Abstract:
The present invention relates to a novel form of pravastatin, notably pravastatin sodium, and a method for the preparation of a novel form of pravastatin.
Field of the invention

The present invention relates to a novel form of pravastatin, notably pravastatin sodium, and a method for the preparation of a novel form of pravastatin.

Background of the invention

Pravastatin is an inhibitor of the enzyme (3S)-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. The sodium salt of pravastatin, pravastatin sodium (1) is a potent cholesterol-lowering agent which is commercially available for the treatment of hyperlipidemia. Pravastatin can be prepared by microbial oxidation of the fermentatively obtained precursor compactin, the sodium salt of which has structure (2), such as described for instance in US 4,346,227. Recently, an improved approach has been described in WO 2007/147827 in which pravastatin is prepared directly in a host cell equipped with genes for compactin biosynthesis and a gene for conversion of compactin into pravastatin.

Despite the progress made thus far in pravastatin production processes, there still remain disadvantages that need to be solved. One such disadvantage of the prior art methods is that during work-up procedures unwanted loss of product occurs as a result of lactonization leading to pravastatin lactone (3). The latter process can occur as a result of certain pH-regimes, elevated temperatures, the presence of traces of other
molecules or combinations of any or all of these circumstances. Any breakthrough approach omitting or suppressing lactonization is therefore of great importance, at least for improving yields but also in order to achieve process simplification, omission of purification steps and/or improvement of product purity.

Many of the approaches used in the art arrive at one or more points in the downstream processing sequence at a point at which a solution comprising pravastatin needs to be treated at elevated temperatures, for instance in order to concentrate and/or in order to eliminate traces of water. The latter usually is quite important when having to crystallize pravastatin sodium from an organic solvent. It is well known that the presence of water in such solutions can lead to impure product and low yields. In the worst case, the presence of certain amounts of water can even be detrimental to the crystallization process to an extent that crystal formation does not occur at all.

**Detailed description of the invention**

Pravastatin sodium and hydrates thereof can exist in several polymorphic forms. Polymorphism is the property to assume more than one crystalline form in the solid state whereas each polymorph can assume distinct physical properties. One of the most widely accepted tools for investigating polymorphic structure is X-ray diffraction, a technique whereby the angles at which X-ray radiation is reflected from a crystal is a measure for a specific crystalline structure. Because of the different orientation and intermolecular interactions of adjacent molecules (complexes) in the bulk solid, different polymorphs have different physical properties and are therefore distinct solids sharing only the same molecular formula but displaying different advantageous and/or disadvantageous physical properties. Some polymorphs of pravastatin sodium are known and have been described as obtainable from a process wherein both an aprotic and a protic solvent are used, for example in US 7,001,919 (EP 1523978) and references cited therein.

In a first aspect, the present invention discloses a form of pravastatin sodium that is hitherto unknown. Noteably, the pravastatin sodium or hydrates thereof of the present invention has an X-ray powder diffraction pattern characterized in that it
comprises peaks at 4.52 ± 0.1, 4.90 ± 0.1, 9.12 ± 0.1, and 9.87 ± 0.1 degrees measured at reflection angle 2 theta. Preferably, the intensity of any of the aforementioned peaks (in cps) is at least 10% of the intensity of the peak with the highest intensity. More preferably, the intensity of the peaks in the region from 5 deg. 2-theta to 9 deg. 2-theta, an area wherein prior art crystalline pravastatin forms show significant peaks, is below 4% of the intensity of the peak with the highest intensity. Smaller but distinct peaks are also present at 10.32 ± 0.1 and 13.71 ± 0.1 degrees measured at reflection angle 2-theta. For reasons of reproducibility and/or confirmation by the skilled person, the X-ray diffraction patterns mentioned above are, in a second embodiment, measured in the presence of an internal standard which preferably is silicon.

The pravastatin sodium of the present invention can be obtained from the process as described in the second aspect wherein only aprotic solvents are used. Advantageously, the product is of high purity resulting from the fact that unwanted lactonization occurs only at very low levels and hence pravastatin lactone (3) is not present, or present in very low levels, as contaminant in the final product.

In a second aspect of the present invention there is disclosed a process for the preparation of pravastatin sodium and hydrates thereof comprising the steps of:

(a) Mixing an aqueous solution of pravastatin or a salt thereof with a first organic solvent at pH 3 to 5 and separating the phases;
(b) Adding a second organic solvent to the organic phase obtained after step (a);
(c) Adding a sodium salt to the mixture obtained in step (b);
(d) Isolating the solids from the mixture obtained in step (c),

wherein said first organic solvent is a ketone with 5 or more carbon atoms such as methyl isobutyl ketone or an ester such as ethyl acetate or methyl acetate and said second organic solvent is a ketone with less than 5 carbon atoms such as acetone or 2-butane. Preferably said first organic solvent is methyl isobutyl ketone and said second organic solvent is acetone. It is noted that when crystallizations are carried out without the use of the second solvent this will lead to the formation of oily substances without any crystal formation. It has been established that distillation can circumvent this problem, perhaps because distilling will remove water remaining from the phase
separation step. Unfortunately distillation also implies elevated temperatures and/or prolonged residence times. Both phenomena promote unwanted lactonization.

From US 7,001,919 it becomes apparent that the choice of solvents for crystal formation is not a simple and obvious one. The rationale behind the relation between the formation of a certain polymorph and the solvent used for crystallization is poorly understood. This is even more valid for mixtures of solvents. The formation of a certain crystalline form is highly dependent upon control of conditions such as the composition of the solvent system employed, the pH of the solvent system and the temperature. Thus, US 7,001,919 teaches that pravastatin sodium Forms A through F may be obtained by re-crystallization from solvent systems having a protic component and an aprotic component. Pravastatin sodium crystallizes as Forms A, B and E from solvent systems having a protic component that is either ethanol or an ethanol/water mixture whereas Forms C, D and F are obtained from solvent systems that have a protic component that is water alone. There is however no indication towards the solvent system of the present invention.

It has been found that the ratio between the first organic solvent and the second organic solvent preferably is between 10:1 to 1:1, more preferably between 5:1 to 2:1. The exact optimum may vary from one solvent mixture to the other. For instance when said first organic solvent is methyl isobutyl ketone and said second organic solvent is acetone said ratio preferably is between 4:1 to 3:2, more preferably from 7:2 to 2:1.

The concentration of pravastatin in the first organic solvent preferably is from 10 g/L to 100 g/L, more preferably from 20 g/L to 80 g/L. Best results were obtained with a concentration from 40 g/L to 65 g/L both from process economics and the finding that at higher concentrations unwanted coloring occurs.

The particular choice of solvents in the process of the second aspect of the invention is surprising. First of all because it was not to be expected from any prior art teaching that such a mixture would lead to a new form of pravastatin sodium, but secondly also because the particular solvent mixtures as proposed are well suited to crystallize without having to perform distillation steps or remove water through other mechanical or chemical approaches.

In a preferred embodiment the final step (d) is followed by a washing step which is followed by a drying step. Washing is preferably carried out using the same second solvent or a mixture of the first and second solvent. This approach will greatly facilitate
solvent recovery and process economics. Nevertheless, alternative solvents used for washing and known to the skilled person may also be employed.

Preferably, a temperature of about 0°C or higher is applied, more preferably a temperature close to ambient temperature (18-25°C i.e. around 20°C). The fact that the process of the present invention may be employed at ambient temperature of course is advantageous as it avoids the use of cooling equipment and cooling materials, conditions that are usually advocated in the prior art. For drying the final product, suitable temperatures are from 25-70°C, preferably from 30-50°C.

The pH-value at which step (a) as described above takes place is from 3 to 5, preferably from 3.5 to 4.5. Depending on the pH-value of the starting aqueous solution comprising pravastatin, pH-adjusted is to be performed using acid or base. In most cases this will be acid such as formic acid, hydrochloric acid, phosphoric acid, sulphuric acid and the like.

Suitable sodium salts for use in step (c) are sodium carbonate, sodium chloride, sodium ethyl hexanoate, sodium hydroxide, sodium nitrate, sodium phosphate and the like. In one embodiment sodium carbonate was successfully replaced or partly replaced with sodium ethyl hexanoate resulting in a lower chance of unwanted inclusion of sodium carbonate particles in the end product. Addition times of sodium carbonate may be from instantaneous to 24 hours; favourable results, i.e. lack of inclusion of sodium carbonate, were observed when addition to place between 10 min and 2 hours.

**Legend to the Figure**

Figure 1 is the XRD spectrum of compound (1). X-axis: 2-theta value (deg). Y-axis: intensity (cps). The following distinct peaks can be discerned:
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<tr>
<th>Peak no.</th>
<th>2-Theta (deg)</th>
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<tbody>
<tr>
<td>1</td>
<td>4.517</td>
</tr>
<tr>
<td>2</td>
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<td>3</td>
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<td>10</td>
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The dotted lines (marked with ■) are the expected positions of the internal calibration standard silicon.
XRD measurements

Powders were used without grinding to minimize water uptake. X-ray diffraction experiments were performed using a BRUKER D8 Advance diffractometer in $\Theta-\Theta$ geometry using a VANTEC line detector; a series of data sets were recorded from each sample to monitor potential changes due to humidity.

Measurements of the powders had been performed at least four times. A first screening was performed using relatively short data collection times in order to gain a rapid overview on the initial diffraction patterns. In a second step a much longer experiment has been performed in order to improve data quality (reliability of rather weak diffraction peaks). From changes among the latter it could be concluded whether and to which extend data acquisition times needed to be adopted in order to minimize changes due to exposure of the samples to humidity. In a second series of experiments all samples were mixed with Si-powder to provide an internal calibration standard. Based on the known peak positions of Si, a correction of the diffraction patterns according to the known values could be performed, thus providing an accuracy in peak positions of less than 0.1°. The data have been corrected for background signal arising from sample holder and peak positions have been assigned via the peak search routine implemented in the data evaluation software EVA. 2$\Theta$ values below 4° were not measured.

HPLC analysis

Pravastatin samples were analyzed using the following HPLC-method.

- Mobile phase A = 1000 ml MilliQ-water + 1 ml formic acid
- Mobile phase B = 1000 ml Methanol + 1 ml formic acid
- Column = Chromolith SpeedROD RP-18° 50-4.6 mm
- Column temperature = 30°C
- Injection volume = 5 µl
- Injection temperature = 10°C
- Flow = 2.0 ml/min
- Wave length = 238 nm
• Gradient:

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<th>Time (min.)</th>
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<th>Mobile phase B (%)</th>
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</tr>
<tr>
<td>6.5</td>
<td>55</td>
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Under the above conditions the following retention times are observed: 0.585 min (hydrolysed pravastatin); 2.137 min (epi-pravastatin); 2.375 min (3-p-iso-pravastatin); 2.607 min (hydrolysed compactin); 2.883 min (3-a-iso-pravastatin); 3.368 min (pravastatin); 4.138 min (pravastatin lactone).

Example 1

Re-crystallization of pravastatin sodium salt

Pravastatin sodium salt (7.96 g, purity 95.1%; 17 mmol) was dissolved in water (120 mL) and the mixture was extracted at pH 4 with methyl isobutyl ketone (120 mL). Acetone (40 mL) was added to the methyl isobutyl ketone extract. Under diligent stirring 2.85 mL of an aqueous solution of 300 g/L sodium carbonate (8.1 mmol) was added in 1 hour at 20°C. A homogeneous suspension was obtained after 1 hour of stirring. Next 2-ethyl hexanoic acid (0.4 mL, 2.5 mmol) was added and stirring at room temperature was continued overnight. The mixture was filtered and the precipitate was washed with 2x30 mL methyl isobutyl ketone/acetone (3/1, v/v) and dried under vacuum at 50°C for 24 hours, yielding pravastatin sodium.

Example 2

Preparation of pravastatin sodium salt from pravastatin 2,2’-(ethylenedioxy)diethyl amine salt

Pravastatin 2,2’-(ethylenedioxy)diethyl amine salt (prepared as described in WO 2009/121869, 4.23 g, purity 98.2%; 8.5 mmol pravastatin) was dissolved in water (35 mL). Methyl isobutyl ketone (55 mL) was added and the pH was adjusted to 4 with 10% sulfuric acid. The phases were separated and the methyl isobutyl ketone phase
was washed with water (25 mL). Next the methyl isobutyl ketone phase was filtered through a 0.45 µm filter and the volume of the filtrate was adjusted to 59 mL with methyl isobutyl ketone and the resulting solution was diluted with acetone (20 mL). Under stirring 1.38 mL of an aqueous solution of 300 g/L sodium carbonate (3.9 mmol) was added in portions of 100 µL in 30 minutes. Next 2-ethyl hexanoic acid (0.27 mL, 1.7 mmol) was added and the mixture was stirred at 20°C overnight. The precipitate was filtered off, washed with 2x40 mL methyl isobutyl ketone/acetone (3/1, v/v) and dried under vacuum at 40°C overnight, yielding 3.53 g pravastatin sodium as a white solid having the XRD spectrum of Figure 1.

The experiment was repeated without addition of 2-ethyl hexanoic acid leading to a similar result.

**Example 3**

**Ratio between methyl isobutyl ketone and acetone in the preparation of pravastatin sodium salt**

To an extract of pravastatin in methyl isobutyl ketone (40 g/L, as obtained in Examples 1 or 2) different percentages (2, 5, 10, 20%) of acetone were added followed by the addition of saturated sodium carbonate. With a low percentage of acetone (2 and 5%) only oil was formed. With 10% acetone initially oil was formed, but it changed into a white solid upon stirring. With 20% acetone a white solid was directly formed. Visually the precipitate looked amorphous (under microscope). The precipitate could easily be filtered off. It was washed with acetone and dried under vacuum at 40°C. The amount of lactone formed during the precipitation step was very small (<0.2 %, completely recovered in mother liquor).
CLAIMS

1. Pravastatin sodium and hydrates thereof having an X-ray powder diffraction pattern characterized in that said X-ray powder diffraction pattern comprises peaks at 4.52 ± 0.1, 4.90 ± 0.1, 9.12 ± 0.1, and 9.87 ± 0.1 degrees measured at reflection angle 2-theta wherein the intensity of any of said peaks is at least 10% of the intensity of the peak with the highest intensity.

2. Pravastatin sodium and hydrates thereof according to claim 1 wherein the intensity of the peaks in the region from 5 deg 2-theta to 9 deg 2-theta is below 4% of the intensity of the peak with the highest intensity.

3. Pravastatin sodium and hydrates thereof according to any one of claims 1 to 2 wherein said X-ray powder diffraction pattern further comprises peaks at 10.32 ± 0.1 and 13.71 ± 0.1 degrees measured at reflection angle 2-theta.

4. Pravastatin sodium and hydrates thereof according to anyone of claims 1 to 3 wherein said X-ray powder diffraction pattern is measured in the presence of silicon powder.

5. Process for the preparation of pravastatin sodium and hydrates thereof comprising the steps of:
   (a) Mixing an aqueous solution of pravastatin or a salt thereof with a first organic solvent at pH 3 to 5 and separating the phases;
   (b) Adding a second organic solvent to the organic phase obtained after step (a);
   (c) Adding a sodium salt to the mixture obtained in step (b);
   (d) Isolating the solids from the mixture obtained in step (c), characterized in that said first organic solvent is chosen from the list consisting of methyl isobutyl ketone, ethyl acetate and methyl acetate and said second organic solvent is chosen from the list consisting of acetone and 2-butanone.

6. Process according to claim 5 wherein said first organic solvent is methyl isobutyl ketone and said second organic solvent is acetone.
7. Process according to any one of claims 5 to 6 wherein said solids obtained in step (d) are washed with said second solvent or a mixture of said first solvent and said second solvent and subsequently dried.
**INTERNATIONAL SEARCH REPORT**

**PCT/EP2011/073767**

**A. CLASSIFICATION OF SUBJECT MATTER**

INV. C07C59/11 C07D309/30

**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 C07C

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

Electronic database consulted during the international search (name of database and, where practical, search terms used)

EPO-Internal, CHEM ABS Data, WPI Data

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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<td>20 April 2005 (2005-04-20) page 2, paragraph 6 - paragraph 7 page 4, paragraphs 20, 24 claim 9</td>
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Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:

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- "O" document referring to an oral disclosure, use, exhibition or other means
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- "Z" document member of the same patent family

Date of the actual completion of the international search

15 February 2012

Date of mailing of the international search report

22/02/2012

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Form PCT/ISA/210 (second sheet) (April 2005)
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