present invention in one aspect relates to a method for the preparation of a (crystalline) succinate of Tenofovir Disoproxil comprising the steps of dissolving or mixing Tenofovir disoproxil free base and succinic acid in a suitable solvent or mixture thereof, preferably methanol, ether, acetone, acetonitrile or mixtures thereof (such as 50/50 v/v methanol-ether), if necessary by warming to about 50- 90, preferably between 55 and 75 , most preferably about 60-65 degrees Celsius for a time between one minute and one day, preferably between two minutes and two hours. After cooling at slow rate of about between 0,5 and 2 degrees/hour, more preferably about between 0.8 and 1.2 degrees/hour Celsius until a temperature of about between 0 and 10 degrees Celsius, more preferably around 5 degrees Celsius has been reached, the mixture can be kept for a prolonged period (between 1 and 48 hours) at that temperature. The solid material can be isolated using techniques known the art such as centrifugation or filtration. The solid material (designated as Suc2) can be treated with water (preferably high purity water), for instance by dissolving or slurrying.

Water can be used in a ratio of about 10% w/w drawn on the solid. The solid can be treated with water (for instance by stirring or shaking for a period of between one hour and one week, preferably about one or two days The solid can be separated, washed with (ice) cold water and dried under vacuum to yield the succinate of tenofovir disoproxil of the invention The succinate (Suc8) is identified by XRPD.

In another aspect of the invention, the invention relates to a method for the preparation of the succinate of tenofovir disoproxil of the invention from tenofovir disoproxil succinate (Suc2) by treating the succinate salt with water (preferably high purity water), for instance by dissolving or slurrying. Water can be used in a ratio of about 10% w/w drawn on the solid. The solid can be treated with water (for instance by stirring or shaking for a period of between one hour and one week, preferably about one or two days. The solid can be separated, washed with (ice)cold water and dried under vacuum to yield the succinate salt of tenofovir disoproxil of the invention. The succinate (Suc8) was identified by XRPD.

In another aspect of the invention, the invention relates to a method for the preparation of the succinate of Tenofovir disoproxil of the invention from Tenofovir disoproxil free base by treating the free base in the presence of succinic acid with water (preferably high purity water), for instance by dissolving or slurrying. Water can be used in a ratio of about 10% w/w drawn on the solid. The solid can be separated, washed, for instance with hexane, and dried under vacuum to yield the succinate salt of tenofovir disoproxil of the invention. The succinate (Suc8) was identified by XRPD.

In another aspect of the invention, the invention relates to a method for the preparation of a pharmaceutical formulation of the succinate of Tenofovir disoproxil comprising a step of wet granulating the succinate of Tenofovir disoproxil in the presence of a pharmaceutically acceptable carrier/excipient and/or another pharmaceutical ingredient, preferably an anti HIV agent, preferably Efavirenz and/or Emtricitabine Preferably, the excipient and/or the other pharmaceutical ingredient are in direct contact with each other, i.e. mixed directly, preferably in a one phase formulation. Preferably the wet granulated product is more stable and develops less degradation products over time compared to a conventional formulations known I the art described herein elsewhere.

In a further aspect, the invention relates to a composition comprising the succinate of Tenofovir disoproxil. In certain embodiments, the composition comprises the succinate of Tenofovir disoproxil, Efavirenz and Emtricitabine in an essentially homogenous composition. In certain embodiments, the succinate of Tenofovir disoproxil, Efavirenz and Emtricitabine are in direct contact with each other. In certain embodiments, the succinate of Tenofovir disoproxil, Efavirenz and Emtricitabine are in a one component formulation and/or a single component dosage form. In certain embodiments, the compositions are produced by wet granulation of the succinate of Tenofovir disoproxil, Efavirenz and Emtricitabine.

Pharmaceutical formulations.

The present invention further relates to pharmaceutical formulations comprising the novel crystalline forms of the succinate of Tenofovir Disoproxil.

Pharmaceutical formulations of the present invention contain the form according to the present invention, as disclosed herein. The invention also provides pharmaceutical compositions comprising the form according to the present invention. Pharmaceutical formulations of the present invention contain the form according to the present invention as active ingredient, optionally in a mixture with other form(s).

The pharmaceutical formulations according to the invention, may further comprise, in addition to the form described herein additional pharmaceutical active ingredients, preferably Anti-HIV agents and more preferably Efavirenz and/or Emtricitabine.

In addition to the active ingredient(s), the pharmaceutical formulations of the present invention may contain one or more excipients. Excipients are added to the formulation for a variety of purposes.

Diluents increase the bulk of a solid pharmaceutical composition, and may make a pharmaceutical dosage form containing the composition easier for the patient and caregiver to handle. Diluents for solid compositions include, for example, microcrystalline cellulose (e.g. Avicel(R)), micro fine cellulose, lactose, starch, pregelatinized starch, calcium carbonate, calcium sulfate, sugar, dextrates, dextrin, dextrose, dibasic calcium phosphate dihydrate, tribasic calcium phosphate, kaolin, magnesium carbonate, magnesium oxide, maltodextrin, mannitol, polymethacrylates (e.g. Eudragit(R)), potassium chloride, powdered cellulose, sodium chloride, sorbitol and talc.

Solid pharmaceutical compositions that are compacted into a dosage form, such as a tablet, may include excipients whose functions include helping to bind the active ingredient and other excipients together after compression. Binders for solid pharmaceutical compositions include acacia, alginic acid, carbomer (e.g. Carbopol), carboxymethylcellulose sodium, dextrin, ethyl cellulose, gelatin, guar gum, hydrogenated vegetable oil, hydroxyethyl cellulose, hydroxypropyl cellulose (e.g. Klucel(R)), hydroxypropyl methyl cellulose (e.g. Methocel(R)), liquid glucose, magnesium aluminum silicate, maltodextrin, methylcellulose, polymethacrylates, povidone (e.g. Kollidon(R), Plasdone(R)), pregelatinized starch, sodium alginate and starch.

The dissolution rate of a compacted solid pharmaceutical composition in the patient's stomach may be increased by the addition of a disintegrant to the composition. Disintegrants include alginic acid, carboxymethylcellulose calcium, carboxymethylcellulose sodium (e.g Ac-Di-Sol(R), Primellose(R)), colloidal silicon dioxide, croscarmellose sodium, crospovidone (e g. Kollidon(R), Polyplasdone(R)), guar gum, magnesium aluminum silicate, methyl cellulose, microcrystalline cellulose, polacrilin potassium, powdered cellulose, pregelatinized starch, sodium alginate, sodium starch glycolate (e.g. Explotab(R)) and starch.

Glidants can be added to improve the flowability of a non-compacted solid composition and to improve the accuracy of dosing. Excipients that may function as glidants include colloidal silicon dioxide, magnesium trisilicate, powdered cellulose, starch, talc and tribasic calcium phosphate

When a dosage form such as a tablet is made by the compaction of a powdered composition, the composition is subjected to pressure from a punch and dye. Some excipients and active ingredients have a tendency to adhere to the surfaces of the punch and dye, which can cause the product to have pitting and other surface irregularities. A lubricant can be added to the composition to reduce adhesion and ease the release of the product from the dye. Lubricants include magnesium stearate, calcium stearate, glyceryl monostearate, glyceryl palmitostearate, hydrogenated castor oil, hydrogenated vegetable oil, mineral oil, polyethylene glycol, sodium benzoate, sodium lauryl sulfate, sodium stearyl fumarate, stearic acid, talc and zinc stearate. Flavoring agents and flavor enhancers make the dosage form more palatable to the patient. Common flavoring agents and flavor enhancers for pharmaceutical products that may be included in the composition of the present invention include maltol, vanillin, ethyl vanillin, menthol, citric acid, fumaric acid, ethyl maltol and tartaric acid. Solid and liquid compositions may also be dyed using any pharmaceutically acceptable colorant to improve their appearance and/or facilitate patient identification of the product and unit dosage level.

In liquid pharmaceutical compositions of the present invention, the crystalline forms according to the present invention and any other solid excipients are suspended in a liquid carrier such as water, vegetable oil, alcohol, polyethylene glycol, propylene glycol or glycerin.

Liquid pharmaceutical compositions may contain emulsifying agents to disperse uniformly throughout the composition an active ingredient or other excipient that is not soluble in the liquid carrier. Emulsifying agents that may be useful in liquid compositions of the present invention include, for example, gelatin, egg yolk, casein, cholesterol, acacia, tragacanth, chondrus, pectin, methyl cellulose, carbomer, cetostearyl alcohol and cetyl alcohol.

Liquid pharmaceutical compositions of the present invention may also contain a viscosity enhancing agent to improve the mouth-feel of the product and/or coat the lining of the gastrointestinal tract. Such agents include acacia, alginic acid bentonite, carbomer, carboxymethylcellulose calcium or sodium, cetostearyl alcohol, methylcellulose,

ethylcellulose, gelatin guar gum, hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, maltodextrin, polyvinyl alcohol, povidone, propylene carbonate, propylene glycol alginate, sodium alginate, sodium starch glycolate, starch tragacanth and xanthan gum

Sweetening agents such as sorbitol, saccharin, sodium saccharin, sucrose, aspartame, fructose, mannitol and invert sugar may be added to improve the taste. Preservatives and chelating agents such as alcohol, sodium benzoate, butylated hydroxyl toluene, butylated hydroxyanisole and ethylenediamine tetraacetic acid may be added at levels safe for ingestion to improve storage stability. According to the present invention, a liquid composition may also contain a buffer such as gluconic acid, lactic acid, citric acid or acetic acid, sodium gluconate, sodium lactate, sodium citrate or sodium acetate Selection of excipients and the amounts used may be readily determined by the formulation scientist based upon experience and consideration of standard procedures and reference works in the field.

For infections of the eye or other external tissues, e g. mouth and skin, the formulations are preferably applied as a topical ointment or cream containing the active ingredient(s) in an amount of, for example, 0.01 to 10% w/w (including active ingredient(s) in a range between 0.1% and 5% in increments of 0.1% w/w such as 0 6% w/w, 0.7% w/w, etc), preferably 0.2 to 3% w/w and most preferably 0.5 to 2% w/w. When formulated in an ointment, the active ingredients may be employed with either a paraffinic or a water-miscible ointment base.

Alternatively, the active ingredients may be formulated in a cream with an oil-in-water cream base.

If desired, the aqueous phase of the cream base may include, for example, at least 30% w/w of a polyhydric alcohol, i.e. an alcohol having two or more hydroxyl groups such as propylene glycol, butane 1,3-diol, mannitol, sorbitol, glycerol and polyethylene glycol (including PEG 400) or mixtures thereof The topical formulations may desirably include a compound which enhances absorption or penetration of the active ingredient through the skin or other affected areas. Examples of such dermal penetration enhancers include dimethyl sulphoxide and related analogs.

The oily phase of the emulsions of this invention may be constituted from known ingredients in a known manner. While the phase may comprise merely an emulsifier (otherwise known as an emulgent), it desirably comprises a mixture of at least one emulsifier with a fat or an oil or with both a fat and an oil. Preferably, a hydrophilic emulsifier is included together with a lipophilic emulsifier which acts as a stabiliser. It is also preferred to include both an oil and a fat. Together, the emulsifier(s) with or without stabiliser(s) make up the emulsifying wax, and the wax together with the oil and fat make up the emulsifying ointment base which forms the oily dispersed phase of the cream formulations.

Emulgents and emulsion stabilisers suitable for use in the formulation of the present invention include Tween8 60, Spans 80, cetostearyl alcohol, benzyl alcohol, myristyl alcohol, glyceryl monostearate and sodium lauryl sulfate.

The choice of suitable oils or fats for the formulation is based on achieving the desired cosmetic properties Thus the cream should preferably be a non-greasy, non-staining and washable product with suitable consistency to avoid leakage from tubes or other containers.

Straight or branched chain, mono- or dibasic alkyl esters such as diisoadipate, isocetyl stearate, propylene glycol diester of coconut fatty acids, isopropyl myristate, decyl oleate, isopropyl palmitate, butyl stearate,2-ethylhexyl palmitate or a blend of branched chain esters known as Crodamol CAP may be used, the last three being preferred esters. These may be used alone or in combination depending on the properties required. Alternatively, high melting point lipids such as white soft paraffin and/or liquid paraffin or other mineral oils can be used

Formulations suitable for topical administration to the eye also include eye drops wherein the active ingredient is dissolved or suspended in a suitable carrier, especially an aqueous solvent for the active ingredient. The active ingredient is suitably present in such formulations in a concentration of 0.01 to 20%, in some embodiments 0.1 to 10%, and in others about 1.0% w/w.

Formulations suitable for topical administration in the mouth include lozenges comprising the active ingredient in a flavored basis, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

Formulations for rectal administration may be presented as a suppository with a suitable base comprising for example cocoa butter or a salicylate.

Formulations suitable for nasal or inhalational administration wherein the carrier is a solid include a powder having a particle size for example in the range 1 to 500 microns (including particle sizes in a range between 20 and 500 microns in increments of 5 microns such as 30 microns, 35 microns, etc) Suitable formulations wherein the carrier is a liquid, for administration as for example a nasal spray or as nasal drops, include aqueous or oily solutions of the active ingredient.

Formulations suitable for aerosol administration may be prepared according to conventional methods and may be delivered with other therapeutic agents Inhalational therapy is readily administered by metered dose inhalers.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

The solid compositions of the present invention include powders, granulates, aggregates and compacted compositions. The dosages include dosages suitable for oral, buccal, rectal, parenteral (including subcutaneous, intramuscular, and intravenous), inhalant and ophthalmic administration. Although the most suitable administration in any given case will depend on the nature and severity of the condition being treated, the most preferred route of the present invention is oral. The dosages may be conveniently presented in unit dosage form and prepared by any of the methods well-known in the pharmaceutical arts.

Dosage forms include solid dosage forms like tablets, powders, capsules, suppositories, sachets, troches and lozenges, as well as liquid syrups, suspensions and elixirs.

The dosage form of the present invention may be a capsule containing the composition, preferably a powdered or granulated solid composition of the invention, within either a hard or soft shell. The shell may be made from gelatin and optionally contain a plasticizer such as glycerin and sorbitol, and an opacifying agent or colorant.

The active ingredient and excipients may be formulated into compositions and dosage forms according to methods known in the art. A composition for tabletting or capsule filling may be prepared by wet granulation. In wet granulation, some or all of the active ingredients and excipients in powder form are blended and then further mixed in the presence of a liquid, typically water, that causes the powders to clump into granules. The granulate is screened and/or milled, dried and then screened and/or milled to the desired particle size. The granulate may then be tabletted/compressed, or other excipients may be added prior to tabletting, such as a glidant and/or a lubricant.

A tabletting composition may be prepared conventionally by dry blending. For example, the blended composition of the actives and excipients maybe compacted into a slug or a sheet and then comminuted into compacted granules. The compacted granules may subsequently be compressed into a tablet.

As an alternative to dry granulation, a blended composition may be compressed directly into a compacted dosage form using direct compression techniques. Direct compression produces a more uniform tablet without granules. Excipients that are particularly well suited for direct compression tableting include microcrystalline cellulose, spray dried lactose, dicalcium phosphate dihydrate and colloidal silica. The proper use of these and other excipients in direct compression tableting is known to those in the art with experience and skill in particular formulation challenges of direct compression tableting.

A capsule filling of the present invention may comprise any of the aforementioned blends and granulates that were described with reference to tableting, however, they are not subjected to a final tableting step

Moreover, the crystalline forms according to the present invention can be formulated for administration to a mammal, preferably a human, via injection. The crystalline forms according to the present invention may be formulated, for example, as a viscous liquid solution or suspension, preferably a clear solution, for injection. The formulation may contain solvents. Among considerations for such solvent include the solvent's physical and chemical stability at various pH levels, viscosity (which would allow for syringeability), fluidity, boiling point, miscibility and purity. Suitable solvents include alcohol USP, benzyl alcohol NF, benzyl benzoate USP and Castor oil USP. Additional substances may be added to the formulation such as buffers, solubilizers, antioxidants, among others. Ansel *et al.*, Pharmaceutical Dosage Forms and Drug Delivery Systems, 7th Ed.

The present invention also provides pharmaceutical formulations comprising the form according to the present invention, optionally in combination with other (polymorphic) forms or co-crystals, to be used in a method of treatment of a mammal, preferably a human, in need thereof. A pharmaceutical composition of the present invention comprises the form. The form according to the present invention may be used in a method of treatment of a mammal comprising administering to a mammal suffering from the ailments described herein before a therapeutically effective amount of such pharmaceutical composition. The invention further relates to the use of the crystalline form of the invention for the preparation of a medicament for the treatment of the ailments described herein before, in particular HIV.

Having described the invention with reference to certain preferred embodiments, other embodiments will become apparent to one skilled in the art from consideration of the specification. The invention is further defined by reference to the following examples describing in detail the preparation of the compounds of the present invention It will be apparent to those skilled in the art that many modifications, both to materials and methods, may be practiced without departing from the scope of the invention.

Examples

The starting material for the crystallisation experiments was obtained as a research sample TDF 1:1 from Tengarden Chemistry HI-TECH Inc.308, North Zhongshan Road Hangzhou zhejiang China and converted to the free base using common procedures. Analytical data of the starting material as purchased are presented in Figs 8, 9, and 10. Analytical data of the free base are presented in Figs 11, 12 and 13.

Tenofovir disoproxil succinate (suc2) from free base

About 100 mg of tenofovir disoproxil free base was solid dosed into an 8ml vial together with about 22.78 mg of succinic acid. The crystallization solvent (acetonitrile) were added so that the concentration with respect to the free base was 100 mg/ml. The vials were heated to 60°C for 60 min. The solutions were cooled with 1.1°C/h to a temperature of 5°C where they remained for 24h. The solids were obtained by centrifugation with 3000 rpm speed for 10 min and dried under vacuum at 40 °C for about 5 h. The solid was labelled Suc2.

The succinate (suc8) of Tenofovir disoproxil

The starting material (Suc2) was weighed in a separate 8ml vial. The high purity water was added with 10% w/w of material and the mixture was stirred for 24 hrs. After 24 h the liquid was separated by centrifugation and the material was washed with 1ml ice cold water. Finally the material was dried under vacuum at 40 °C, analysed using XRPD and labelled Suc8.

Tenofovir disoproxil succinate (Suc2) gram scale

Tenofovir disoproxil succinate is prepared by placing about 997 mg of tenofovir disoproxil free base in a 50 ml glass reactor together with succinic acid at about 1.1:1 counter-ion:free-base molecular ratio. The crystallization solvent (methanol, acetonitrile) were added so that the concentration with respect to the free base was 100 mg/ml. The reactor was heated to 60°C with a heating rate of 5° C/min and maintain at 60 °C for 60 min. Subsequently, the solutions were cooled with 1.1° C/h to a temperature of 5 where they remained for 24h. At the end the solutions were filtered by using Buckner Filter with 0.5 micron filter mesh, dried at room temperature under vacuum and measured by XRPD. The X-ray powder diffraction peaks were 4.9, 9.5, 10.3, 11.5, 13.3, 14.7, 17.9, 18.2, 19.1, 24.7, 29.8 degrees two-theta, DSC with an onset at 102.0 °C and a characterising peak at 111.0 °C.

The succinate of Tenofovir disoproxil (Suc 8) from Tenofovir disoproxil succinate Suc2

In an 8 ml vial, high purity water was added to Tenofovir disoproxil succinate Suc2 to a ratio of 10% w/w and the mixture was stirred for 24 hrs. After 24 h the liquid was separated by centrifugation and the material was washed with 1ml ice cold water. The material was dried under vacuum at 40 °C and measured by XRPD.

The succinate of Tenofovir disoproxil (Suc 8) from Tenofovir disoproxil free base

About 200 mg of Tenofovir disoproxil free base was solid dosed into an 8ml vial together with about 45.40 mg of Succinic acid. To this mixture water was added so that the concentration with respect to the free base was 100 mg/ml. The vials were stirred by using magnetic stirrer for about 4 hrs. The solids were obtained by centrifugation with 3000 rpm speed for 10 min and washed with 1 ml of n-hexane. The material was dried for 5 hrs under vacuum at 40 °C and measured on XRPD.

Experimental conditions

X-ray Powder Diffraction:

XRPD patterns were obtained using a T2 high-throughput XRPD set-up by Avantium technologies, The Netherlands. The plates were mounted on a Bruker GADDS diffractometer equipped with a Hi-Star area detector. The XRPD platform was calibrated using Silver Behenate for the long d-spacings and Corundum for the short d-spacings.

Data collection was carried out at room temperature using monochromatic CuK(alpha)radiation in the two-theta region between 1.5 ° and 41.5 °. The diffraction pattern of each well is collected in two two-theta ranges (1.5 ° $\leq 2\theta \leq$ 21.5 ° for the first frame, and 19.5 ° $\leq 2\theta \leq$ 41.5 ° for the second) with an exposure time of 120 s for each frame. One of ordinary skill in the art understands that experimental differences may arise due to differences in instrumentation, sample preparation, or other factors. Typically, XRPD data are collected with a variance of about 0.3 degrees two-theta, preferable about 0.2 degrees, more preferably 0.1 degrees, even more preferable 0.05 degrees. This has consequences for when X-ray peaks are considered overlapping.

Thermal analysis:

Mass loss due to solvent or water loss from the crystals was determined by TGA/SDTA. Monitoring of the sample weight, during heating in a TGA/SDTA851e instrument (Mettler-Toledo GmbH, Switzerland), resulted in a weight vs. temperature curve. The TGA/SDTA851e was calibrated for temperature with indium and aluminium. Samples were weighed into 100 microliter aluminium crucibles and sealed. The seals were pin-holed and the crucibles heated in the TGA from 25°C to 300°C at a heating rate of 20°C/min. Dry N_2 gas is used for purging. Melting point determinations based on DSC have a variability of +/- 2.0 degrees Celsius, preferably 1.0 degrees Celsius.

Dynamic Vapour Sorption (DVS)

Moisture sorption isotherms were measured using a DVS-1 system of Surface Measurement Systems (London, UK). Differences in moisture uptake of a solid material indicate differences in the relative stabilities of the various solid forms for increasing relative humidity. The experiment was carried out at a constant temperature of 25°C.

A sample of about 2.4 mg of the succinate of the invention was spread in the DVS pan. The sample was dried at 0% RH for 7 h. Subsequently the relative humidity of the chamber was increased in steps of 5% units from 0% to 95% in order to monitor the sorption of water vapours. The samples remained in each of the steps for 1 h. Following, desorption was monitored by decreasing the relative humidity to 0% in steps of 5% units and remaining at each step for 1 h. The total uptake of water vapors was about 0.3% demonstrating good stability of the material and no hygroscopicity. At the end of the experiment, the solid material was measured by XRPD which showed that there were no any changes in the structure.

HPLC

Column:	SunFire C18 (100 x 4.6mm; 3.5um).
Column temp:	40 °C
Mobile phase: Gradient mode	
Eluent A:	10mM Ammonium Acetate (not pH adjusted) in water.
Eluent B:	Acetonitrile
Flow:	1.0 ml/min

Gradient:	Time:	Eluent A:	Eluent B:
	0	90%	10%
	16	10%	90%
	20	10%	90%
	21	90%	10%
	25	90%	10%

UV-Detector:	DAD
Wavelengths:	265 nm
Time:	0-20 min
MS-Detector:	MSD
Scan:	positive
Mass Range:	70 – 1000 amu
Time:	0-20 min
Sample:	
Injection mode:	Full loop
Injection volume:	5 ul
Dilution solvent:	1:1 Eluent-A/Eluent-B
HPLC Equipment:	LC-MS
Manufacturer:	Agilent
HPLC:	HP1100
UV-detector:	HP DAD
MS-detector:	HP1100 API-ES MSD VL-type

1H-NMR

^{1}H NMR spectroscopy in DMSO-d^6 was performed for compound integrity characterization. The spectra were recorded at room temperature on a Bruker 400 MHz instrument.

Dissolution Instrument: pION µDiss profiler, at a wavelength of 260nm

A tablet of 10mg of the succinate of Tenofovir disoproxil succinate was pressed on tablet machine and placed along with a magnetic stirrer in a 25 ml vial in the micro-dissolution thermal block. The probe, which is connected with DAD (Diode Array Detector), was placed along with the 5mm path length tip. Thereafter, 20 ml of high pure water was added to the sample by using a 20 ml volumetric pipette. The solution was stirred with a speed of 100 rpm and absorbance or the optical density was determined with respect to time by UV spectrometer. The intrinsic dissolution rate was determined by plotting concentration versus time and calculating the slope of the curve. The 100% transmittance and dark spectra was collected by using high pure water The experiments for TDF 1:1 and Tenofovir disoproxil succinate were performed in an identical protocol in buffered media of pH values of 1.5, 3.0, 4.5, 6.4 and 7.8 The following table shows the intrinsic dissolution rate of Tenofovir disoproxil fumarate (TDF), Tenofovir disoproxil succinate Suc2 and the succinate of Tenofovir disoproxil (Suc8)

Results of intrinsic dissolution rate measurements of suc 2 and suc 8

medium	**TDF-Dissolution rate (mgmin^{-1}cm^{-2})**	**Succinate Dissolution rate (mgmin^{-1}cm^{-2}) suc8**	**Succinate Dissolution rate (mgmin^{-1}cm^{-2}) suc2**
Buffer pH 1.5	3.29	3.40	10.00
Buffer pH 3.0	1.36	1.37	1.78
Buffer pH 4.5	0.99	0.85	3.55
Buffer pH 6.8	0.98	1.19	1.88
Buffer pH 7.4	0	1.19	1.09
water	1.12	0.85	1.42

Solubility of SUC8 in buffer solutions

According to USP (United State Pharmacopeia) the list of buffers to carry out the solubility of SUC8 are as follows

- pH 1.5: USP SGF without pepsin (0.05M sodium chloride adjusted to pH 1.5 with HCl)
- pH 3.0: 0.05 sodium di-hydrogen phosphate buffer adjusted to pH 6.8 with HCl
- pH 4.5: 0.05M sodium di-hydrogen phosphate buffer adjusted to pH 4.5 with NaOH
- pH 6.8: USP SIF without pancreatic (0.05M sodium di-hydrogen phosphate buffer adjusted to pH 6.8 with NaOH)

- pH 7 4: 0 05M sodium di-hydrogen phosphate adjusted to pH 7 4 with NaOH

Results of solubility of SUC8 in pH buffers (FB=Free Base)

starting Solid Forms	Medium	Solubility (mg/ml)	Solid Form After 1day slurry
SUC8	water	12.7	**SUC8**
SUC8	buffer 1.5	25.7	**SUC8**
SUC8	buffer 3.0	8.76	**SUC8**
SUC8	buffer 4.5	13.2	**SUC8**
SUC8	buffer 6.8	5.3	**SUC8**
SUC8	buffer 7.4	4.7	**SUC8+FB**

The table shows the solubility of SUC8 in water and 5 different pH buffers and the solid materials measured by XRPD, which showed that there were no any changes in the structure except the pH buffer 7.4 At pH 7.4 part of the SUC8 converts to free base of Tenofovir disoproxil which leads to the mixture of SUC8 and free base. Quantitative solubility of Tenofovir disoproxil succinate (SUC8) as compared to Suc2 and TDF is displayed in Fig 14.

Particle size distribution

The particle size distribution of Tenofovir disoproxil fumarate and Tenofovir disoproxil succinate (SUC8) were measured on MALVERN Mastersiezer 2000^{TM}. The average particle size distribution of Tenofovir disoproxil fumarate was in between 240-1150 um which is shown in figure 16. The average particle size distribution of SUC8 is more uniform as compare to Tenofovir disoproxil fumarate which is shown in figure 17. (Size distribution 250-536 um)

Bulk density, Tap density and compressibility index of SUC8

Compressibility and compactability of a powder are influenced by the flow properties, and at microscale, by the adhesion forces between particles. Compressibility is the ability to reduce the volume under pressure and compactability is the ability to build a solid "agglomerate" under pressure with sufficient strength and stability

The bulk density and tap density of Tenofovir disoproxil fumarate and succinate was measured by a graduated cylinder method which was mentioned in USP The bulk density was calculated by the following formula

$$Bulk\_Density = \left(\frac{Mass}{Volume}\right)$$

Tap density was measured by tapping a measuring cylinder containing a powder. After observing the initial volume, the cylinder is mechanically tapped, and volume readings are taken until little further volume change is observed. After measuring tap density the compressibility was measured by the following formula.

$$\%compressibility = \left[\frac{Tap\_density - Bulk\_density}{Tap\_density}\right] * 100$$

The Table shows the bulk density, tap density and compressibility of Tenofovir disoproxil fumarate and Tenofovir disoproxil succinate (SUC8).

Bulk density, tap density and compressibility of Tenofovir disoproxil.

Material	**Bulk Density (g/ml)**	**Tap Density (g/ml)**	**% compressibility**
TDF	0.447	0.499	10.42
SUC8	0.232	0.439	47.15

The compressibility of Tenofovir disoproxil succinate (SUC8) is 4.5 times higher than the Tenofovir disoproxil fumarate. This may be attributed to the uniform particle size distribution of SUC8 which allows for the most efficient coverage of voids between the particles upon tapping. The table shows the compressibility values of Tenofovir disoproxil succinate in comparison with Tenofovir disoproxil fumarate.

Stability of Tenofovir disoproxil succinate under elevated humidity and temperature

The physical and chemical stability of Tenofovir disoproxil succinate (Suc8) was checked under elevated humidity (75% RH) and temperature (40°C). The material was analyzed by XRPD and HPLC. In all cases the XRPD indicated that the solid form did not change compared to the initial solid form. The purity as indicated by HPLC is shown in the table below.

Sampling time	Purity by HPLC
2 Weeks	99.47
4 Weeks	99.83
8 Weeks	99.05
13 Weeks	99.23
40 Weeks	98.17
45 Weeks	98.01

Example:

wet granulation of tenofovir disoproxil succinate (suc8)

The wet granulation of Tenofovir disoproxil succinate was carried out by Viread, Truvada and Atripla formulation methods described in US patent 6475491 and US2007/0077295.

Analytical method: HPLC assay of degradation product (Viread formulation)

Tenofovir disoproxil succinate (SUC8) granules are assayed by HPLC for Tenofovir disoproxil succinate (SUC8) using external references as described in US patent 6475491. The presence of degradation products are determined by area normalization with the application of relative response factor. The identities of Tenofovir disoproxil succinate are confirmed by comparison of their retention times with those of the reference standards.

Analytical method: HPLC assay of degradation product (Truvada and Atripla formulation)

Efavirenz/Emtricitabine/Tenofovir disoproxil succinate (SUC8) granules are assayed by HPLC for Efavirenz, Emtricitabine and Tenofovir disoproxil succinate (SUC8) using external references as described in US patent US2007/0077295. The presence of degradation products are determined by area normalization with the application of relative response factor. The identities of Emtricitabine and Tenofovir disoproxil succinate are confirmed by comparison of their retention times with those of the reference standards.

Standard and sample solvent: 25 mM phosphate buffer pH 3

3.4 g of potassium phosphate monobasic, anhydrous is weighed and transferred into a 1 L volumetric flask. About 800 mL of water is added and mixed until dissolved. The pH to 3.0 ± 0.1 is adjusted with phosphoric acid, and then diluted to volume with water. Sample solvent (mixture of 25 mM phosphate buffer pH 3 40%: Acetonitrile 30%: methanol 30%): 400 mL of 25 mM phosphate buffer pH 3, 300 mL acetonitrile, 300 mL methanol is combined, mixed and allowed to equilibrate to ambient temperature. 50:50 Acetonitrile: methanol: Combine 500 mL acetonitrile and 500 mL methanol is combined, mixed and allowed to equilibrate to ambient temperature. Standard solution: 20 mg of Efavirenz, 20 mg of Emtricitabine reference standard and 30 mg of Tenofovir disoproxil reference standard was weighed and transferred into a 100 mL volumetric flask. Approximately 80 mL of sample solvent was added (as prepared in step 2) to the flask and mixed or sonicated until dissolved Diluted to volume with sample solvent (40:30:30) and mixed well. The final concentration of each component is approximately 0.2 mg/mL of Emtricitabine and 0.3 mg/mL Tenofovir disoproxil.

Sample preparation for Efavirenz/Emtricitabine/Tenofovir disoproxil succinate granules.

Approximately 6520 mg of Efavirenz/Emtricitabine/Tenofovir disoproxil succinate granules was weighted into a 1 L volumetric flask. Added was 400 mL 25 mM phosphate buffer, pH 3 to the volumetric flask Mixing by stirring vigorously for about 75 minutes. 50:50 acetonitrile: methanol was added to the flask to approximately 2 cm below the 1 L mark. The solution was equilibrated to ambient temperature by mixing for 1 hour. The volume was diluted to 1L with 50:50 acetonitrile: methanol and mixed well by stirring with a magnetic stirring bar. Using a 0.45 µm syringe filter with a syringe, approximately 10 mL for the next dilution was filtered. The first 2 mL of the filtrate was discarded. A class A pipette was used to transfer 5.0 mL of the filtrate into a 50 mL volumetric flask and dilute the to volume with sample solvent (40:30:30).

Chromatography

An LCMS system with UV detector, HP1100 API-ES MSD VL-type detector and electronic data acquisition system was used. An HPLC column, 4.6 mm i.d. by 100 mm long, packed with C18 reversed phase, 3.5 µm particle size 80 Å pore size material was used Mobile phase buffer: a 20 mM ammonium acetate buffer pH 4.6; adjust pH with acetic acid Mobile phase gradient: mobile phase buffer: acetonitrile from 99:1 to 1:99 over 67 minutes. Peak detection: UV at 265 nm Injection volume 5 µL. Under the stated chromatographic conditions the retention times of Emtricitabine is 7.5 minutes. The retention time of he Tenofovir disoproxil is around 25 minutes.

Experimental procedure

Table I, table II and table III show the amounts of components used in the Viread, Truvada and Atripla formulations of Tenofovir disoproxil succinate, respectively.

Table I. Viread method for Tenofovir disoproxil succinate

Components	**%w/w**	**mg/ tablet**

Tenofovir disoproxil succinate (SUC8-SU375)	34.0	74.79
Lactose monohydrate	54.0	109.24
Croscarmellose sodium	2.0	6.16
Magnesium stearate	1.0	2.15
Starch pregelatinized	5.0	11.0
Total		203.34

The components of mass of 15 tablets (mentioned in table 5) were mixed in a fluidized bed. The high pure water was sprayed and the granules were analyzed visually. The mixture was than dried and analyzed by HPLC for the purity and stability of Tenofovir disoproxil succinate (SUC8).

Table II shows the list of ingredients used in the formulation of Tenofovir disoproxil succinate by using Truvada formulation method.

Table II. Truvada formulation for Tenofovir disoproxil succinate.

Ingredient	**%w/w of total**	**Unit formula (mg/tablet)**
Emtricitabine	30.68	200.33
Tenofovir disoproxil succinate	46.00	300.05
Micro crystalline cellulose	13.72	90
Croscarmellose sodium	7.37	48.9
Magnesium stearate	2.23	14.5

A composition comprising the ingredients and ratios as listed in table II are subjected to a standard wet granulation process with respectively 30% and 40% w/w water. The quality of the granulation was assessed visually. The experiments were carried out by using fluidized bed which was designed to carry out the wet granulation experiments of Tenofovir disoproxil succinate and Emtricitabine.

Table III shows the number of components used for wet granulation of Tenofovir disoproxil succinate with reference to the Atripla formulation technique.

Table III. Atripla formulation for Tenofovir disoproxil succinate.

Ingredient	%w/w of total	Unit formula (mg/tablet)
Emtricitabine	13.07	400
Tenofovir disoproxil (Fumarate or Hemifumarate)	19.59	600
Efavirenz	39.17	1201.3
Micro crystalline cellulose	17.08	523.3
Croscarmellose sodium	6.27	192
Magnesium stearate	1.58	48.4
Hydroxy propyl cellulose	2.53	76.8
Sodium lauryl sulphate	0.78	24

A composition comprising the ingredients and ratios as listed in table III are subjected to a standard wet granulation process with respectively 30% and 40% w/w water. The quality of the granulation was assessed visually. The experiments were carried out by using fluidized bed which was designed to carry out the wet granulation experiments of Tenofovir disoproxil succinate, Emtricitabine and Efavirenz.

Results

The level of each degradation product observed in the chromatogram was determined by the following formula:

$$\boxed{\%Degradation\_product = \left(\frac{I}{TPA}\right) \times RRF \times 100}$$

Where: I= Area of the degradation product peak

TPA= Total area peak (area of the SUC8 main peak, all related degradation products, and all unassigned peaks, excluding impurities and artifacts

RRF=Relative response factor with respect to SUC8.

The following table IV shows the %degradation of the impurities obtained during the wet granulation of Tenofovir disoproxil succinate by using Truvada formulation technique.

Table IV. The amount of degradation product obtained during the Truvada formulations.

Samples	% water	Impurities	Area of product	Area of Impurity (I)	Total peak area (TPA)	RRF	% degradation
TDF	30	Dimer	3980.860	13.510	3994.370	0.900	**0.304**
		Mixed dimer	3980.860	0.000	3980.860	1.000	-
		impurity 3	3980.860	0.000	3980.860	0.600	-
	40	Dimer	15297.000	27.490	15324.490	0.900	**0.161**
		Mixed dimer	15297.000	17.860	15314.860	1.000	**0.117**
		impurity 3	15297.000	22.340	15319.340	0.600	**0.087**
SUC8	30	Dimer	6583.490	0.000	6583.490	0.900	-
		Mixed dimer	6583.490	0.000	6583.490	1.000	-
		impurity 3	6583.490	0.000	6583.490	0.600	-
	40	Dimer	9813.840	8.250	9822.090	0.900	**0.076**
		Mixed dimer	9813.840	0.000	9813.840	1.000	-
		impurity 3	9813.840	0.000	9813.840	0.600	-

The Dimer is the impurity of Tenofovir disoproxil with a mass of 1051.2, the mixed dimer is the impurity of mass 936.3 and the impurity 3 is the impurity of Tenofovir disoproxil with a mass of 606.7.

Table V shows the % degradation impurity obtained during the Atripla formulation of Tenofovir disoproxil succinate

Table V. The amount of degradation product obtained during the Atripla formulations.

Samples	% water	Impurities	Area of product	Area of Impurity (I)	Total peak area (TPA)	RRF	%degradation
TDF	30	Dimer	7220.210	58.530	7278.740	0.900	**0.72**

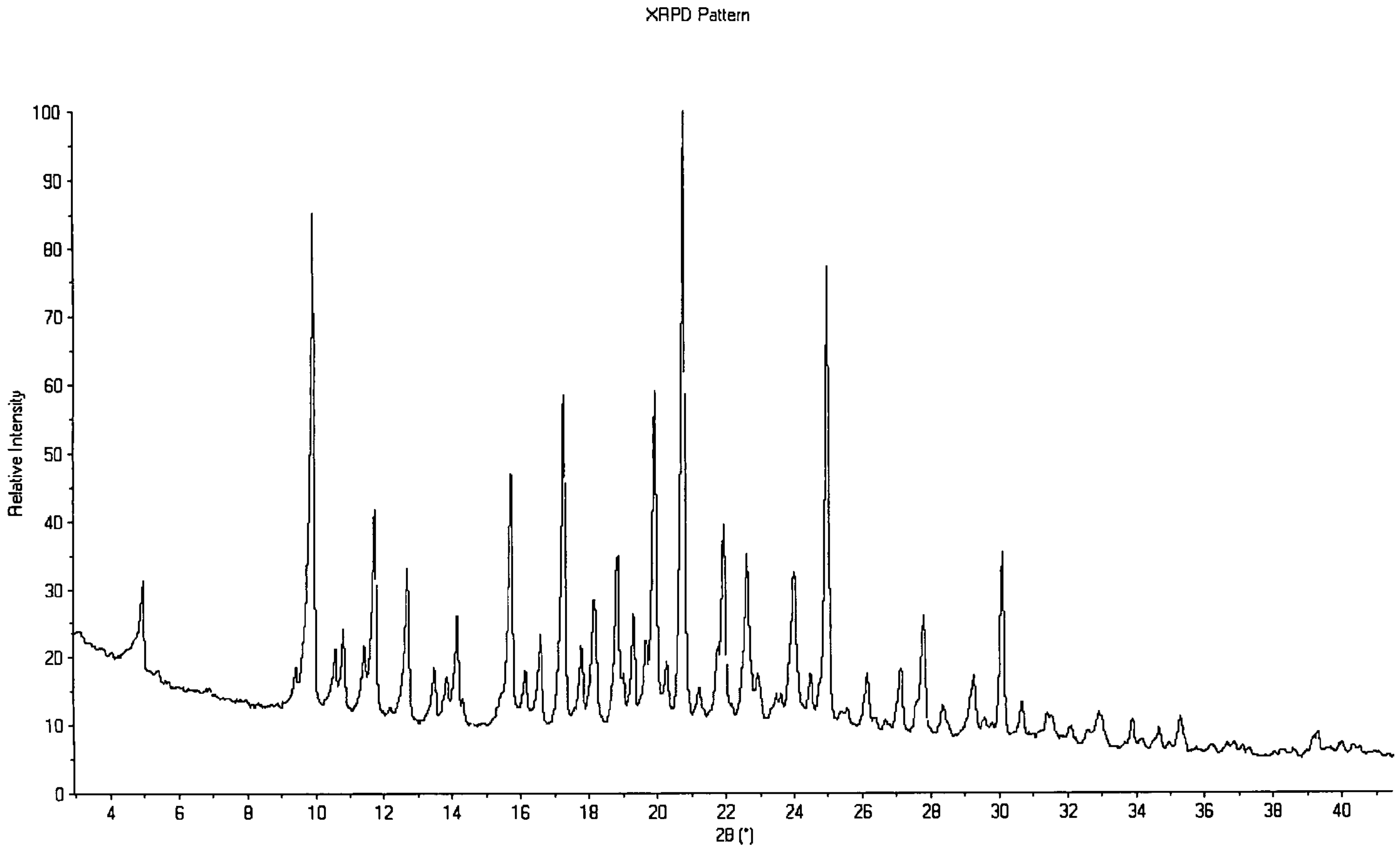
		Mixed dimer	7220.210	10.310	7230.520	1.000	**0.14**
		impurity 3	7220.210	11.720	7231.930	0.600	**0.10**
	40	Dimer	9553.620	32.200	9585.820	0.900	**0.30**
		Mixed dimer	9553.620	11.390	9565.010	1.000	**0.12**
		impurity 3	9553.620	13.149	9566.769	0.600	**0.08**
SUC8	30	Dimer	8363.750	7.900	8371.650	0.900	**0.08**
		Mixed dimer	8363.750	0.000	8363.750	1.000	**0.00**
		impurity 3	8363.750	0.000	8363.750	0.600	**0.00**
	40	Dimer	9813.840	8.250	9822.090	0.900	**0.08**
		Mixed dimer	9813.840	0.000	9813.840	1.000	**0.00**
		impurity 3	9813.840	0.000	9813.840	0.600	**0.00**

Claims

1. The succinate of Tenofovir disoproxil.
2. The succinate of Tenofovir disoproxil, characterised by one or more of:
   a. at least one, preferably at least two, more preferably at least three, even more preferably at least four, particularly preferred at least five and most preferred six X-ray powder diffraction peaks selected from the group consisting of 5.0, 9.9, 11.7, 12.7, 14.1, 15.7, 17.3, 18.2, 18.9, 19.9, 20.8, 25.0, 30.1 degrees two-theta +/- 0.3 degrees two-theta, preferably +/- 0.2 degrees two-theta, more preferably +/- 0.1 degrees two-theta, most preferably +/- 0.05 degrees two-theta;
   b. FTIR having characterising peaks selected from the group consisting of 634, 950, 1027, 1255, 1623, 1669, 1744, 1759 cm^{-1} +/- 0.3 cm^{-1}, preferably +/- 0.2 cm^{-1}, more preferably +/- 0.1 cm^{-1}, most preferably +/- 0.05 cm^{-1}
3. Method for the preparation of the succinate of tenofovir disoproxil comprising the steps of
   a. dissolving or mixing Tenofovir disoproxil free base and succinic acid in a suitable solvent or mixture thereof, preferably acetonitrile or methanol, and crystallising a product
   b. contacting the product of step (a) with water and crystallising the succinate of Tenofovir disoproxil
4. Method for the preparation of the succinate of Tenofovir disoproxil comprising the steps of contacting tenofovir disoproxil succinate with water and obtaining the succinate of Tenofovir Disoproxil.
5. Method for the preparation of the succinate of Tenofovir disoproxil comprising the steps of contacting Tenofovir disoproxil free base with succinic acid in the presence of water and obtaining the succinate of Tenofovir Disoproxil.
6. Pharmaceutical formulation comprising the succinate of Tenofovir disoproxil.
7. Method for the preparation of a pharmaceutical formulation of the succinate of Tenofovir disoproxil comprising a step of wet granulating the succinate of Tenofovir disoproxil in the presence of a pharmaceutically acceptable carrier/excipient and/or another pharmaceutical ingredient, preferably an anti HIV agent, preferably Efavirenz and/or Emtricitabine.
8. Use of the succinate of Tenofovir disoproxil as a medicament.
9. Use of the succinate of Tenofovir disoproxil in the preparation of a medicament for the treatment of HIV
10. Use of the succinate of Tenofovir disoproxil in the treatment of HIV.

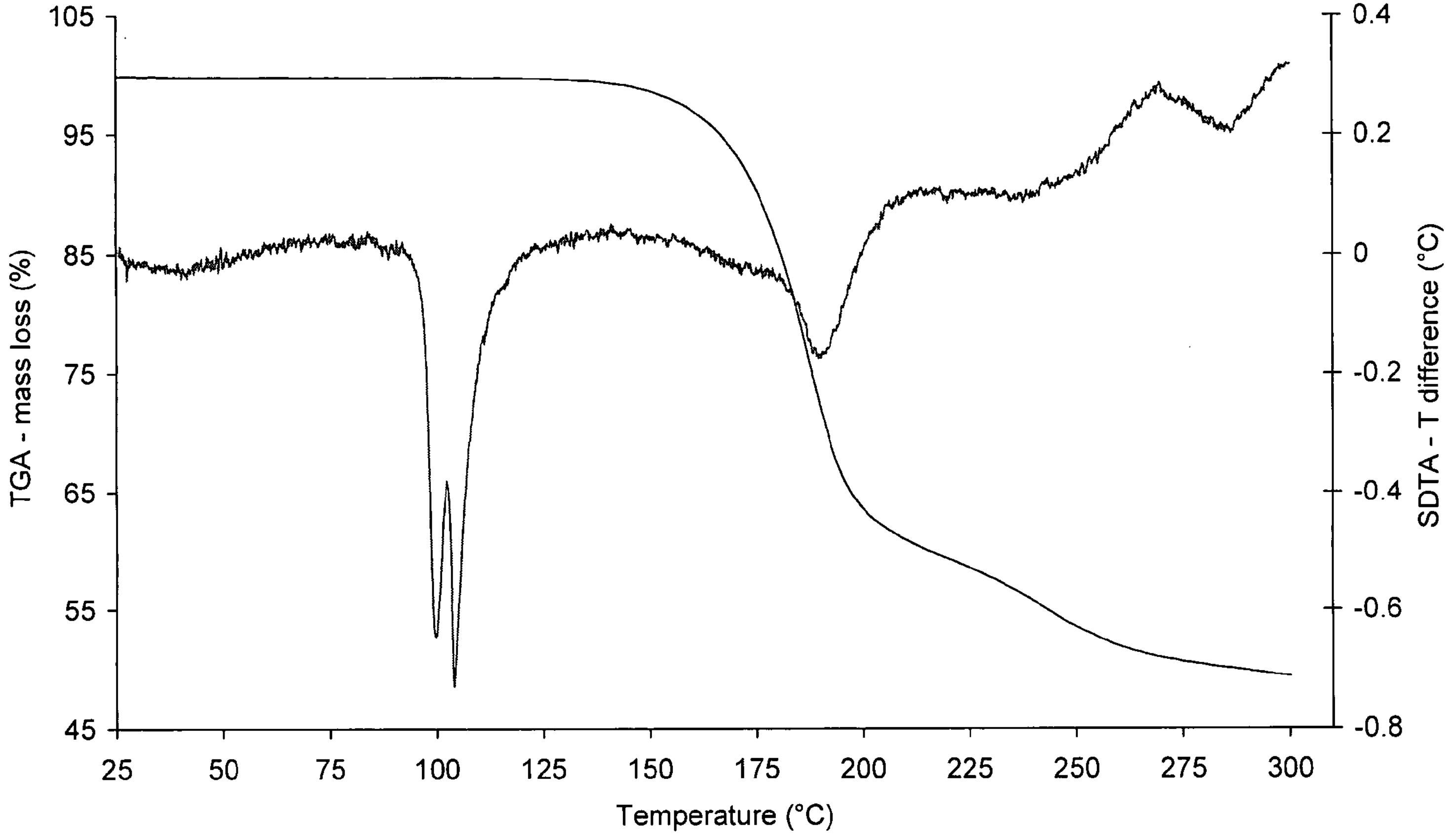
11 Use of the succinate of Tenofovir disoproxil in combination with another pharmaceutical ingredient, preferably an anti HIV agent, preferably Efavirenz and/or Emtricitabine.

FIG 1 Succinate of Tenofovir Disoproxil XRPD



Form Suc8 (1HR_B1)

FIG 2 Succinate of Tenofovir Disoproxil TGA



SU53_296
TGA (10 ºC/min)
Mass loss ~0.2% for T<115 ºC
No mass loss is observed thus the solid form is anhydrous/unsolvated

FIG 3 Succinate of Tenofovir Disoproxil DVS

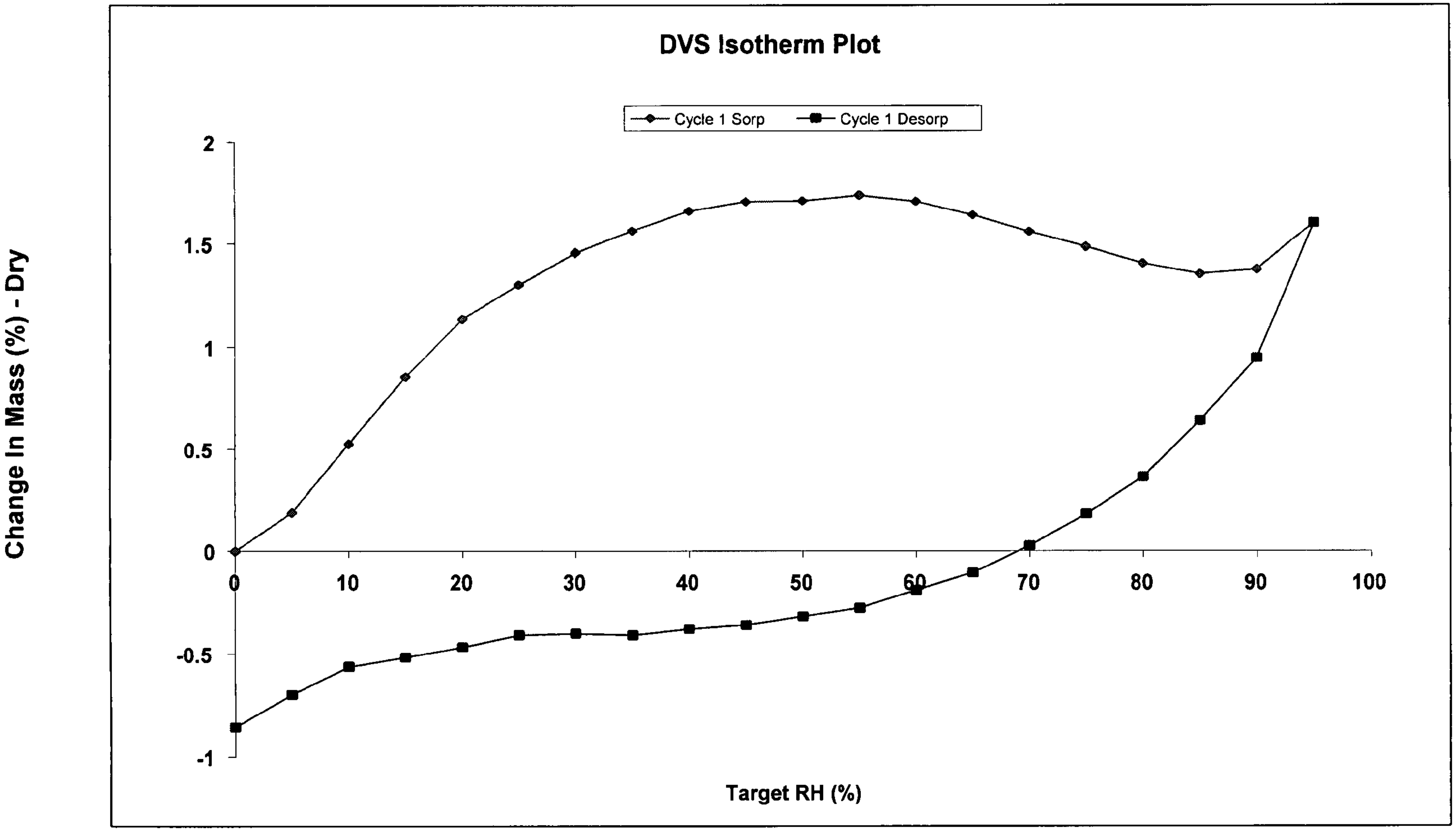
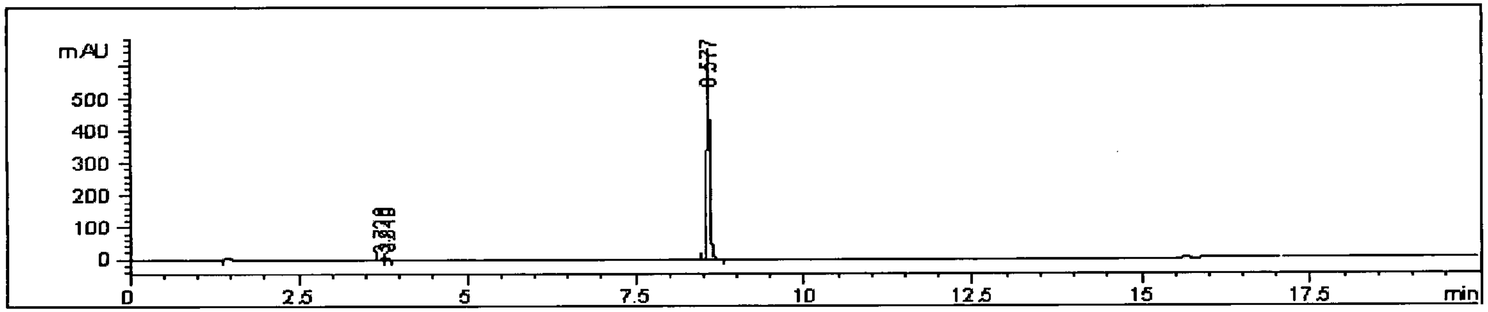


FIG 4 Succinate of Tenofovir Disoproxil HPLC



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=====================================================================
                         Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=265,4 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   3.728 BV    0.0573    5.94411    1.56589   0.3026
   2   3.810 VB    0.0429   10.81518    3.72495   0.5506
   3   8.577 BB    0.0460 1947.33142  648.23218  99.1467

Totals :                  1964.09071  653.52302
```

FIG 5 Succinate of Tenofovir Disoproxil FTIR

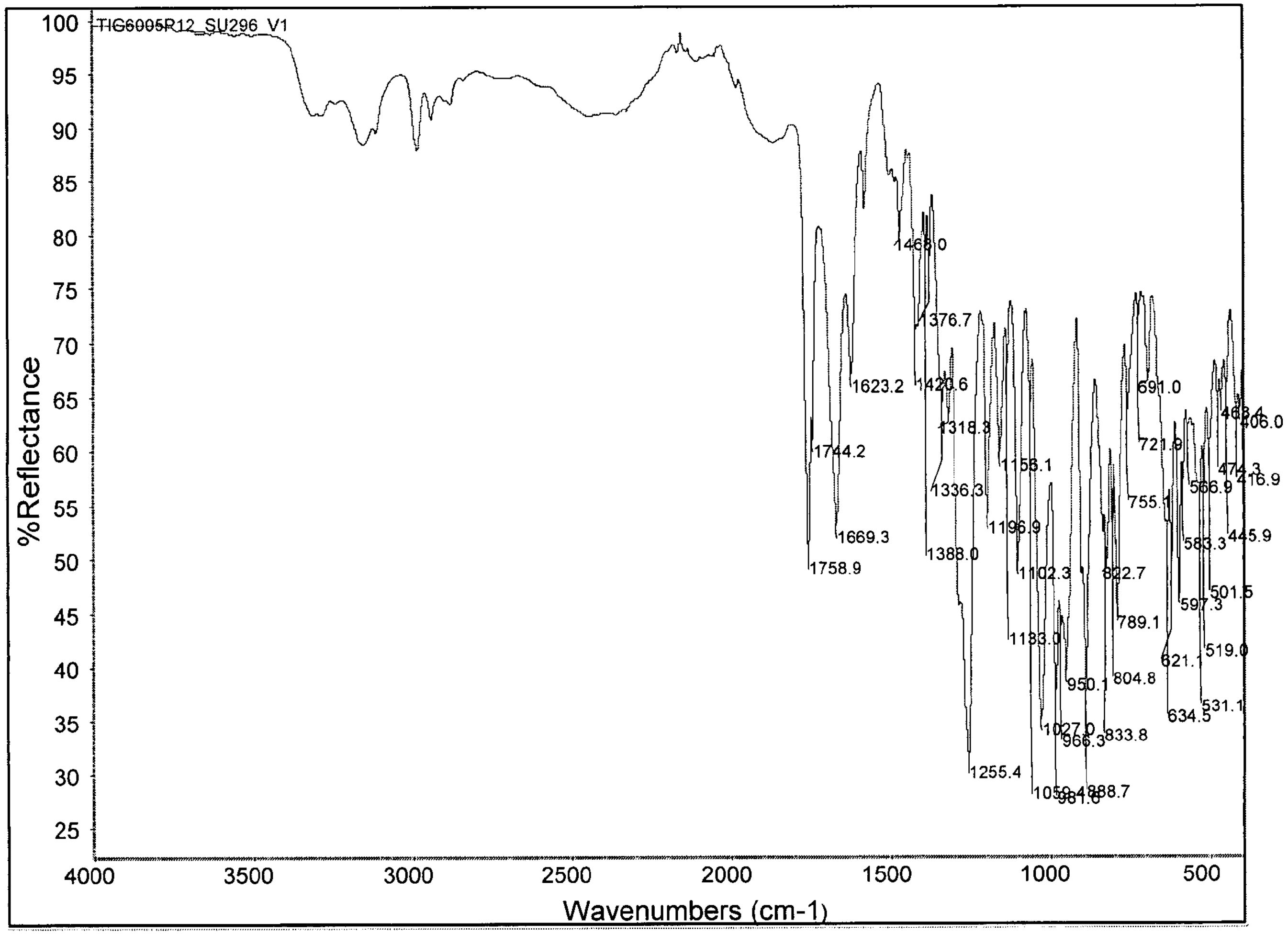


FIG 6 Succinate of Tenofovir Disoproxil ^{1}H NMR

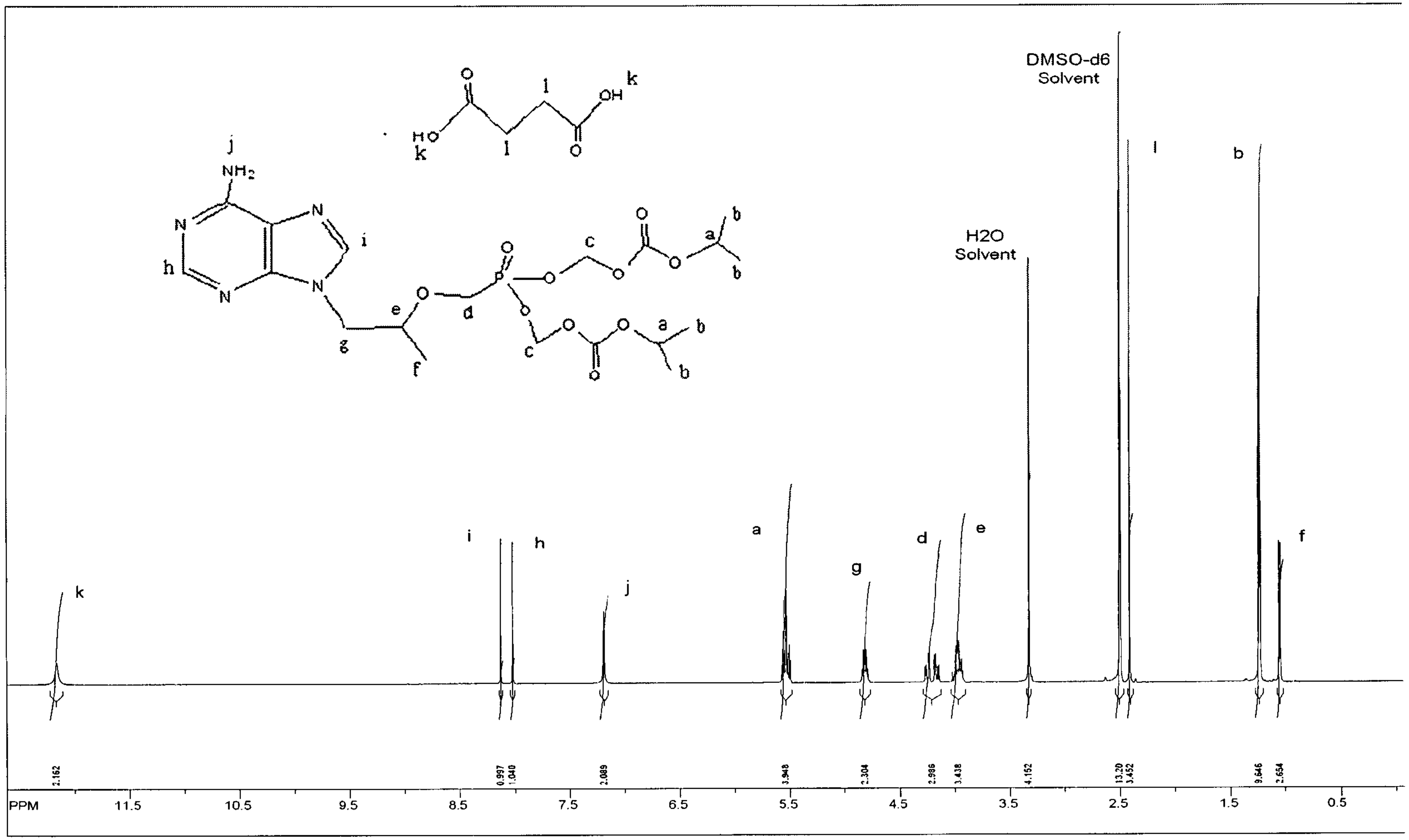


FIG 7 Succinate of Tenofovir Disoproxil

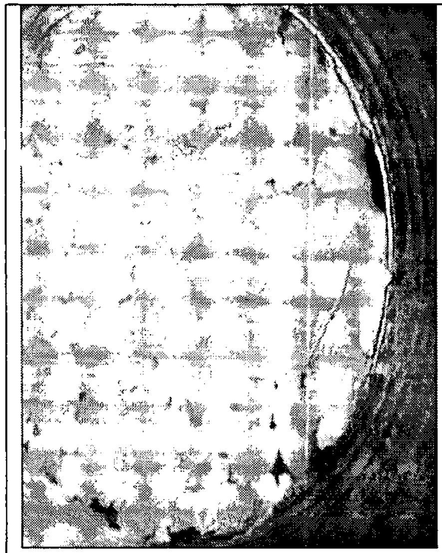
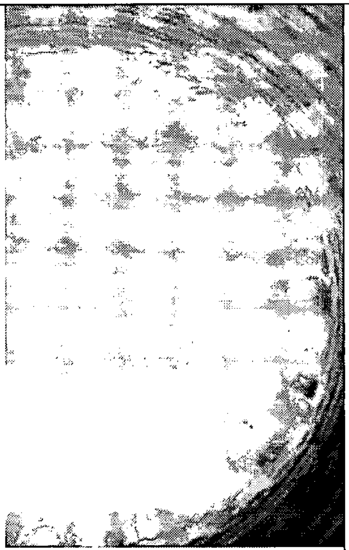
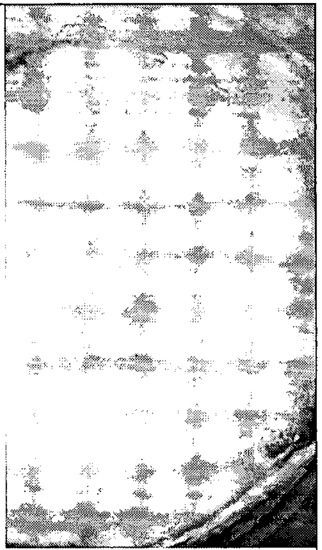
 Tenofovir Disoproxil succinate suc2	 the succinate of Tenofovir Disoproxil succinate Suc8	 TDFA 2:1 (WO2008143500)

FIG 8 Tenofovir Disoproxil Fumarate starting material XRPD

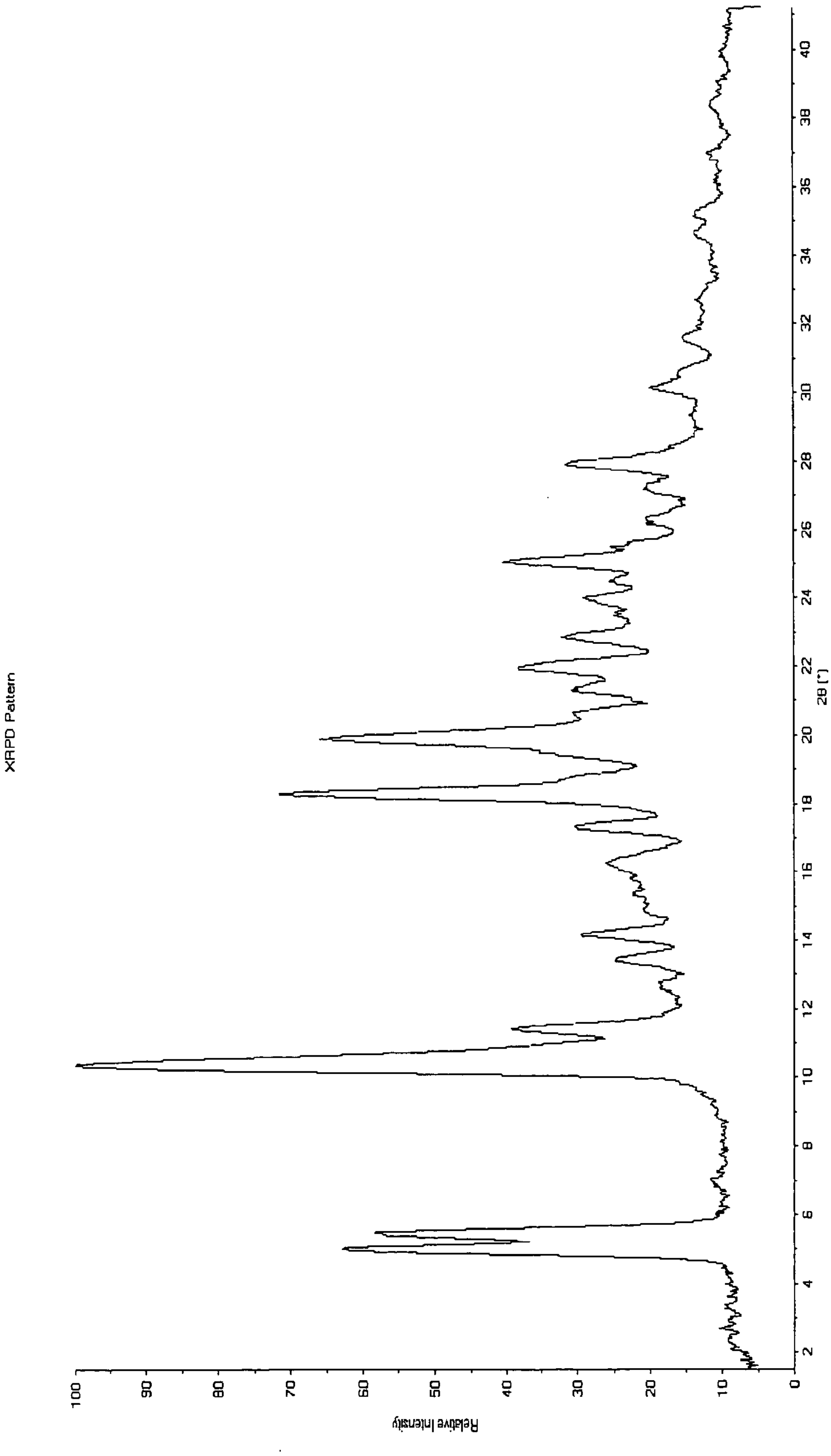


FIG 9 Tenofovir Disoproxil Fumarate starting material FTIR

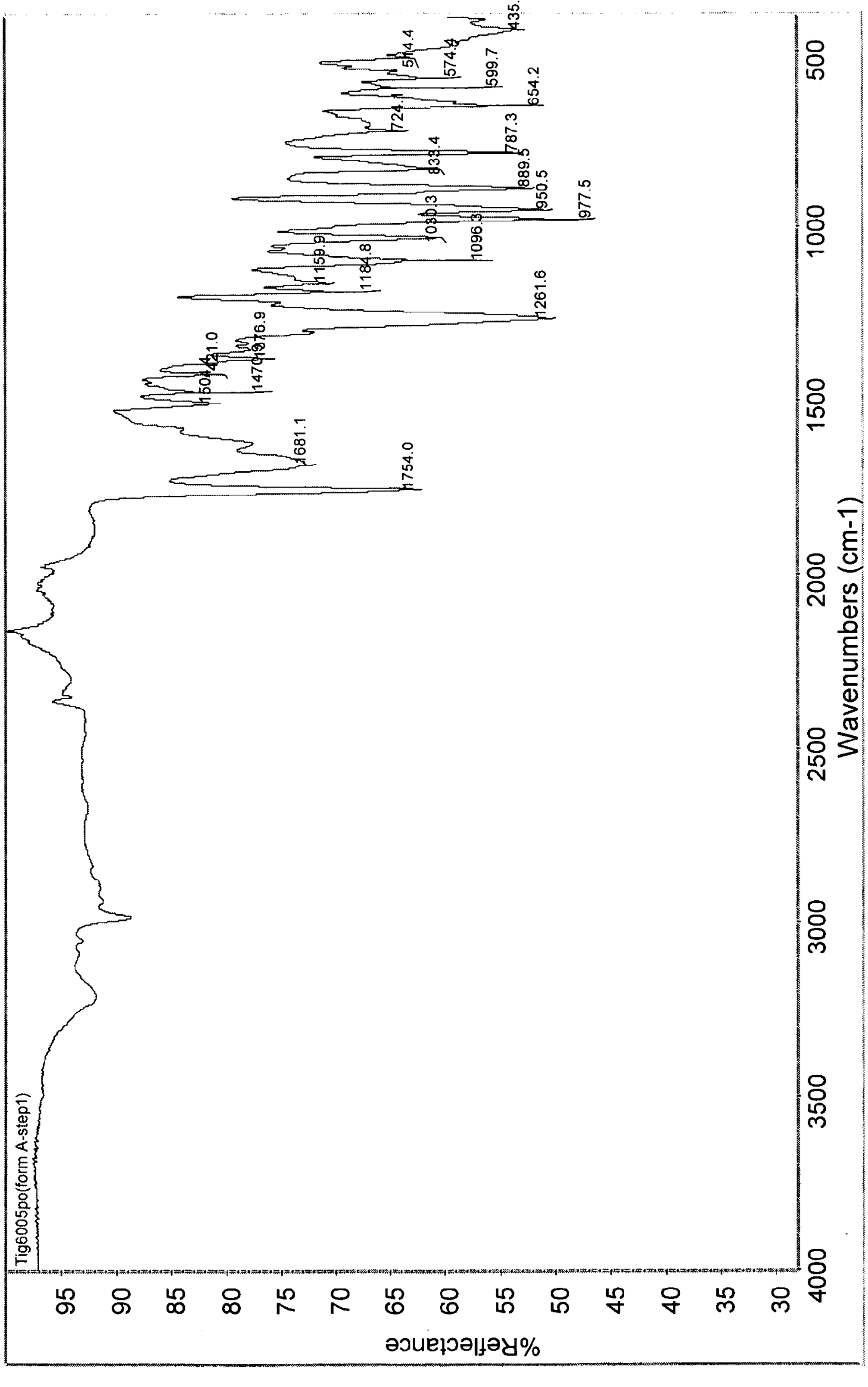
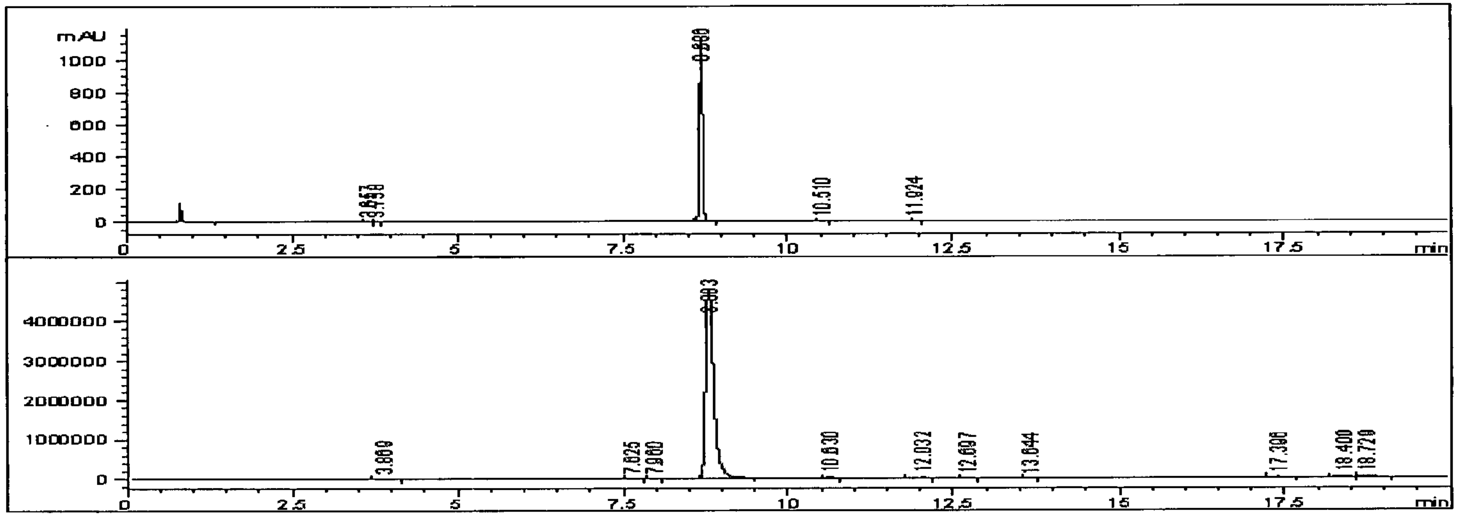


FIG 10 Tenofovir Disoproxil Fumarate starting material HPLC



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=====================================================================
                         Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=265,4 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
   1   3.657 BV    0.0584   11.92325    3.13247   0.3172
   2   3.758 VB    0.0422   17.45088    6.12761   0.4643
   3   8.686 BB    0.0509 3711.60205 1141.64978  98.7495
   4  10.510 BB    0.0554    7.66080    2.15944   0.2038
   5  11.924 BB    0.0544    9.96809    2.87917   0.2652

Totals :                  3758.60507 1155.94847
```

FIG 11 Tenofovir Disoproxil Free base XRPD

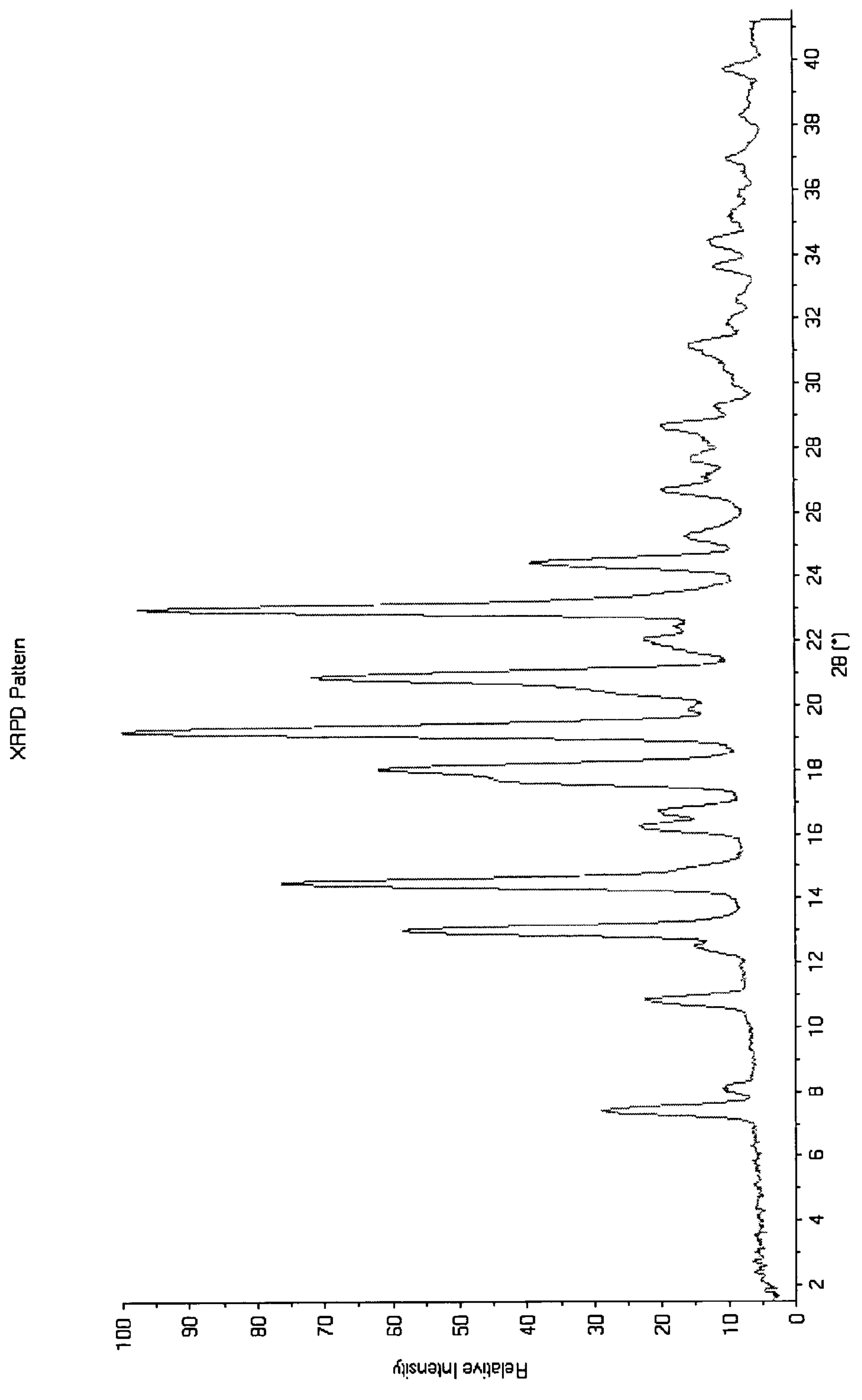


FIG 12 Tenofovir Disoproxil Free base FTIR

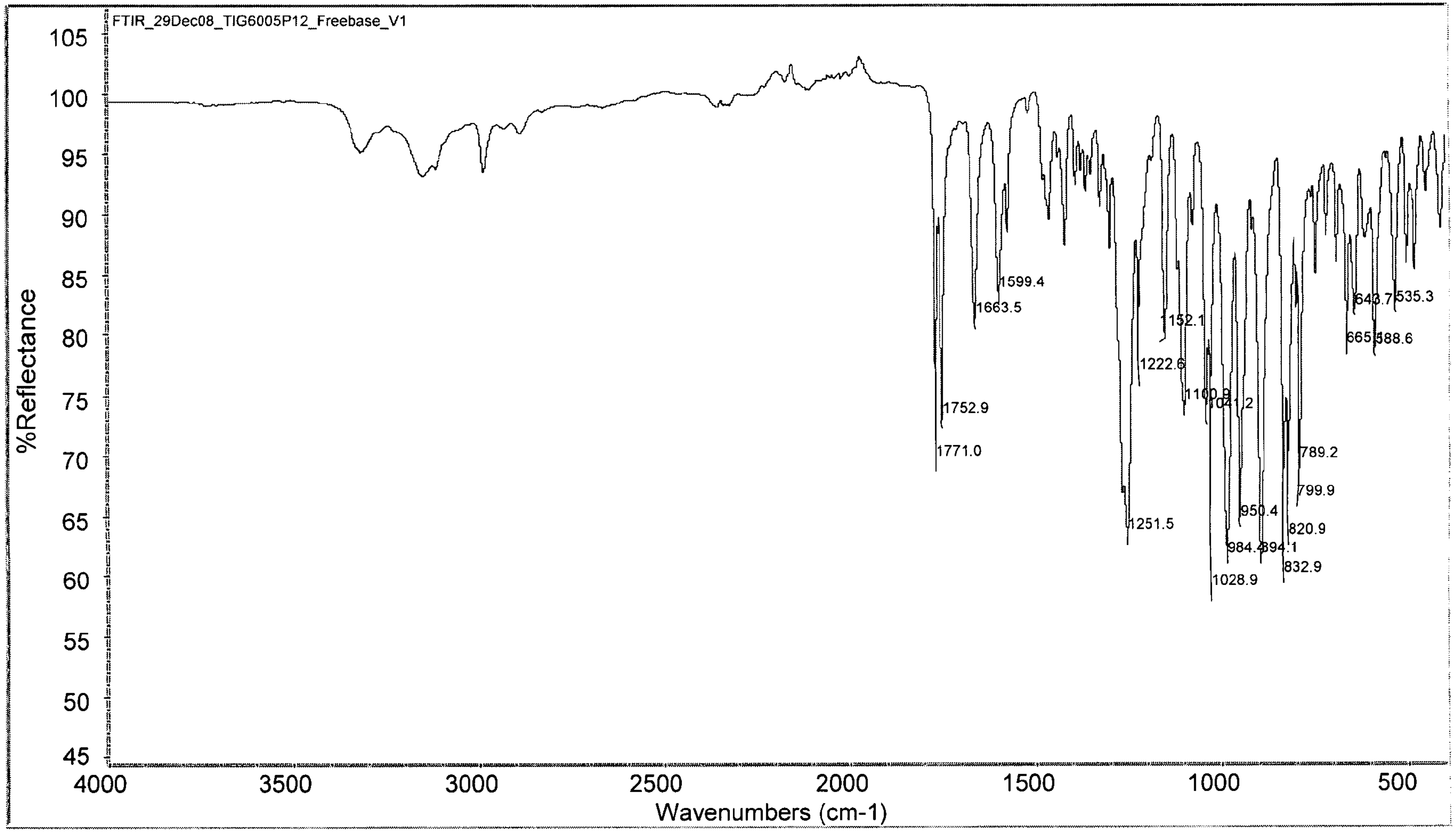
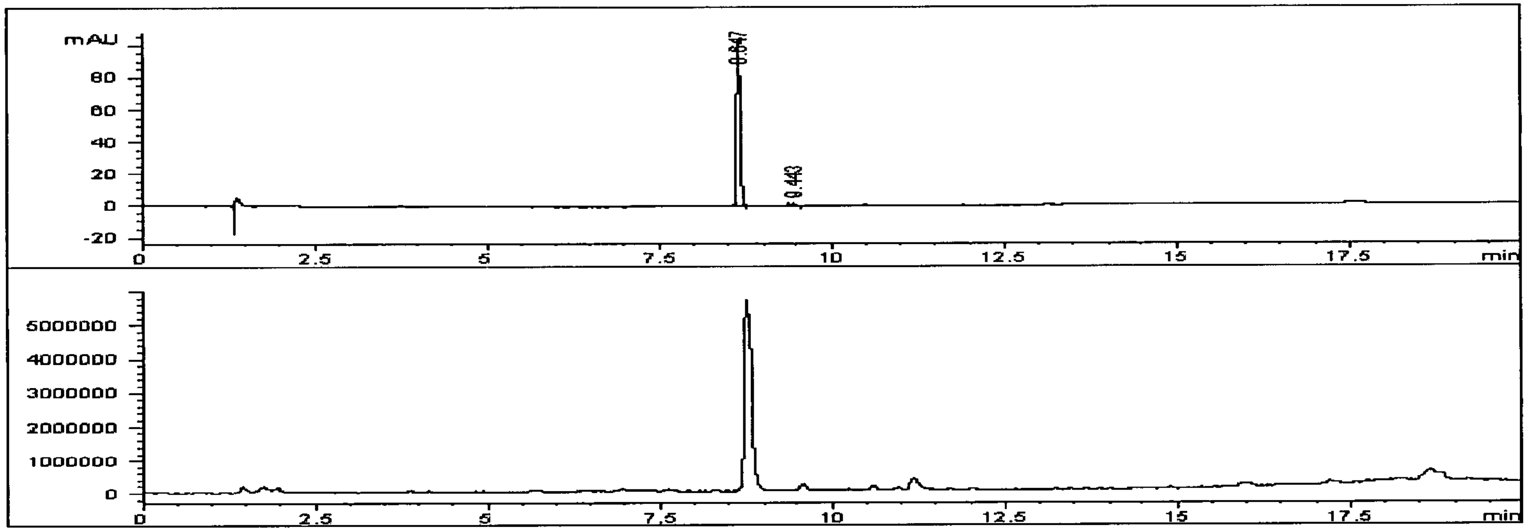


FIG 13 Tenofovir Disoproxil Fumarate Free base HPLC



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=====================================================================
                     Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=265,4 Ref=off

Peak RetTime Type  Width     Area       Height      Area
  #   [min]        [min]   [mAU*s]      [mAU]        %
----|-------|----|-------|----------|----------|--------|
   1   8.647 BB    0.0513  328.81082  102.77548  98.1524
   2   9.443 BB    0.0531    6.18951    1.84758   1.8476

Totals :                   335.00033  104.62306

Signal 2: MSD1 TIC, MS File

=====================================================================
                      *** End of Report ***
```

FIG 14

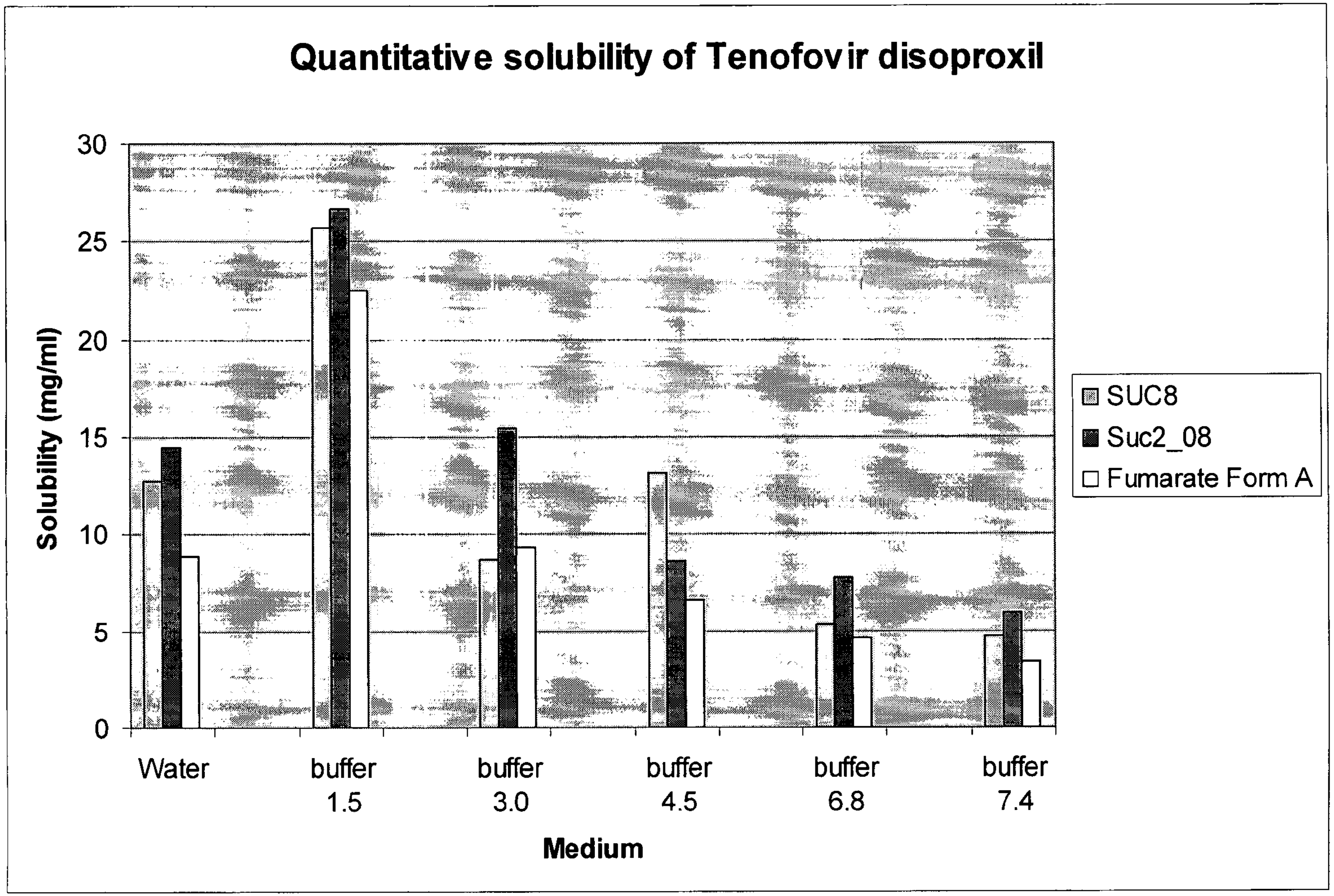
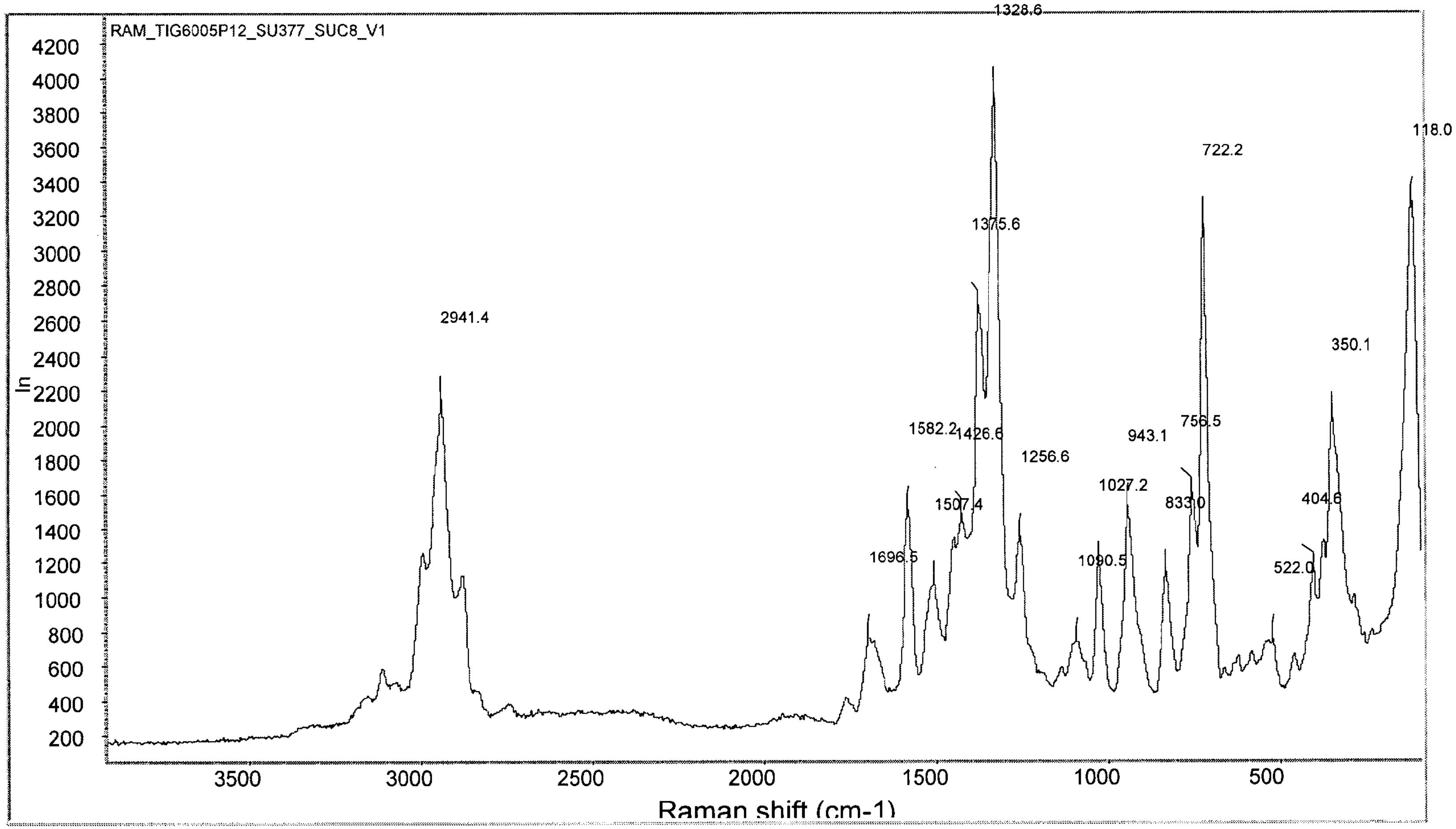

Quantitative solubility of Tenofovir disoproxil
Solubility (mg/ml)
30
25
20
15
10
5
0
Water
buffer 1.5
buffer 3.0
buffer 4.5
buffer 6.8
buffer 7.4
Medium
▨ SUC8
■ Suc2_08
☐ Fumarate Form A

FIG 15 Raman spectrum of the succinate of Tenofovir Disoproxil.



FIND PEAKS:
Spectrum: RAM_TIG6005P12_SU377_SUC8_V1
Region: 3914.1-93.8
Absolute threshold: 507.578
Sensitivity: 50

fig 15 cont'd

Peak ID	Position (cm-1)	Intensity
1	118.0	H
2	350.1	M
3	404.6	L
4	522.0	L
5	722.2	H
6	756.5	L
7	833.0	L
8	943.1	L
9	1027.2	L
10	1090.5	L
11	1256.6	L
12	1328.6	H
13	1375.6	M
14	1426.6	L
15	1507.4	L
16	1582.2	L
17	1696.5	L
18	2941.4	M

Fig 16. Particle size distribution of Tenofovir disoproxil fumarate.

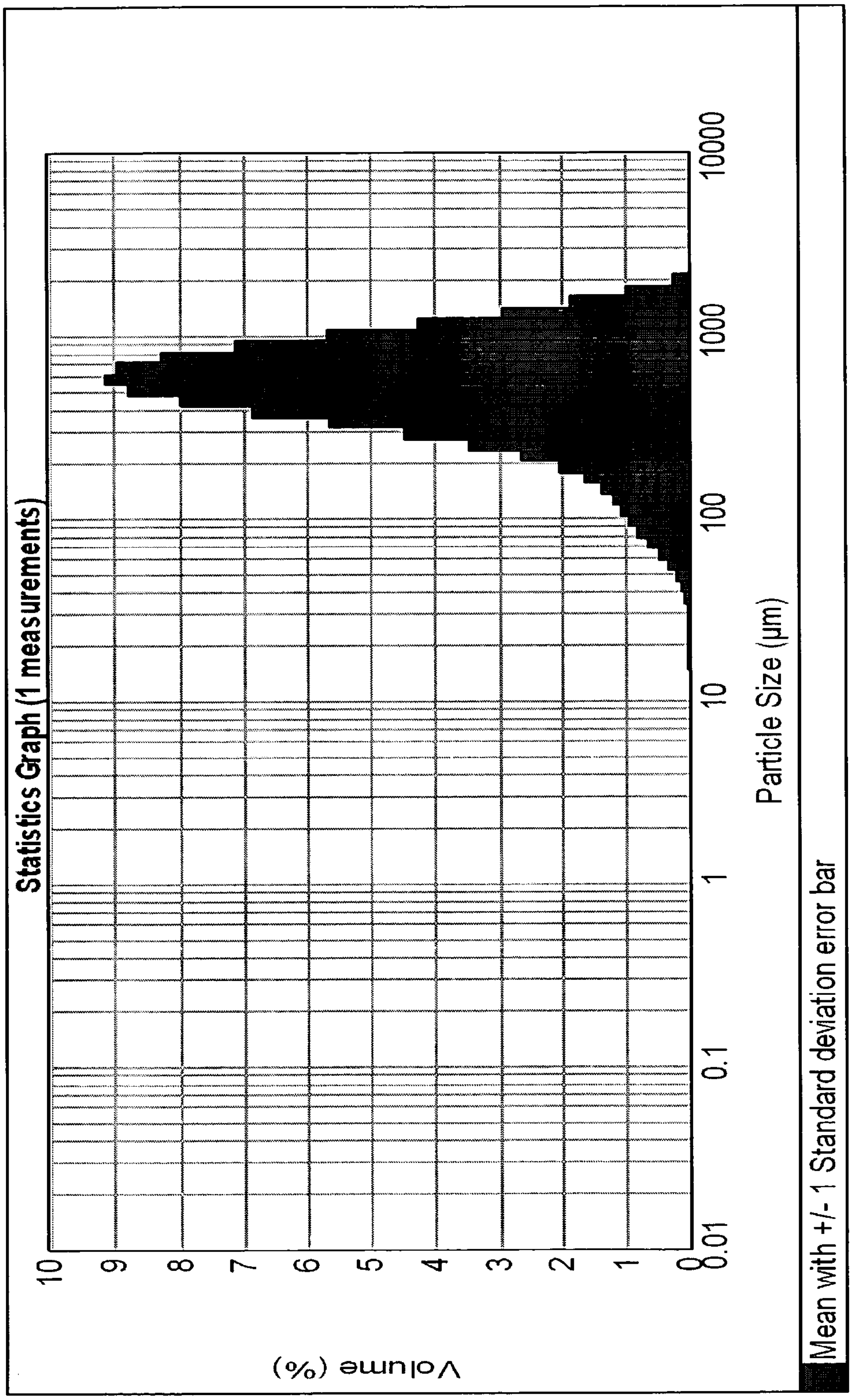


Fig 17. Particle size distribution of Tenofovir disoproxil succinate (SUC8).

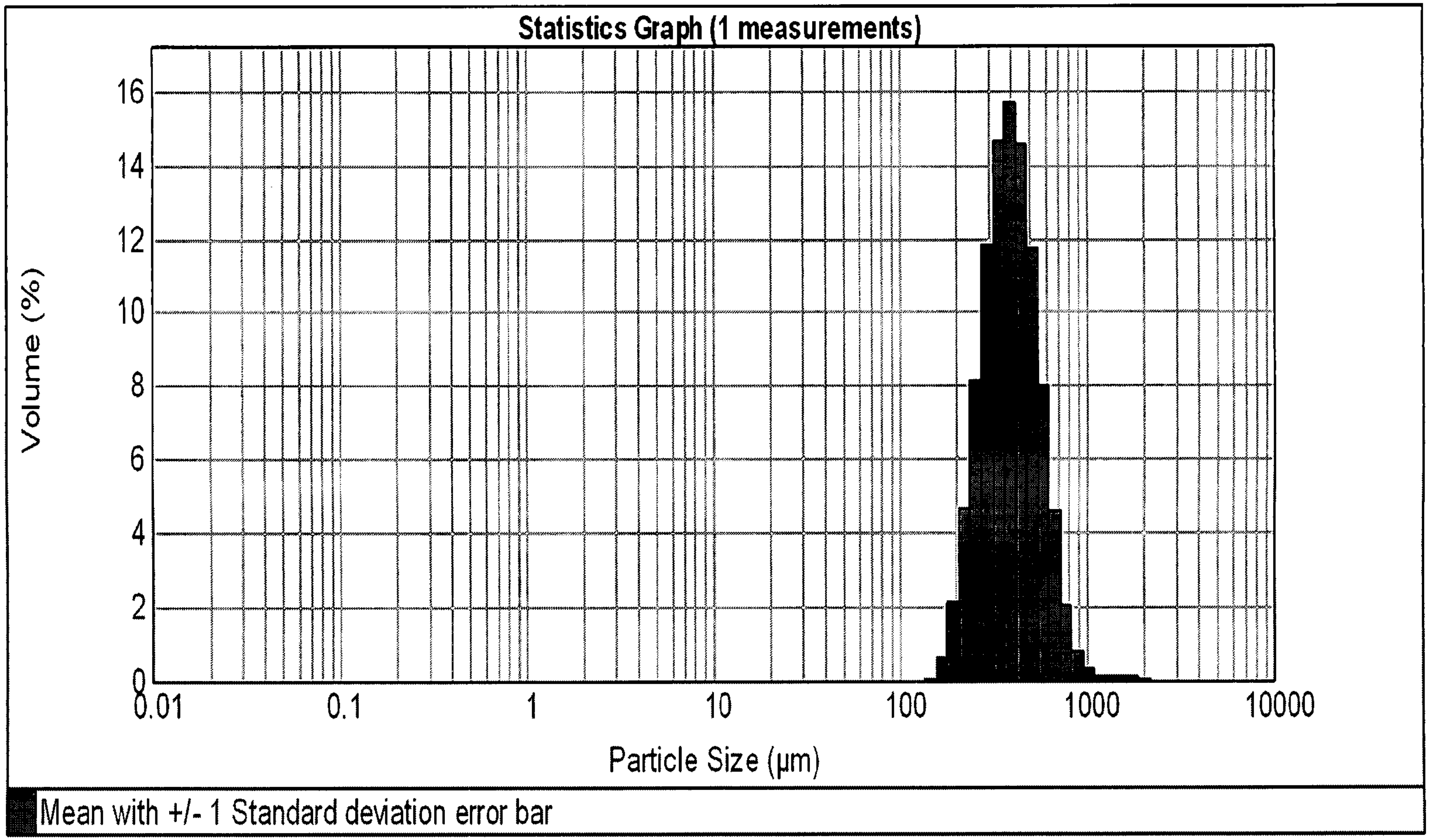
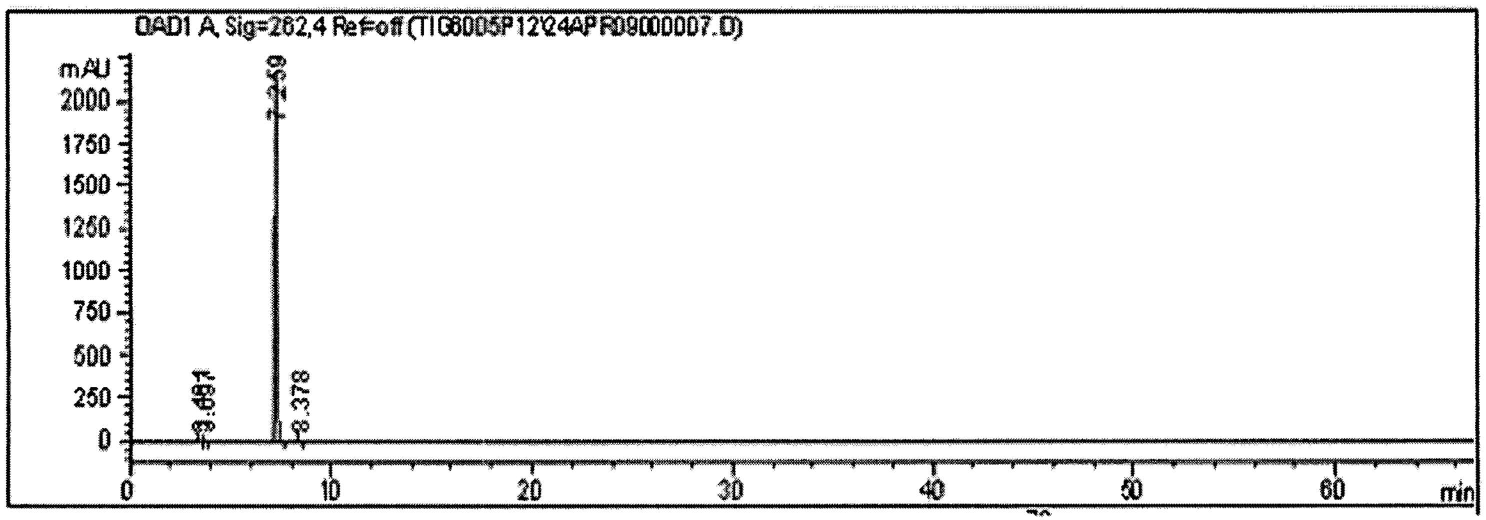


FIG 18

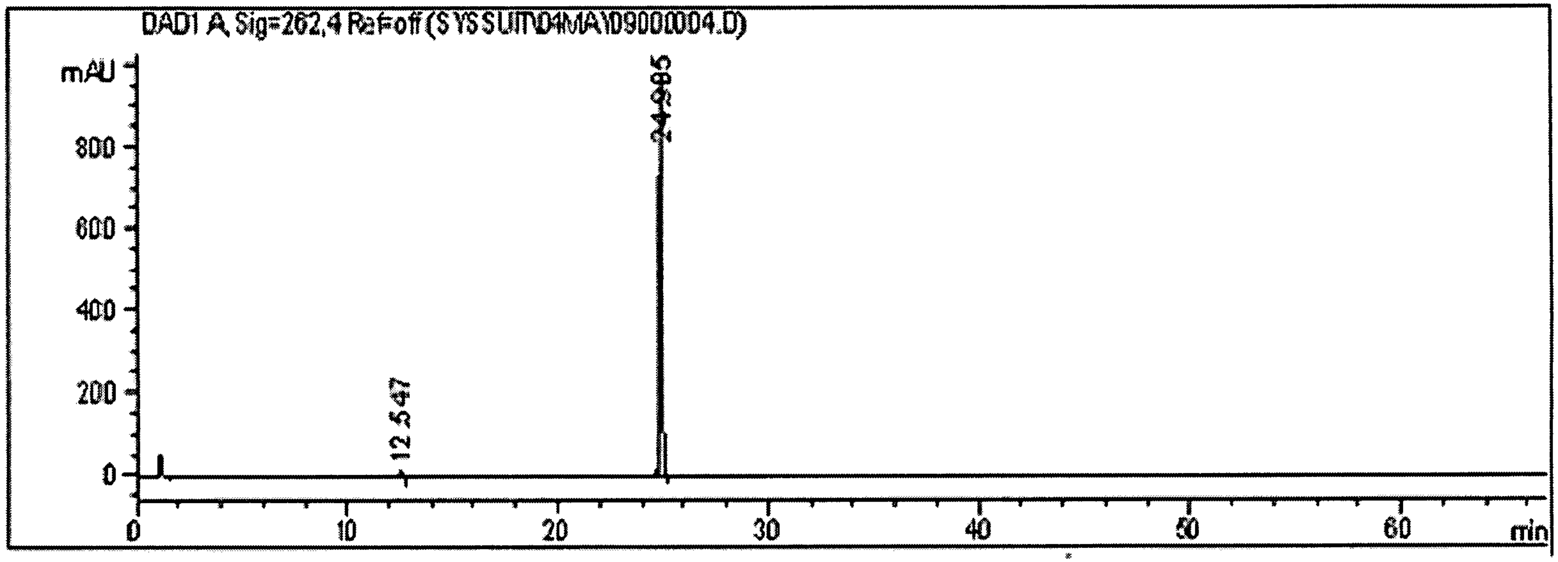


```
Signal 1: DAD1 A, Sig=262,4 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
   1   3.481 BB    0.0716   22.18937    4.65495   0.1952
   2   3.697 BB    0.0737    8.99866    1.78847   0.0792
   3   7.259 BB    0.0819 1.13222e4 2167.47046  99.5999
   4   8.378 BB    0.0794   14.29602    2.80694   0.1258

Totals :                  1.13677e4 2176.72082
```

FIG 19

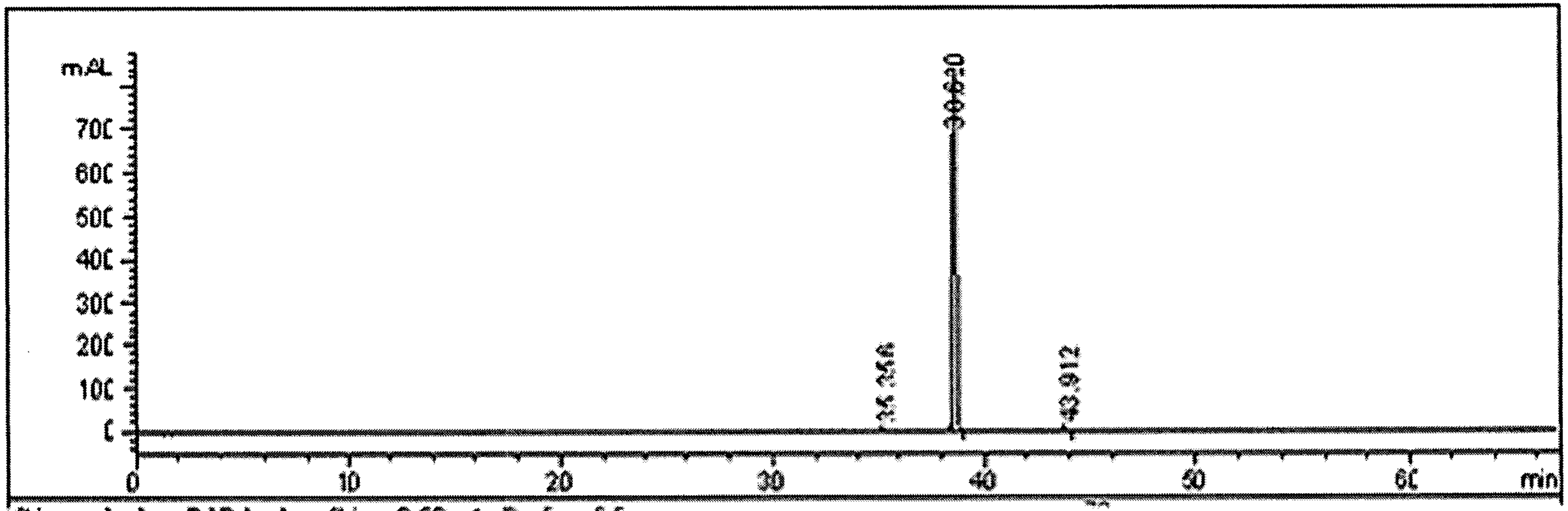


```
Signal 1: DAD1 A, Sig=262,4 Ref=off

Peak RetTime Type  Width     Area       Height     Area
  #   [min]        [min]   [mAU*s]      [mAU]        %
----|-------|----|-------|----------|----------|--------|
   1  12.547 BB    0.0707   47.63638   10.35634   0.7740
   2  24.905 BB    0.0968 6106.68750  978.38525  99.2260

Totals :                  6154.32388  988.74159
```

FIG 20

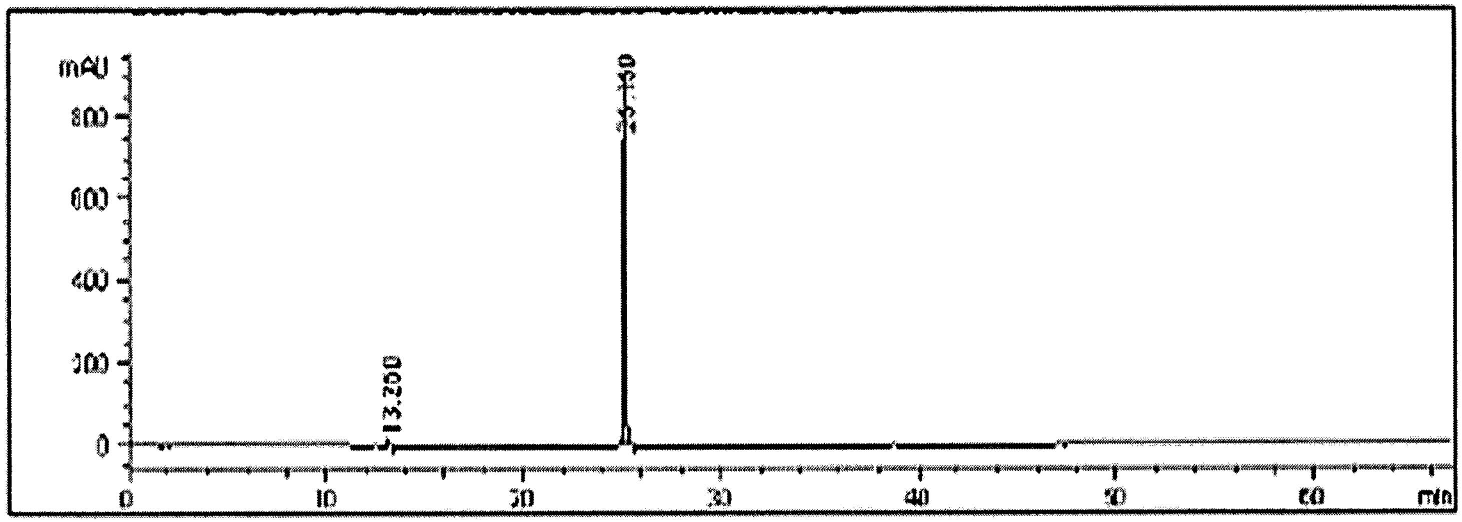


```
Signal 1: DAD1 A, Sig=262,4 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
   1  35.356 BB    0.1718   53.40126    3.69185   0.6608
   2  38.620 BB    0.1487 8009.47559  844.10809  99.1072
   3  43.912 BB    0.1358   18.75134    2.08756   0.2320

Totals :                  8081.62819  849.88751
```

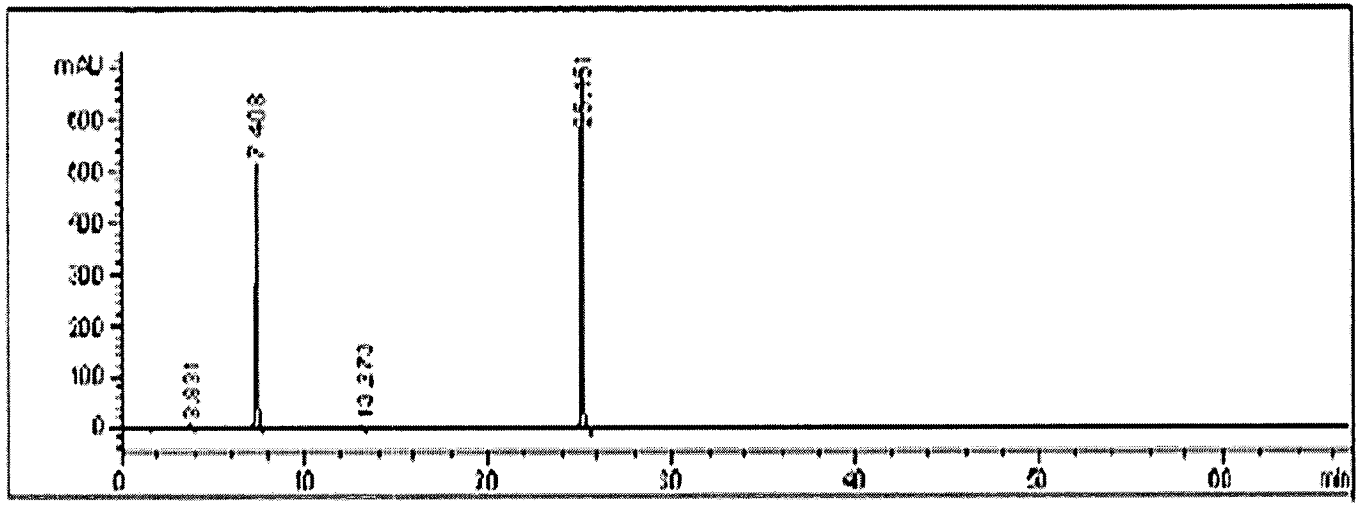
FIG 21



```
Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
   1  13.260 BB    0.0674   20.46399    4.73806   0.3750
   2  25.150 BB    0.0922 5437.15723  916.48595  99.6250

Totals :                  5457.62121  921.22342
```

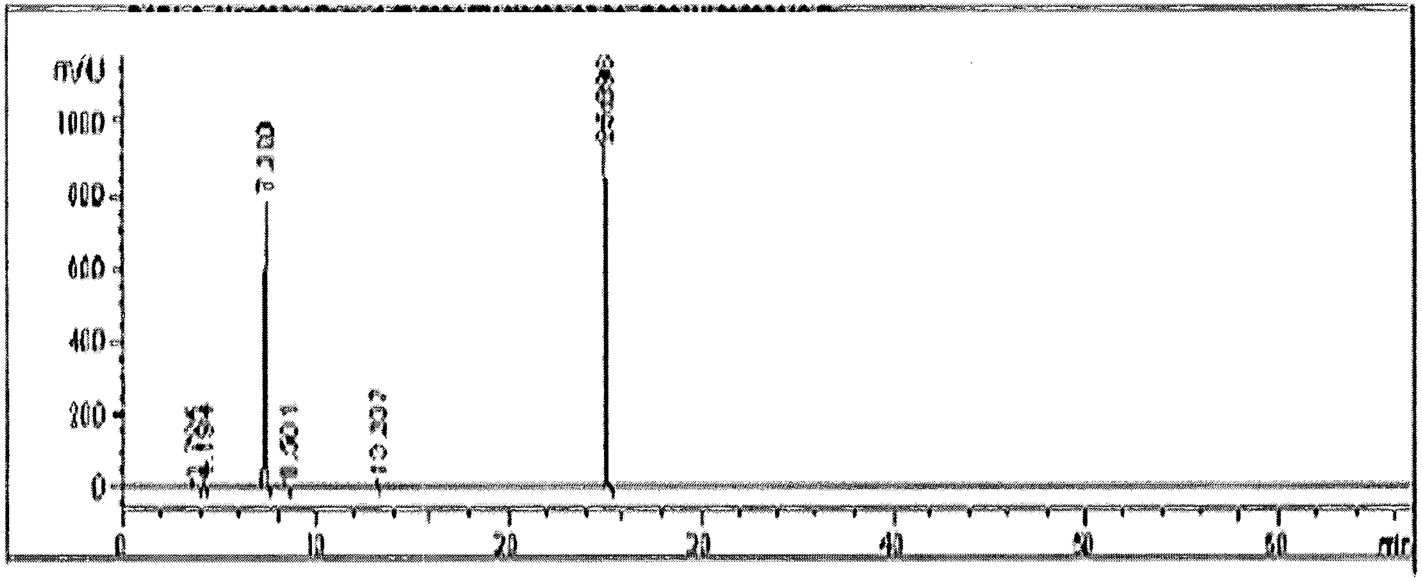
FIG 22



```
Peak RetTime Type  Width     Area       Height     Area
 #    [min]        [min]   [mAU*s]      [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   3.631 BB   0.0892     6.70370    1.16448   0.0846
   2   7.408 BB   0.1010  3748.38672  514.69763  47.3073
   3  13.273 BB   0.0679    17.68693    4.05254   0.2232
   4  25.151 BB   0.0920  4150.70752  702.15118  52.3849

Totals :                  7923.48487 1222.06584
```

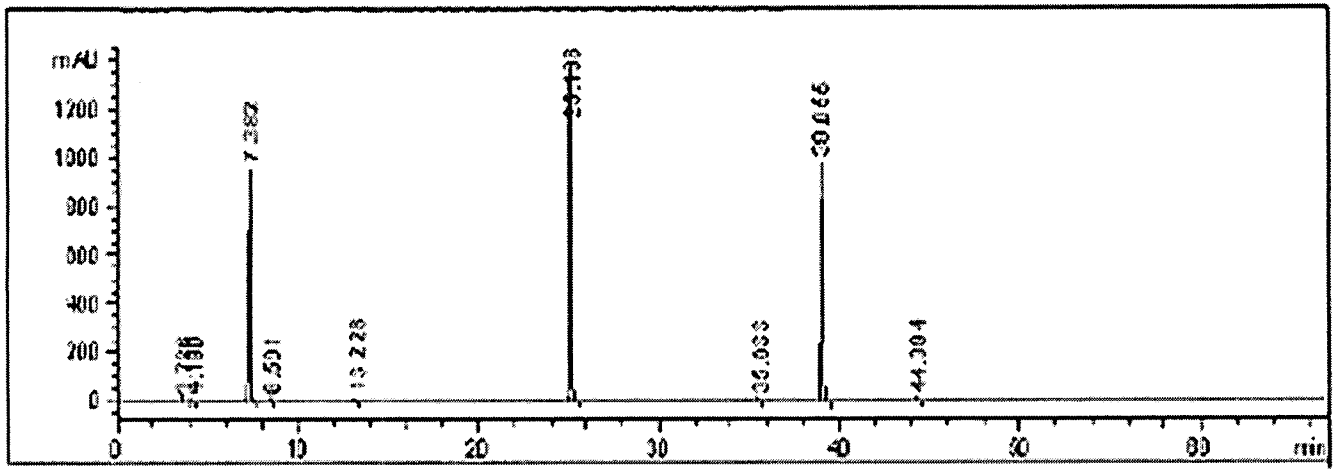
FIG 23



```
Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   3.785 BB    0.0894   10.10649    1.72408   0.0811
   2   4.194 BB    0.0773    6.02177    1.12682   0.0483
   3   7.380 BB    0.0986 5558.33987  785.35278  44.5765
   4   8.501 BB    0.0810    6.92383    1.36892   0.0555
   5  13.237 BB    0.0672   32.56467    7.57127   0.2613
   6  25.096 BB    0.0946 6855.23633 1133.48879  54.9773

Totals :                  1.24692e4  1930.62667
```

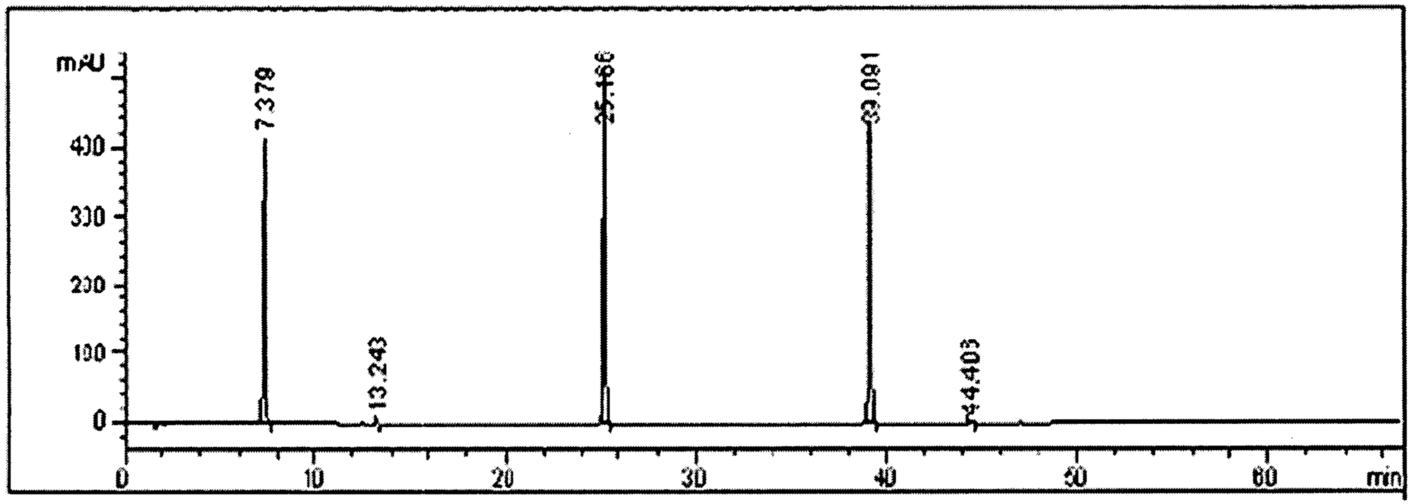
FIG 24



```
Peak RetTime Type  Width     Area       Height     Area
  #   [min]        [min]   [mAU*s]      [mAU]       %
----|-------|----|-------|----------|----------|--------|
  1   3.786 BB   0.0998   12.58698    1.91193   0.0517
  2   4.190 BB   0.0836    7.28159    1.23877   0.0299
  3   7.382 BB   0.1000 6913.70557  960.10931  28.3736
  4   8.501 BB   0.0797    8.55316    1.69904   0.0351
  5  13.228 BB   0.0674   61.51737   14.23254   0.2525
  6  25.138 BB   0.0941 8363.74902 1392.85522  34.3246
  7  35.633 BV   0.1069    7.90763    1.15460   0.0325
  8  39.055 BB   0.1437 8970.14844  980.80731  36.8133
  9  44.394 BB   0.1305   21.20167    2.48490   0.0870

Totals :                2.43667e4  3356.49363
```

FIG 25



```
Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]        %
----|-------|----|-------|----------|----------|--------|
   1   7.379 BB    0.0994 2976.03979  416.14221  29.9159
   2  13.243 BB    0.0664   24.56032    5.68232   0.2469
   3  25.166 BB    0.0919 3071.88916  520.36066  30.8794
   4  39.091 BB    0.1390 3867.06543  437.86914  38.8727
   5  44.406 BB    0.1272    8.45678    1.00494   0.0850

Totals :                  9948.01149 1381.05926
```

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2010/058146

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D473/34 A61K31/685 C07C51/41 C07F9/6512 C07F9/6561
A61P31/00 A61P43/00
ADD.

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 C07C C07F

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, BIOSIS, CHEM ABS Data, EMBASE, WPI Data, BEILSTEIN Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6 043 230 A (ARIMILLI MURTY N [US] ET AL) 28 March 2000 (2000-03-28) cited in the application	1,6-11
A	column 3, lines 51,64; claims; example 1	2-5
A	WO 2007/013086 A1 (HETERO DRUGS LTD [IN]; PARTHASARADHI REDDY BANDI [IN]; RATHNAKAR REDDY) 1 February 2007 (2007-02-01) claims; examples	1-11
X,P	WO 2009/074351 A2 (ULTIMORPHIX TECHNOLOGIES B V [NL]; DOVA EVANTHIA [NL]) 18 June 2009 (2009-06-18) page 44; claims; examples	1-11

☐ 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
"E" earlier document but published on or after the international filing date
"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 1 September 2010	Date of mailing of the international search report 22/09/2010
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Härtinger, Stefan

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2010/058146

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 6043230 A	28-03-2000	US 5977089 A US 5922695 A	02-11-1999 13-07-1999
WO 2007013086 A1	01-02-2007	NONE	
WO 2009074351 A2	18-06-2009	AU 2008334924 A1 CA 2709219 A1 EP 2220098 A2	18-06-2009 18-06-2009 25-08-2010