Title: AMORPHOUS MIXTURE OF LOPINAVIR AND RITONAVIR CO-PRECIPITATED ON COPovidONE

Abstract: The present invention relates to amorphous mixture of lopinavir and ritonavir co-precipitated on copovidone, process for its preparation and pharmaceutical compositions comprising it.
AMORPHOUS MIXTURE OF LOPINAVIR AND RITONAVIR CO-
PRECIPITATED ON COPOVIDONE

This application claims the benefit of Indian Provisional patent Application No. 2828/CHE/2011, filed on Aug 18, 2011, which is incorporated herein by reference.

Filed of the Invention

The present invention relates to amorphous mixture of lopinavir and ritonavir co-
precipitated on copovidone, process for its preparation and pharmaceutical compositions
comprising it.

Background of the Invention

Inhibitors of human immunodeficiency virus (HIV) protease have been approved
for use in the treatment of HIV infection for several years. A particularly effective HIV
protease inhibitor was (2S,3S,5S)-2-(2,6-dimethylphenoxyacetyl)amino-3-hydroxy-5-(2-
l-tetrahydropyrimid-2-onyl)-3-methylbutanoyl)amino-1,6-diphenylhexane, also known
as lopinavir.

Lopinavir was known to have ability of inhibiting HIV protease and the HIV
infection. Lopinavir was particular effective for the inhibition of HIV protease and for the
inhibition of HIV infection when co-administered with Ritonavir.

The combination of lopinavir and ritonavir is marketed in the dosage strength
133.3:33.3; 80:20; 100:25; and 200:50 under the brand name of KALETRA®.

Ritonavir was chemically, (5S,8S,10S,11S)-10-Hydroxy-2-methyl-5-(1-
methylethyl)-1-[2-(1-methylethyl)-4-thiazolyl]-3,6-dioxo-8,1 1-bis(phenylmethyl)-
2,4,7,12-tetraazatridecan-13-oic acid 5-thiazolylmethyl ester.

Lopinavir and its process were disclosed in U.S. patent no. 5,914,332. According
to the patent, amorphous lopinavir can be prepared by dissolving lopinavir in a solvent
such as absolute ethanol, isopropanol, acetone or acetonitrile and then adding the solution
to water.

Ritonavir and its process were disclosed in U.S. patent no. 5,541,206.

Polymorphism is defined as "the ability of a substance to exist as two or more
crystalline phases that have different arrangement and/or conformations of the molecules
in the crystal Lattice. Thus, in the strict sense, polymorphs are different crystalline structures of the same pure substance in which the molecules have different arrangements and/or different configurations of the molecules". Different polymorphs may differ in their physical properties such as melting point, solubility, X-ray diffraction patterns, etc. Although those differences disappear once the compound is dissolved, they can appreciably influence pharmaceutically relevant properties of the solid form, such as handling properties, dissolution rate and stability. Such properties can significantly influence the processing, shelf life, and commercial acceptance of a polymorph. It is therefore important to investigate all solid forms of a drug, including all polymorphic forms, and to determine the stability, dissolution and flow properties of each polymorphic form. Polymorphic forms of a compound can be distinguished in the laboratory by analytical methods such as X-ray diffraction (XRD), Differential Scanning Calorimetry (DSC) and Infrared spectrometry (IR).

Solvent medium and mode of crystallization play very important role in obtaining one polymorphic Form over the other.

A mixture of lopinavir and ritonavir can exist in different polymorphic Forms, which may differ from each other in terms of stability, physical properties, spectral data and methods of preparation.

PCT Publication No. WO 2001/74787 described various polymorphic Forms of lopinavir and processes for their preparation. The Publication described the formation of several polymorphic Forms of lopinavir, which were designated lopinavir crystal Form of Type I hydrated, Type I higher hydrated, Type II isopropanol hemisolvate, Type II isopropanol solvate, Type II ethyl acetate hemisolvate, Type II ethyl acetate solvate, Type II chloroform hemisolvate, Type III ethyl acetate solvated, Type III de-solvated and Type IV non-solvated.

A process for the preparation of lopinavir amorphous Form was disclosed in PCT publication nos. WO 2009/004653 and WO 2009/019661.

PCT publication no. WO 2010/089753 disclosed a de-solvated crystalline Form HI and cyclohexane solvate Form of lopinavir.
An unpublished application, IN 303/CHE/2011 assigned to Hetero research foundation discloses a process for the preparation of lopinavir amorphous Form, lopinavir de-solvated crystalline Form H2 and lopinavir de-solvated crystalline Form H3.

An unpublished application, IN 665/CHE/2011 assigned to Hetero research foundation discloses a novel amorphous Form of lopinavir and ritonavir mixture.

Crystalline Form II of ritonavir was disclosed in U.S. patent no. 6,894,171. According to the patent also described crystalline form I of ritonavir.

U.S. patent no. 7,205,413 disclosed crystalline Form III, Form IV and Form V of ritonavir.

U.S. patent no. 7,148,359 disclosed a substantially pure amorphous ritonavir.

Process for the preparation of substantially pure amorphous ritonavir was disclosed in U.S. patent no. 7,183,416. According to the patent, substantially pure amorphous ritonavir can be prepared by adding a solution of ritonavir containing methanol or methylene chloride to an anti-solvent such as hexane or methyl t-butyl ether and isolating.

Process for the preparation of amorphous ritonavir was described in Journal of Pharmaceutical Sciences, Vol. 90, No. 8, P. 1015-1025 by heating the ritonavir to 135°C in oil bath, followed by rapid cooling using liquid nitrogen or cold water.

Process for the preparation of amorphous ritonavir was described in Journal of Pharmaceutical Sciences, Vol. 91, No. 8, P. 1863-1872 by freeze-drying.

U.S. patent no. 5,559,158 disclosed a solid pharmaceutical composition of ritonavir having the composition is encapsulated in a hard gelatin capsule.

U.S. patent no. 5,948,436 disclosed pharmaceutical composition comprising a solution of ritonavir having the solution is encapsulated in a hard gelatin capsule or a soft elastic gelatin capsule.

U.S. patent no. 7,364,752 disclosed compositions of ritonavir prepared by solid dispersion technique.

Preparation of amorphous Form of ritonavir was described in Journal of the American association of pharmaceutical scientists, Vol. 13, No. 9, P. 7473-7476, September 1996 by spray drying of the ritonavir on polyvinylpyrillodone (PVP) and...
ethanol. Similarly, preparation of amorphous Form of ritonavir was described by layering of the ritonavir on microcrystalline cellulose and silicon dioxide.

It was observed that the amorphous material obtained by the process described in U.S. patent no. 7,181,416 was changed into molten state within about 30 minutes. Thus, the amorphous form obtained by the process of U.S. patent no. 7,181,416 was shown to be unstable.

The amorphous mixture of lopinavir and ritonavir co-precipitated on copovidone has been found to be stable over the time and reproducible and so, suitable for pharmaceutical preparations.

Thus, an object of the present invention is to provide amorphous mixture of lopinavir and ritonavir co-precipitated on copovidone, process for its preparation and pharmaceutical compositions comprising it.

**Summary of the Invention**

In one aspect, the present invention provides amorphous mixture of lopinavir and ritonavir co-precipitated on copovidone.

In another aspect, the present invention provides a process for the preparation of amorphous mixture of lopinavir and ritonavir co-precipitated on copovidone, which comprises:

i) dissolving a mixture of lopinavir, ritonavir, copovidone, aerosil and span-20 in an alcoholic solvent; and

ii) removing the solvent by drying at about 50 to 80°C to obtain amorphous mixture of lopinavir and ritonavir co-precipitated on copovidone.

Yet in another aspect, the present invention provides a pharmaceutical composition comprising amorphous mixture of lopinavir and ritonavir co-precipitated on copovidone and pharmaceutically acceptable excipients.

**Brief Description of the Drawings**

Figure 1 is an X-ray powder diffraction spectrum of amorphous mixture of lopinavir and ritonavir co-precipitated on copovidone.
X-ray powder diffraction spectrum was measured on a bruker axs D8 advance X-ray powder diffractometer having a copper-Kα radiation. Approximately 1 gm of sample was gently flattered on a sample holder and scanned from 2 to 50 degrees two-theta, at 0.019 degrees two theta per step and a step time of 119 seconds. The sample was simply placed on the sample holder. The sample was rotated at 30 rpm at a voltage 40 KV and current 35 mA.

**Detailed Description of the Invention**

The term "room temperature" refers to temperature at about 25 to 35°C.

According to one aspect of the present invention, there is provided amorphous mixture of lopinavir and ritonavir co-precipitated on copovidone. The powdered x-ray diffractogram (PXRD) of amorphous mixture of lopinavir and ritonavir co-precipitated on copovidone is shown in figure 1.

Amorphous mixture of lopinavir and ritonavir co-precipitated on copovidone is found to be stable.

According to another aspect of the present invention, there is provided a process for the preparation of amorphous mixture of lopinavir and ritonavir co-precipitated on copovidone, which comprises:

i) dissolving a mixture of lopinavir, ritonavir, copovidone, aerosol and span-20 in an alcoholic solvent; and

ii) removing the solvent by drying at about 50 to 80°C to obtain amorphous mixture of lopinavir and ritonavir co-precipitated on copovidone.

Lopinavir and ritonavir used in step (a) may be any known crystalline or amorphous Forms.

The alcoholic solvent used in step (a) may preferably be a solvent or a mixture of solvents selected from methanol, ethanol, isopropyl alcohol and n-butanol, and more preferably the alcoholic solvent is ethanol.

The dissolution in step (a) may be performed, for example, by heating the mixture of lopinavir, ritonavir and copovidone in the solvent.

Drying in step (b) may preferably be carried out at about 60 to 70°C under high vacuum.
According to another aspect of the present invention, there is provided a pharmaceutical composition comprising amorphous mixture of lopinavir and ritonavir co-precipitated on copovidone and pharmaceutically acceptable excipients, and optionally other therapeutic ingredients. The amorphous mixture of lopinavir and ritonavir co-precipitated on copovidone may preferably be formulated into tablets, capsules, suspensions, dispersions, injectables or other pharmaceutical forms.

The invention will now be further described by the following examples, which are illustrative rather than limiting.

**Examples**

**Example 1:**

Preparation of amorphous mixture of lopinavir and ritonavir co-precipitated on copovidone

A mixture of lopinavir de-solvated crystalline Form H1 (75 gm), ritonavir crystalline Form I (18.75 gm), copovidone (5.68 gm), aerosil (0.0066 gm) and span-20 (0.55 gm) was dissolved in ethanol (400 ml) under stirring at room temperature. The solution was then heated to 40 to 45°C to obtain a clear solution. The resulting solution was subjected to dry under high vacuum at 60 to 65°C for 10 hours to obtain amorphous mixture of lopinavir and ritonavir co-precipitated on copovidone.

**Example 2:**

Preparation of amorphous mixture of lopinavir and ritonavir co-precipitated on copovidone

Example 1 was repeated using ritonavir crystalline Form II instead of ritonavir crystalline Form I to obtain amorphous mixture of lopinavir and ritonavir co-precipitated on copovidone.

**Example 3:**

Preparation of amorphous mixture of lopinavir and ritonavir co-precipitated on copovidone

Example 1 was repeated using methanol solvent instead of ethanol solvent to obtain amorphous mixture of lopinavir and ritonavir co-precipitated on copovidone.
Example 4:
Preparation of amorphous mixture of lopinavir and ritonavir co-precipitated on copovidone

Example 1 was repeated using lopinavir de-solvated crystalline Form H2 instead of lopinavir de-solvated crystalline Form H1 to obtain amorphous mixture of lopinavir and ritonavir co-precipitated on copovidone.

Example 5:
Preparation of amorphous mixture of lopinavir and ritonavir co-precipitated on copovidone

Example 1 was repeated using lopinavir de-solvated crystalline Form H3 instead of lopinavir de-solvated crystalline Form H1 to obtain amorphous mixture of lopinavir and ritonavir co-precipitated on copovidone.

Example 6:
Preparation of amorphous mixture of lopinavir and ritonavir co-precipitated on copovidone

Example 1 was repeated using lopinavir type I hydrated instead of lopinavir de-solvated crystalline Form H1 to obtain amorphous mixture of lopinavir and ritonavir co-precipitated on copovidone.

Example 7:
Preparation of amorphous mixture of lopinavir and ritonavir co-precipitated on copovidone

Example 1 was repeated using lopinavir type I higher hydrated instead of lopinavir de-solvated crystalline Form H1 to obtain amorphous mixture of lopinavir and ritonavir co-precipitated on copovidone.

Example 8:
Preparation of amorphous mixture of lopinavir and ritonavir co-precipitated on copovidone
Example 1 was repeated using lopinavir amorphous Form instead of lopinavir desolvated crystalline Form H1 to obtain amorphous mixture of lopinavir and ritonavir co-precipitated on copovidone.

Example 9:

Tablet composition comprising Ritonavir and Lopinavir prepared by hot-melt extrusion method:

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Ingredients</th>
<th>Mg/tablet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Dry mix</strong></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Amorphous mixture of Ritonavir and Lopinavir*</td>
<td>266.6</td>
</tr>
<tr>
<td>2.</td>
<td>Copovidone</td>
<td>827.2</td>
</tr>
<tr>
<td>3.</td>
<td>Colloidal silicon dioxide</td>
<td>12.0</td>
</tr>
<tr>
<td></td>
<td><strong>Addition of surfactant</strong></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Sorbitan monolaurate</td>
<td>83.9</td>
</tr>
<tr>
<td></td>
<td><strong>Lubrication</strong></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Colloidal silicon dioxide</td>
<td>18.0</td>
</tr>
<tr>
<td>6.</td>
<td>Sodium stearyl fumarate</td>
<td>12.3</td>
</tr>
<tr>
<td></td>
<td><strong>Core tablet weight</strong></td>
<td>1220.0</td>
</tr>
<tr>
<td></td>
<td><strong>Film-coating</strong></td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Opadry yellow</td>
<td>30.0</td>
</tr>
<tr>
<td>8.</td>
<td>Purified water</td>
<td>274.5</td>
</tr>
<tr>
<td></td>
<td><strong>Coated tablet weight</strong></td>
<td>1250.0</td>
</tr>
</tbody>
</table>

* Amorphous mixture of lopinavir and ritonavir was prepared as per any of the examples 1-8.

Each 266.6 mg of amorphous mixture of lopinavir and ritonavir contains 200 mg of lopinavir and 50 mg of ritonavir.

**Manufacturing process:**

i) Amorphous mixture of Ritonavir and Lopinavir, copovidone and colloidal silicon dioxide were sifted through mesh # 30.
ii) the sifted material of step no. (i) were loaded into rapid mixer granulator and mixed for 10 minutes,

iii) surfactant was added to the material of step no. (ii) while mixing for 6-7 minutes,

iv) the blend of step no. (iii) was passed through hot melt extruder to form extrudes,

ev) the extrudes of step no. (iv) were milled using pulverizer and the milled extrudes were sifted through mesh # 30,

vi) milled extrudes of step no. (v) were lubricated with colloidal silicon dioxide and sodium stearyl fumarate and finally compressed into tablets and

vii) the tablets of step no. (vi) were film coated using opadry yellow.

Example 10:
Tablet composition comprising Ritonavir and Lopinavir prepared by hot-melt extrusion method:

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Ingredients</th>
<th>Mg/ tablet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry mix</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Amorphous mixture of Ritonavir and Lopinavir</td>
<td>133.3</td>
</tr>
<tr>
<td>2.</td>
<td>Copovidone</td>
<td>413.6</td>
</tr>
<tr>
<td>3.</td>
<td>Colloidal silicon dioxide</td>
<td>6.0</td>
</tr>
<tr>
<td>Addition of surfactant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Sorbitan monolaurate</td>
<td>42.0</td>
</tr>
<tr>
<td>Lubrication</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Colloidal silicon dioxide</td>
<td>9.0</td>
</tr>
<tr>
<td>6.</td>
<td>Sodium stearyl fumarate</td>
<td>6.1</td>
</tr>
<tr>
<td>Core tablet weight</td>
<td>610.0</td>
<td></td>
</tr>
<tr>
<td>Film-coating</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Opadry yellow</td>
<td>15.0</td>
</tr>
<tr>
<td>8.</td>
<td>Purified water</td>
<td>137.0</td>
</tr>
</tbody>
</table>

Coated tablet weight 625.0
mixture of Ritonavir and Lopinavir was prepared as per any of the examples 1-7.
Each 133.3mg of ηοφ ηοφ mixture of Ritonavir and Lopinavir contains 100mg lopinavir and 25mg Ritonavir.

Manufacturing process:

i) Amorphous mixture of Ritonavir and Lopinavir, copovidone and colloidal silicon dioxide were sifted through mesh # 30,
ii) the sifted material of step no. (i) were loaded into rapid mixer granulator and mixed for 10 minutes,
iii) surfactant was added to the material of step no. (ii) while mixing for 6-7 minutes,
iv) the blend of step no. (iii) was passed through hot melt extruder to form extrudes,
v) the extrudes of step no. (iv) were milled using pulverizer and the milled extrudes were sifted through mesh # 30,
vi) milled extrudes of step no. (v) were lubricated with colloidal silicon dioxide and sodium stearyl fumarate and finally compressed into tablets and
vii) the tablets of step no. (vi) were film coated using opadry yellow.
We claim:

1. Amorphous mixture of lopinavir and ritonavir co-precipitated on copovidone.

2. The amorphous mixture of lopinavir and ritonavir co-precipitated on copovidone of claim 1, having a powder X-ray diffractogram as shown in figure 1.

3. A process for the preparation of amorphous mixture of lopinavir and ritonavir co-precipitated on copovidone, which comprises:
   i) dissolving a mixture of lopinavir, ritonavir, copovidone, aerosol and span-20 in an alcoholic solvent; and
   ii) removing the solvent by drying at about 50 to 80°C to obtain amorphous mixture of lopinavir and ritonavir co-precipitated on copovidone.

4. The process as claimed in claim 3, wherein the alcoholic solvent used in step (a) is a solvent or a mixture of solvents selected from methanol, ethanol, isopropyl alcohol and n-butanol.

5. The process as claimed in claim 4, wherein the alcoholic solvent is ethanol.

6. The process as claimed in claim 3, wherein the drying in step (b) is carried out at about 60 to 70°C under high vacuum.

7. A pharmaceutical composition comprising amorphous Form of a mixture of lopinavir and ritonavir co-precipitated on copovidone and pharmaceutically acceptable excipients, and optionally other therapeutic ingredients.

8. A pharmaceutical composition comprising i) amorphous mixture of lopinavir and ritonavir co-precipitated on copovidone, ii) a water-soluble polymer and iii) a surfactant wherein the composition is prepared by hot melt extrusion method.

9. The pharmaceutical composition according to claim 8, selected from a tablet, a capsule and a granule.

10. A process for preparing compositions of amorphous mixture of lopinavir and ritonavir co-precipitated on copovidone by hot melt extrusion method involves:
   i) sifting and blending of amorphous mixture of lopinavir and ritonavir co-precipitated on copovidone, water soluble polymer and one or more pharmaceutically acceptable excipients to form a dry mix,
   ii) blending the dry mix of step no. (i) with surfactant,
iii) passing the material of step no. (ii) through hot melt extruder to form extrudes followed by milling and sifting and,
iv) blending the milled extrudes of step no. (iii) with remaining portion of excipients and finally compressing into tablets.

11. The pharmaceutical composition according to claim 8 and 10, wherein said water-soluble polymer is selected from copovidone and polyethylene oxide and said surfactant is selected from sorbitan monolaurate and polyoxyl 35 castor oil.