ESTETROL POLYMORPHIC FORM AND PRODUCTION THEREOF

FIELD OF THE INVENTION

The present invention relates to a specific polymorphic form of estetrol and production methods thereof.

BACKGROUND OF THE INVENTION

Estrogenic substances are commonly used in methods of Hormone Replacement Therapy (HRT) and methods of female contraception. Estetrol (E4) is a biogenic oestrogen that is endogeneously produced by the foetal liver during human pregnancy. Recently, estetrol has been approved as the estrogenic component in a combined oral contraceptive. Other important applications of estetrol may be the fields of HRT, therapy of auto-immune diseases, prevention and therapy of breast and colon tumours, enhancement of libido, skin care, and wound healing.

An important aspect of the industrial production of estetrol for pharmaceutical applications is the identification and provision of crystalline form(s) of estetrol and solvates thereof. Especially, because different crystal forms may exhibit distinct physical, chemical and pharmacological properties. However, present synthesis processes of estetrol do not allow to obtain a desired polymorphic form with high predictability, yield and/or purity. Typically, the specific polymorphic form is obtained by additional work-up and purification steps to isolate said polymorph from a mixture of different polymorphs, which is time-consuming and labour/resource intensive.

Monari et al. (Crystals 2023, 13, 1211) prepared Estetrol monohydrate powder via precipitation, induced by adding water to a clear solution of Estetrol in a 2-propanol-water mixture at room temperature. The XRPD pattern and detailed solid-state characterization verified that the resulting powder corresponds to the same polymorphic form of Estetrol monohydrate described in WO2015/086643, herein referred to as Form 1. Additionally, Monari et al. obtained single crystals of Estetrol monohydrate through slow evaporation of the solvent from a clear 2-propanol-water solution. The process of growing single crystals requires slow and controlled changes under quasi-stationary conditions, often taking days or weeks to produce a few well-formed crystals from highly dilute solutions with low API content. However, scaling this process to laboratory or commercial levels is impractical due to its lengthy duration, high solvent consumption, and the difficulty of maintaining precise quasi-stationary conditions in larger volumes. Moreover, altering the parameters of this method risks yielding a different polymorphic form. Consequently, single-crystal growth is not considered a commercially viable crystallization technique.

A detailed crystallization process for producing Estetrol monohydrate Form 1, as disclosed in WO2015/086643, is further described in US 2023/287036 A1. This crystalline form can be

obtained from a clear solution in methanol through the addition of water as an antisolvent.

In Example 5 of US 2023/287036 A1, Estetrol monohydrate Form 1 is prepared by means of mixing the solution of Estetrol in methanol with water as antisolvent and eliminating methanol at reduced pressure. The driving force of the process is the high supersaturation created by the addition of water as antisolvent to the clear solution or, in some cases the removal of the methanol as solvent from the clear solution. In either case the process lacks a cooling step for the initiation of crystallization and results in Estetrol monohydrate Form 1.

In view of the above, there exists a need for improved preparation methods to obtain a specific polymorphic form of estetrol with high predictability, yield and/or purity.

SUMMARY OF THE INVENTION

It has now surprisingly been found that some or all of the above demands and objectives can be attained either individually or in any combination by using a process as defined herein. The process of the invention relies on dissolving crude estetrol in a solvent mixture of water and at least one alcohol to obtain a solution, and gradually cooling said solution to obtain crystalline estetrol monohydrate in good yield and with an excellent degree of purity.

In particular, the present inventors have advantageously found that slow cooling of the solution in at least two cooling intervals may improve both the yield and quality of the estetrol monohydrate, while a certain polymorphic form is selectively obtained. In a first cooling step according to the invention, the solution is cooled to a temperature 2. In a second cooling step, the solution is further cooled to a cooling temperature 3 to form a suspension comprising (purified) estetrol monohydrate.

Advantageously, the process of the present invention is characterized in that gradual cooling of the solution comprising dissolved estetrol substantially avoids the formation of undesired polymorphs. Moreover, the optimized crystallization process as described herein allows for the formation of larger crystals, which improves filtration, handling, and downstream processing. In addition, it has been found that the present process is very amenable for industrial adaptation.

Also advantageously, the process of the present invention may avoid the inclusion of undesired impurities or solvents in the crystal lattice, which reduces the need for additional purification steps to meet regulatory requirements. In addition, the process of the present invention provides the selective production of a particular polymorphic form, which is in line with ICH guidelines of the pharmaceutical industry.

A first aspect of the present invention relates to estetrol monohydrate identified by characteristic XRPD peaks at 11.9, 12.2, 12.4, 12.8, 13.4, 19.9, 20.7, 20.8, 21.6, 21.7 and 24.5 °(±0.1°) 2θ using CuKα radiation.

According to a second aspect of the present invention, a process is provided for the production of estetrol monohydrate comprising the steps of:

a) dissolving estetrol in a solvent mixture of water and at least one alcohol;
b) cooling the solution to a cooling temperature 2 to obtain a mixture;
c) maintaining the cooled mixture at the cooling temperature 2;
d) further cooling the mixture to a cooling temperature 3 to form a suspension;
e) maintaining the cooled suspension at the cooling temperature 3; and
f) isolating estetrol monohydrate from the suspension.

According to a third aspect, the present invention also encompasses estetrol monohydrate obtained or obtainable by the process according to the second aspect identified by characteristic XRPD peaks at 11.9, 12.2, 12.4, 12.8, 13.4, 19.9, 20.7, 20.8, 21.6, 21.7 and 24.5 °(±0.1°) 2θ using CuKα radiation.

According to a fourth aspect, the present invention also encompasses a pharmaceutical composition comprising estetrol monohydrate according to the first aspect or the third aspect as described herein, or obtained or obtainable by the process according to the second aspect as described herein; and at least one pharmaceutically acceptable excipient.

The above and other characteristics, features and advantages of the present invention will become apparent from the following detailed description, which illustrate, by way of example, the principles of the invention.

DETAILED DESCRIPTION OF THE FIGURES

The teaching of the application is illustrated by the following Figures which are to be considered as illustrative only and do not in any way limit the scope of the claims.

FIG.1 is a graph showing an XPRD diffractogram of estetrol monohydrate according to embodiments of the invention.

FIG.2 is a graph showing an XPRD diffractogram of estetrol monohydrate (Form 12) according to embodiments of the invention and another estetrol monohydrate (Form 1).

FIG.3 is a graph showing a zoomed image of the XPRD diffractogram of FIG.2.

DETAILED DESCRIPTION OF THE INVENTION

When describing the invention, the terms used are to be construed in accordance with the following definitions, unless a context dictates otherwise.

Unless otherwise defined, all terms used in disclosing the invention, including technical and scientific terms, have the meaning as commonly understood by one of ordinary skill in the art

to which this invention belongs. By means of further guidance, term definitions are included to better appreciate the teaching of the present invention.

In the following passages, various aspects of the invention are defined in more detail. Each aspect so defined may be combined with any other aspect or aspects unless clearly indicated to the contrary. In particular, any feature indicated as being preferred or advantageous may be combined with any other feature or features indicated as being preferred or advantageous.

Reference throughout this specification to “one embodiment” or “an embodiment” means that a particular feature, structure, or characteristic described in connection with the embodiment is included in at least one embodiment of the present invention. Thus, appearances of the phrases “in one embodiment” or “in an embodiment” in various places throughout this specification are not necessarily all referring to the same embodiment, but may. Furthermore, the particular features, structures or characteristics may be combined in any suitable manner, as would be apparent to a person skilled in the art from this disclosure, in one or more embodiments. Furthermore, while certain embodiments described herein include some but not other features included in other embodiments, combinations of features of different embodiments are meant to be within the scope of the invention, and form different embodiments, as would be understood by those in the art.

The terms "comprising", "comprises" and "comprised of" as used herein are synonymous with "including", "includes" or "containing", "contains", and are inclusive or open-ended and do not exclude additional, non-recited members, elements, or method steps. It will be appreciated that the terms "comprising", "comprises" and "comprised of" as used herein comprise the terms "consisting of", "consists" and "consists of".

As used in the specification and the appended claims, the singular forms "a", "an," and "the" include plural referents unless the context clearly dictates otherwise. By way of example, "a step" means one step or more than one step.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art.

The recitation of numerical ranges by endpoints includes all integer numbers and, where appropriate, fractions subsumed within that range (e.g. 1 to 5 can include 1, 2, 3, 4 when referring to, for example, a number of elements, and can also include 1.5, 2, 2.75 and 3.80, when referring to, for example, measurements). The recitation of endpoints also includes the end point values themselves (e.g. from 1.0 to 5.0 includes both 1.0 and 5.0). Any numerical range recited herein is intended to include all sub-ranges subsumed therein.

The term "about" as used herein when referring to a measurable value such as a parameter,

an amount, a temporal duration, and the like, is meant to encompass variations of +/-10% or less, preferably +/-5% or less, more preferably +/-1% or less, of and from the specified value, insofar such variations are appropriate to perform in the disclosed invention. It is to be understood that the value to which the modifier "about" refers is itself also specifically, and preferably, disclosed.

The terms “wt%,” “vol%”, or “mol%” refers to a weight percentage of a component, a volume percentage of a component, or molar percentage of a component, respectively, based on the total weight, the total volume of material, or total moles, which includes the component.

The terms “polymorph”, or “polymorphic form”, as used herein refers to different crystalline forms or crystal structures of a chemical compound, wherein the arrangement of molecules within the crystal lattice varies while maintaining the same chemical composition. In other words, polymorphs are isomeric structures of a chemical compound, which encompass different thermodynamic solid-state arrangements or modifications of the compound, each representing a unique crystallographic structure.

The term “suspension” as used herein refers to a heterogeneous mixture in which solid particles or droplets are dispersed within a liquid medium. The solid particles or droplets are generally insoluble or sparingly soluble in the liquid medium and remain dispersed throughout the mixture.

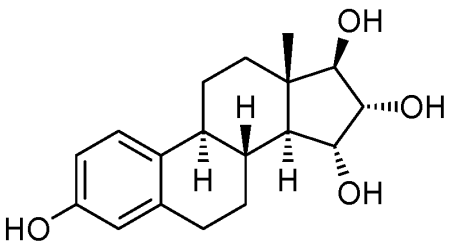
The term “solvate” as used herein refers to crystals formed by an active compound and a second component (solvent) which, in isolated form, is liquid at room temperature. Such solvates may be formed with common organic solvents, e.g., hydrocarbon solvents such as benzene or toluene; chlorinated solvents such as chloroform or dichloromethane; alcoholic solvents such as methanol, ethanol, or isopropanol; ethereal solvents such as diethyl ether or tetrahydrofuran; or ester solvents such as ethyl acetate. Alternatively, the second component may be water, in which case the crystals are referred to as “hydrate”. When a hydrate contains one water molecule, the crystals are referred to as “monohydrate”.

The term “alkyl” by itself or as part of another substituent, refers to a straight or branched saturated hydrocarbon group joined by single carbon-carbon bonds having 1 to 6 carbon atoms, for example 1 to 5 carbon atoms, for example 1 to 4 carbon atoms, preferably 1 to 3 carbon atoms. When a subscript is used herein following a carbon atom, the subscript refers to the number of carbon atoms that the named group may contain. Thus, for example, $C_{1\text{-}6}$alkyl means an alkyl of one to six carbon atoms. Examples of alkyl groups are methyl, ethyl, propyl, isopropyl, butyl, isobutyl, *sec*-butyl, *tert*-butyl, 2-methylbutyl, pentyl iso-amyl and its isomers, hexyl and its isomers.

The term "C_{3-6}cycloalkyl", as a group or part of a group, refers to a saturated or partially saturated cyclic alkyl radical containing from about 3 to about 6 carbon atoms. Examples of monocyclic C_{3-6}cycloalkyl radicals include cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl.

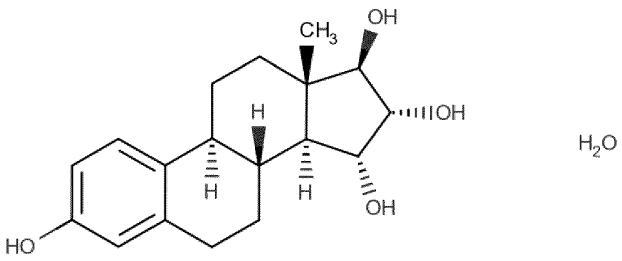
The term "estetrol" as used herein refers to 1,3,5 (10)-estratrien-3,15alpha,16alpha,17beta-tetrol or 15alpha-hydroxyestriol. "Estetrol", or short "E4" is an estrogen steroid produced by the foetal human liver (PubChem CID: 27125). Estetrol may be described as a 3-hydroxy steroid corresponding to 17beta-estradiol wherein the 15α and 16α positions are substituted for two additional hydroxy groups. It is known that estetrol is an estrogen receptor agonist (Coelingh Bennink et al., Climacteric, 2008). The estetrol may be chemically synthesized, synthesized by the use of (mutant) recombinant enzymes, or synthesized by any combination thereof. Estetrol may be indicated in the art by its molecular formula: $C_{18}H_{24}O_4$, or by structural formula (I).

Formula (I)



In the context of the present disclosure, a particularly preferred estetrol (component) is estetrol monohydrate. Estetrol monohydrate is a white to off-white crystalline solid that is poorly soluble in water and aqueous solutions. It is soluble in methanol, ethanol, sparingly soluble in acetone, and slightly soluble in ethyl acetate and acetonitrile. A skilled person appreciates that estetrol monohydrate corresponds to estetrol containing one molecule of water, and that the core structural formula of estetrol does not differ from Formula (I). By means of illustration and not limitation, the structural formula of estetrol monohydrate is indicated by Formula (II):

Formula (II)



When describing the present invention, the terms used are to be construed in accordance with

the following definitions, unless a context dictates otherwise.

Preferred statements (features) and embodiments and uses of this invention are set herein below. Each statement and embodiment of the invention so defined may be combined with any other statement and/or embodiment unless clearly indicated to the contrary. In particular, any feature indicated as being preferred or advantageous may be combined with any other feature or features or statements indicated as being preferred or advantageous. Hereto, the present invention is in particular captured by any one or any combination of one or more of the below numbered statements and embodiments, with any other aspect and/or embodiment.

1. Estetrol monohydrate identified by characteristic XRPD peaks at 11.9, 12.2, 12.4, 12.8 13.4, 19.9, 20.7, 20.8, 21.6, 21.7 and 24.5°(±0.1°) 2θ using CuKα radiation .
2. Process for producing estetrol monohydrate comprising the steps of:
   a) dissolving estetrol in a solvent mixture of water and at least one alcohol;
   b) cooling the solution to a cooling temperature 2 to obtain a mixture;
   c) maintaining the cooled mixture at the cooling temperature 2;
   d) further cooling the mixture to a cooling temperature 3 to form a suspension;
   e) maintaining the cooled suspension at the cooling temperature 3; and
   f) isolating estetrol monohydrate from the suspension.
3. The process according to statement 2, wherein the estetrol monohydrate is according to statement 1.
4. The process according to any one of statements 2 or 3, wherein step b) further comprises the steps of:
   b1) cooling the solution to a cooling temperature 1 to obtain a mixture;
   b2) maintaining the cooled mixture at the cooling temperature 1;
   b3) cooling the mixture to cooling temperature 2.
5. The process according to any one of statements 2 to 4, wherein step a) is performed at a temperature of at least 55.0 °C to at most 100.0 °C, preferably at least 56.0 °C to at most 98.0 °C, preferably a temperature of at least 57.0 °C to at most 96.0 °C, preferably a temperature at least 58.0 °C to at most 90.0 °C, preferably a temperature at least 55.0 °C to at most 90.0 °C.
6. The process according to any one of statements 2 to 6, wherein the cooling temperature 1 is at most 75.0 °C; preferably cooling temperature 1 is at most 70.0 °C; preferably cooling

temperature 1 is at most 65.0 °C; preferably cooling temperature 1 is at most 60.0 °C; preferably cooling temperature 1 is at most 55.0 °C; preferably cooling temperature 1 is at most 50.0 °C; preferably cooling temperature 1 is at most 45.0 °C.

7. The process according to any one of statements 2 to 7, wherein the cooling temperature 1 is at least 70.0 °C, preferably cooling temperature 1 is at least 60.0 °C, preferably cooling temperature 1 is at least 50.0 °C, preferably cooling temperature 1 is at least 45.0 °C.
8. The process according to any one of statements 2 to 8, wherein the cooling temperature 1 is at least 45.0 °C to at most 75.0 °C; preferably cooling temperature 1 is at least 45.0 °C to at most 70.0 °C; preferably cooling temperature 1 is at least 50.0 °C to at most 70.0 °C; preferably cooling temperature 1 is at least 50.0 °C to at most 65.0 °C.
9. The process according to any one of statements 2 to 8, wherein cooling of the solution in step b1) is performed over a period of at least 30.0 minutes to at most 6.0 hours, preferably at least 45.0 minutes to at most 5.0 hours; preferably at least 1.0 hour to at most 6.0 hours, preferably at least 1.0 hour to at most 4.0 hours, preferably at least 2.0 hours to at most 4.0 hours, preferably at least 2.0 hours to at most 3.0 hours.
10. The process according to any one of statements 2 to 9, wherein the solution is stirred in step b2).
11. The process according to any one of statements 2 to 10, wherein step b2) is performed over a period of at least 5.0 minutes to at most 10.0 days, preferably over a period of at least 10.0 minutes to at most 8.0 days, preferably over a period of at least 30.0 minutes to at most 5.0 days, preferably over a period of at least 30.0 minutes to at most 1.0 day, preferably over a period of at least 30.0 minutes to at most 12.0 hours.
12. The process according to any one of statements 2 to 11, wherein step b2) further comprises the addition of seeding compound.
13. The process according to any one of statements 2 to 12, wherein the cooling temperature 2 is at most 40.0 °C; preferably cooling temperature 2 is at most 35.0 °C; preferably cooling temperature 2 is at most 30.0 °C; preferably cooling temperature 2 is at least 20.0 °C.
14. The process according to any one of statements 2 to 13, wherein the cooling temperature 2 is at least 10.0 °C, preferably cooling temperature 2 is at least 12.5 °C, preferably cooling temperature 2 is at least 15.0 °C preferably cooling temperature 2 is at least 20.0 °C.
15. The process according to any one of statements 2 to 14, wherein the cooling temperature 2 is at least 10.0 °C to at most 40.0 °C; preferably cooling temperature 2 is at least 12.5 °C to at most 40.0 °C; preferably cooling temperature 2 it is at least 15.0 °C to at most 35.0 °C; preferably cooling temperature 2 it is at least 15.0 °C to at most 30.0 °C.
16. The process according to any one of statements 2 to 15, wherein cooling of the solution to

a cooling temperature 2 is performed over a period of at least 30.0 minutes to at most 6.0 hours, preferably at least 45.0 minutes to at most 5.0 hours; preferably at least 1.0 hour to at most 6.0 hours, preferably at least 1.0 hour to at most 4.0 hours, preferably at least 2.0 hours to at most 4.0 hours, preferably at least 2.0 hours to at most 3.0 hours.

17. The process according to any one of statements 2 to 16, wherein step c) is performed over a period of at least 5.0 minutes to at most 10.0 days, preferably over a period of at least 10.0 minutes to at most 8.0 days, preferably over a period of at least 20.0 minutes to at most 5.0 days, preferably over a period of at least 20.0 minutes to at most 1.0 day, preferably over a period of at least 20.0 minutes to at most 12.0 hours
18. The process according to any one of statements 2 to 17, wherein in step c) the mixture is stirred.
19. The process according to any one of statements 2 to 18, wherein the cooling temperature 3 is at least -10.0 °C; preferably cooling temperature 3 is at least -8.0 °C; preferably cooling temperature 3 is at least -6.0 °C; preferably cooling temperature 3 is at least -4.0 °C; preferably cooling temperature 3 is at least -2.0 °C.
20. The process according to any one of statements 2 to 19, wherein the cooling temperature 3 is at most 7.5 °C, preferably cooling temperature 3 is at most 5.0 °C, preferably cooling temperature 3 is at most 2.5 °C preferably cooling temperature 3 is at most 1.0 °C.
21. The process according to any one of statements 2 to 20, wherein the cooling temperature 3 is at least -10.0 °C to at most 7.5 °C; preferably cooling temperature 3 is at least -8.0 °C to at most 7.5 °C; preferably cooling temperature 3 is at least -6.0 °C to at most 7.5 °C; preferably cooling temperature 3 is at least -4.0 °C to at most 7.5 °C; preferably cooling temperature 3 is at least -2.0 °C to at most 7.0 °C; preferably cooling temperature 3 is at least -2.0 °C to at most 5.0 °C, preferably cooling temperature 3 is at least -2.0 °C to at most 2.5 °C.
22. The process according to any one of statements 2 to 21, wherein further cooling of the solution in step d) is performed over a period of at least 30.0 minutes to at most 6.0 hours, preferably at least 45.0 minutes to at most 5.0 hours; preferably at least 1.0 hour to at most 6.0 hours, preferably at least 1.0 hour to at most 4.0 hours, preferably at least 2.0 hours to at most 4.0 hours, preferably at least 2.0 hours to at most 3.0 hours.
23. The process according to any one of statements 2 to 22, wherein in step e) the suspension is stirred.
24. The process according to any one of statements 2 to 23, wherein step e) is performed over a period of at least 30.0 minutes to at most 24.0 hours; preferably over a period of at least 45.0 minutes to at most 23.0 hours, preferably over a period of at least 50.0 minutes to at most 22.0 hours; preferably over a period of at least 50.0 minutes to at most 21.0 hours.

25. The process according to any one of statements 2 to 24, wherein the at least one alcohol is a water miscible alcohol.
26. The process according to any one of statements 2 to 25, wherein the alcohol is a C_1-C_3 alcohol; preferably the alcohol is selected from the group consisting of isopropanol, n-propanol, ethanol and methanol; preferably the alcohol is isopropanol.
27. The process according to any one of statements 2 to 26, wherein the solvent mixture comprises water and an alcohol in a ratio of from 5:1 to 1:7; preferably a ratio of from 4:1 to 1:6; preferably a ratio of from 3:1 to 1:6; preferably a ratio of from 2:1 to 1:6.
28. The process according to any one of statements 2 to 27, wherein step c) further comprises the addition of seeding compound.
29. The process according to any one of statements 2 to 28, wherein step f) further comprises the steps of:

    f1) washing the isolated estetrol monohydrate with a solvent; and/or

    f2) drying the isolated estetrol monohydrate.
30. The process according to statement 29, wherein the solvent in step f1) is water.
31. The process according to any one of statements 29 or 30, wherein the solvent in step f1) is water at a temperature of at least -1.0 ºC and at most 0.0 °C.
32. Estetrol monohydrate obtained or obtainable by the process according to any one of statements 2 to 31 identified by characteristic XRPD peaks at 11.9, 12.2, 12.4, 12.8 13.4, 19.9, 20.7, 20.8, 21.6, 21.7, and 24.5°(±0.1°) 2θ using CuKα radiation.
33. Estetrol monohydrate according to statement 1 or 32 having a purity of at least 95.0%, preferably having a purity of at least 96.0%, preferably having a purity of at least 97.0%, preferably having a purity of at least 97.5%, preferably having a purity of at least 98.0%, preferably having a purity of at least 99.0%, preferably having a purity of at least 99.5% as determined by High-performance liquid chromatography (HPLC) analysis under the conditions disclosed in the Example section of the application.
34. A pharmaceutical composition comprising estetrol monohydrate according to statements 1 or 32-33, or obtained or obtainable by the process according to any one of statements 2 to 31; and at least one pharmaceutically acceptable excipient.

In the following passages, different aspects or embodiments of the invention are defined in more detail. Each aspect or embodiment so defined may be combined with any other aspect(s) or embodiment(s) unless clearly indicated to the contrary. In particular, any feature indicated as being preferred or advantageous may be combined with any other feature or features indicated as being preferred or advantageous.

The first aspect of the present invention refers to estetrol monohydrate identified by

characteristic XRPD peaks at 11.9, 12.2, 12.4, 12.8 13.4, 19.9, 20.7, 20.8, 21.6, 21.7 and 24.5 °(±0.1°) 2θ using CuKα radiation.

According to the second aspect of the present invention, a process is provided for the production of estetrol monohydrate. Said method preferably comprises the steps of:

a) dissolving estetrol in solvent mixture of water and at least one alcohol;

b) cooling the solution to a cooling temperature 2 to obtain a mixture;

c) maintaining the cooled mixture at the cooling temperature 2;

d) further cooling the mixture to a cooling temperature 3 to form a suspension;

e) maintaining the cooled suspension at the cooling temperature 3; and

f) isolating estetrol monohydrate from the suspension.

Preferably, the isolated estetrol monohydrate is a crystalline compound. In the context of the present invention, the terms "crystal", or “crystalline compound” refer to a solid material characterized by a highly ordered and repeating three-dimensional arrangement of atoms, ions, or molecules in a well-defined geometric pattern. The crystalline structure typically imparts distinct physical and chemical properties to the material, such as a specific melting point, optical properties, and X-ray diffraction patterns.

The present process and embodiments thereof have the advantage that estetrol monohydrate may be obtained in good yield and with an excellent degree of purity. Another advantage is that the present process and embodiments thereof may avoid the formation of undesired polymorphs and may avoid the inclusion of undesired impurities and/or solvents in the crystal lattice.

Hereunder, particularities and properties of the process steps of the invention, the starting materials applied therein, and the resulting estetrol polymorph will be discussed in more detail.

A first step (step a) described herein) in the process as described herein involves the formation of a solution by dissolving estetrol in a solvent mixture of water and at least one alcohol. Preferably, the solution is a “homogeneous” solution or “uniform” solution, which means that the individual components of the solution are uniformly distributed at the molecular or microscopic level to form a single-phase liquid.

The present process is not limited to a particular form of the (to be dissolved) estetrol. The present process may advantageously be applied starting from any crude estetrol. Said crude estetrol may comprise polymorphic forms of estetrol, such as amorphous estetrol, anhydrous estetrol, and/or crystalline estetrol.

In some embodiments, dissolving of estetrol in a solvent mixture of water and at least one alcohol may involve any form of mixing of the solvent (i.e., the solvent mixture of water and at least one alcohol) and solute (i.e., estetrol). For instance, the solution may be stirred or mechanically mixed to increase the solubility of estetrol in said solution. Alternatively, the solution may be sonicated to increase the solubility of estetrol in said solution.

Preferably, dissolving of estetrol in the solvent mixture of water and at least one alcohol further comprising heating of the solution. In some embodiments, step a) is performed at a temperature of at least 55.5 °C to at most 100.0 °C, preferably at least 56.5 °C to at most 98.0 °C, preferably a temperature of at least 57.5 °C to at most 96.0 °C, preferably a temperature at least 55.0 °C to at most 90.0 °C, preferably a temperature at least 56.5.0 °C to at most 90.0 °C. Advantages of dissolving estetrol at an such temperature may be increased solubility, allowing for the dissolution of a larger quantity of estetrol, and faster dissolution rates, which is particularly useful for achieving large-scale production.

The “solvent mixture of water and at least one alcohol” as used herein refers to a mixture or blend of substances comprising water and at least one alcohol. The mixture may further comprise one or more solutes, additives, or other solvents that are, preferably soluble or dispersible in water. The (aqueous) solvent mixture may be a solution, suspension, emulsion, or colloidal system, and preferably is a homogeneous solution. It should be understood that in the process as described herein, the (aqueous) solvent mixture may comprise one or more alcohol compound (i.e., a chemical compound comprising one or more hydroxyl (-OH) functional group).

In some embodiments of the present process, the at least one alcohol comprised in the solvent mixture, may be a C_1-C_3 alcohol and mixtures thereof. More in particular, the at least one alcohol may be a substituted or unsubstituted C_1, C_2, and/or C_3 alcohol comprising at least one hydroxyl group. Said at least one alcohol may be classified on the number and arrangement of hydroxyl groups, such as primary alcohol, secondary alcohol, or tertiary alcohol.

In some embodiments of the present process, the at least one alcohol is selected from the group consisting of methanol, ethanol, isopropanol, n-propanol, and mixtures thereof.

In some embodiments, the solvent mixture comprises water and an alcohol in a ratio of from 5:1 to 1:7; preferably a ratio of from 4:1 to 1:7; preferably a ratio of from 3:1 to 1:7. It has been advantageously found that a solvent mixture of water and at least one alcohol comprising a specified amount of alcohol(s) may significantly aid in the dissolution of estetrol in said mixture, and subsequent precipitation of estetrol monohydrate upon gradual cooling of the solution. In addition, the present process may avoid the inclusion of said alcohol(s) in the crystalline structure, resulting in a substantially pure polymorphic species.

The present inventors have found that the cooling method may be optimized to obtain a desired crystal structure with a high purity. More in particular, by cooling the solution obtained in step a) of the present process in at least two cooling steps, estetrol monohydrate may be obtained in good yield and with an excellent degree of purity. Said at least two cooling steps are characterized by cooling to a target temperature, and optionally keeping the solution at said target temperature to promote isothermal crystallization.

In accordance with step b) of the present process, the solution is cooled to cooling temperature 2.

Cooling the solution may be achieved through gradual or slow cooling, which allows the solution to cool naturally over an extended period of time. Alternatively, temperature control equipment may be used, which allows for a controlled cooling rate with reproducible crystals of a specific size. This is in contrast to flash cooling or rapid cooling using a cryogenic fluid, which typically produces amorphous forms and/or a mixture of polymorphic forms. It will be understood that the skilled person can correctly set the cooling conditions.

In some embodiments of the present process, the cooling of the solution in step b) may comprise the sub-steps:

b1) cooling the solution to a cooling temperature 1, to obtain a mixture;

b2) maintaining the cooled mixture at the cooling temperature 1;

b3) cooling the mixture to cooling temperature 2.

It is understood that additional steps can be provided and that some of the steps described can be replaced or eliminated for other implementations of the process as described herein.

In other words, in some embodiments, the solution may be gradually cooled from the dissolution temperature to the cooling temperature 2 in cooling sub-steps. Said cooling sub-steps involve cooling the solution to a desired temperature, and preferably keeping the solution at said desired temperature for a desired period of time. This has the advantage that dissolved estetrol molecules and/or water molecules may start to gather into stable clusters or nuclei, which will define the final crystal structure. Furthermore, it has been found that said cooling in sub-steps may avoid the inclusion of undesired solvates or solutes.

In some embodiments of the present process, the cooling temperature 1 is at least 45.0 °C to at most 75.0 °C; preferably cooling temperature 1 is at least 40.0 °C to at most 70.0 °C; preferably cooling temperature 1 is at least 55.0 °C to at most 70.0 °C; preferably cooling temperature 1 is at least 50.0 °C to at most 75.0 °C.

In some embodiments of the present process, the cooling of the solution in step b1) is performed

over a period of at least 35.0 minutes to at most 6.0 hours, preferably at least 45.0 minutes to at most 5.5 hours; preferably at least 1.5 hour to at most 6.0 hours, preferably at least 55.0 minutes to at most 4.0 hours, preferably at least 1.5 hour to at most 4.0 hours, preferably at least 1.0 hours to at most 3.0 hours. It should be understood that the period for cooling the solution in step b) refers to the total period of time needed to cool the solution to cooling temperature 2 as disclosed herein. In some embodiments, the total time may include any intermediate cooling sub-steps used for reaching the cooling temperature 2.

In some embodiments of the present process, the solution is agitated in step b2), preferably wherein agitation comprises isothermal stirring.

In some embodiments step b2) is performed over a period of at least 5.0 minutes to at most 10.0 days, preferably over a period of at least 15.0 minutes to at most 8.0 days, preferably over a period of at least 35.0 minutes to at most 7.0 days, preferably over a period of at least 35.0 minutes to at most 6.0 days, preferably over a period of at least 35.0 minutes to at most 20.0 hours.

In some embodiments of the present process, step b2) further comprises the addition of seeding compound.

In some embodiments, seeding compound or seeds comprise pre-formed crystals (e.g., obtained during a previous crystallization process) of a desired polymorph of estetrol. Said pre-formed crystals may act as a template for crystal growth. It has been found that the addition of pre-formed crystals may help to ensure the formation of a specific crystal structure and a more homogeneous crystal growth.

In some embodiments, the weight ratio of seeding compound and estetrol (dissolved in step a) of the present process) is at least 0.05 and at most 0.40, preferably at least 0.05 and at most 0.35, preferably at least 0.05 and at most 0.30, preferably at least 0.05 and at most 0.25, preferably at least 0.05 and at most 0.20.

Accordingly, in some embodiments, estetrol monohydrate, obtained from a previous crystallization process, preferably a process according to the present invention, may be added in step b2) of the present process to optimize crystal size, morphology, and polymorphism.

In some embodiments of the present process, the cooling temperature 2 is at least 10.0 °C to at most 40.0 °C; preferably cooling temperature 2 is at least 13.5 °C to at most 40.0 °C; preferably cooling temperature 2 it is at least 14.0 °C to at most 35.0 °C; preferably cooling temperature 2 it is at least 15.0 °C to at most 35.0 °C. In some embodiments the cooling of the solution to a cooling temperature 2 is performed over a period of at least 35.0 minutes to at most 6.0 hours, preferably at least 45.0 minutes to at most 5.5 hours; preferably at least 1.5 hour to

at most 6.0 hours, preferably at least 55.0 minutes to at most 4.0 hours, preferably at least 1.5 hour to at most 4.0 hours, preferably at least 1.0 hours to at most 3.0 hours.

The cooled mixture obtained in step b)/b3) may be maintained at the cooling temperature 2. In other words, the present process may provide that the solution is cooled to the cooling temperature 2 and subsequently kept at said cooling temperature 2 for a desired period of time. In some embodiments, the cooled mixture is kept at cooling temperature 2 under continuous stirring. It has been found to be advantageous to maintain the cooled mixture at a constant temperature before further cooling to promote the formation of a highly supersaturated solution. Without wishing to be bound by theory it is rationalized that at cooling temperature 2, the concentration of estetrol may greatly exceed its equilibrium concentration in the solution (supersaturation), which provides for a more homogeneous nucleation (i.e., spontaneous formation of clusters or aggregated compounds without the influence of external surfaces or impurities) and crystal growth.

Under isothermal conditions a “steady-state” may eventually be reached where the rate of nucleation and crystal growth becomes balanced. At this point, the system may maintain a consistent population of crystals, and further changes occur at a relatively constant rate. This has the advantage that the production of a desired polymorphic form may be optimized.

In some embodiments of the present process, step c) is performed over a period of at least 5.0 minutes to at most 10.0 days, preferably over a period of at least 15.0 minutes to at most 8.0 days, preferably over a period of at least 25.0 minutes to at most 7.0 days, preferably over a period of at least 20.0 minutes to at most 6.0 days, preferably over a period of at least 20.0 minutes to at most 20.0 hours.

In some embodiments of the present process, step c) further comprises the addition of seeding compound.

In some embodiments, seeding compound or seeds comprise pre-formed crystals (e.g., obtained during a previous crystallization process) of a desired polymorph of estetrol. Said pre-formed crystals may act as a template for crystal growth. It has been found that the addition of pre-formed crystals may help to ensure the formation of a specific crystal structure and a more homogeneous crystal growth.

In some embodiments, the weight ratio of seeding compound and estetrol (dissolved in step a) of the present process) is at least 0.05 and at most 0.40, preferably at least 0.05 and at most 0.35, preferably at least 0.05 and at most 0.30, preferably at least 0.05 and at most 0.25, preferably at least 0.05 and at most 0.20.

Accordingly, in some embodiments, estetrol monohydrate, obtained from a previous

crystallization process, preferably a process according to the present invention, may be added in step c) of the present process to optimize crystal size, morphology, and polymorphism.

A next step of the present process (i.e., step d)) involves further cooling of the (supersaturated) mixture to a cooling temperature 3 to obtain a slurry or suspension comprising crystals of a desired purity (and size).

In some embodiments of the present process, the cooling temperature 3 is at least -9.0 °C to at most 7.5 °C; preferably cooling temperature 3 is at least -7.0 °C to at most 7.5 °C; preferably cooling temperature 3 is at least -5.0 °C to at most 7.5 °C; preferably cooling temperature 3 is at least -5.5 °C to at most 7.5 °C; preferably cooling temperature 3 is at least -3.0 °C to at most 8.5 °C; preferably cooling temperature 3 is at least -2.0 °C to at most 5.0 °C, preferably cooling temperature 3 is at least -2.0 °C to at most 2.0 °C.

In some embodiments of the present process, further cooling of the mixture in step d) is performed over a period of at least 35.0 minutes to at most 6.0 hours, preferably at least 45.0 minutes to at most 5.5 hours; preferably at least 1.5 hour to at most 6.0 hours, preferably at least 55.0 minutes to at most 4.0 hours, preferably at least 1.5 hour to at most 4.0 hours, preferably at least 1.0 hours to at most 3.0 hours.

Cooling the mixture may be achieved through gradual or slow cooling, which allows the mixture to cool naturally over an extended period of time. Alternatively, temperature control equipment may be used, which allows for a controlled cooling rate with reproducible crystals.

In accordance with step e) of the present process, the cooled suspension may be maintained at the cooling temperature 3. In some embodiments, the cooled mixture is kept at cooling temperature 3 under continuous stirring.

In some embodiments, step e) is performed over a period of at least 35.0 minutes to at most 24.0 hours; preferably over a period of at least 40.0 minutes to at most 22.0 hours, preferably over a period of at least 55.0 minutes to at most 21.0 hours. More particularly, the suspension may be stirred at a constant cooling temperature 3 to further grow crystals.

In accordance with step f) of the present process, the formed crystals of estetrol monohydrate may be isolated from the suspension.

The term “isolating” as used herein refers to separating the solid crystalline material from the liquid or solution in which it was formed. Suitable non-limiting methods for isolating the crystals include filtration, centrifugation, decantation, and flotation. Alternatively, the crystals may be grown on a filter medium, creating a filter cake that may be separated from the liquid medium. It should be clear that the choice of the isolation method may depend on the characteristics of the crystalline estetrol monohydrate (e.g., crystal size and crystal structure) and the nature of

the aqueous mixture. The collected estetrol monohydrate crystals may be further processed downstream.

In some embodiments of the present process, the step f) further comprises the steps of:

f1) washing the isolated crystalline estetrol monohydrate with a solvent; and/or

f2) drying the isolated crystalline estetrol monohydrate.

In some embodiments, washing of the isolated crystalline estetrol monohydrate may comprise rinsing the collected crystals on a filter or membrane (e.g., porous material) to remove any residual impurities or contaminants that may be present on the crystal surface.

In some embodiments, the suspension obtained in step e) is filtered to isolate the solid crystalline estetrol monohydrate from the liquid portion. The retained solid crystals on the filter medium may form a cake or layer on the filter. Subsequently, a suitable washing solvent is poured or sprayed over the collected crystals to remove any remaining impurities or contaminants. The choice of washing solvent may depend on the solubility of impurities and the crystals.

In some embodiments, the washing solvent may be the solvent mixture comprising water and at least one alcohol as disclosed herein. Alternatively, a different solvent or solvent mixture is chosen that may selectively dissolve impurities without substantially dissolving the crystals. A non-limiting example of a suitable washing solvent is water.

In some embodiments, drying of the isolated crystalline estetrol monohydrate may comprise air drying, vacuum drying, and/or oven drying. Drying of the collected crystals ensures the removal of any remaining liquid after isolation from the solution and/or washing the collected crystals with a solvent.

In certain embodiments, the washing solvent is cooled prior to washing, preferably to a temperature of at least 0.0 °C to at most 5.0 °C.

In some embodiments, the suspension obtained in step e) is filtered to isolate the solid crystalline estetrol monohydrate from the liquid portion. The retained solid crystals on the filter medium may form a cake or layer on the filter. Subsequently, the cake or layer is vacuum dried at a temperature between 30.0 °C and 60.0 °C, preferably at 45.0 °C, and reduced pressure to remove residual liquids that may be retained. The term “reduced pressure” as used herein refers to a pressure lower than 50 kPa, such as 40 kPa, or 30 kPa, or 20 kPa, or 10 kPa or 5 kPa.

In some embodiments, the suspension obtained in step e) is filtered to isolate the solid crystalline estetrol monohydrate from the liquid portion. The retained solid crystals on the filter medium may form a cake or layer on the filter. Subsequently, a suitable washing solvent is

poured or sprayed over the collected crystals to remove any remaining impurities or contaminants. The collected crystals are then vacuum dried at a temperature between 30.0 °C and 60.0 °C, preferably at 45.0 °C, and reduced pressure to remove residual liquids that may be retained.

Advantageously, the present process has the advantage that crystalline estetrol monohydrate, can be obtained in a reduced number of steps compared to prior art processes, which is more convenient for an economical and industrial synthesis.

According to a third aspect, the present invention also encompasses estetrol monohydrate. The estetrol monohydrate is preferably obtained or obtainable by the process according to the second aspect as described herein.

The estetrol monohydrate may be characterized by powder X-ray diffraction (XRPD), wherein an X-ray tube emits X-rays, which interact with the crystalline sample. In particular, X-rays are typically directed onto the crystalline sample, preferably a fine powder of the crystalline estetrol monohydrate, at various angles. The resulting diffraction pattern is analysed using Bragg's Law, which relates the angle of diffraction (θ), the wavelength of X-rays (λ), and the interplanar spacing (d) in the crystal lattice. The equation is given by $n\lambda = 2d \sin(\theta)$, where n is the order of diffraction. The diffracted X-rays are detected, and the resulting diffraction pattern is recorded as a series of peaks. The positions and intensities of these peaks provide information about the crystal lattice and spacing.

Preferably, the estetrol monohydrate is identified by characteristic XRPD peaks at 2θ of 11.9, 12.2, 12.4, 12.8 13.4, 19.9, 20.7, 20.8, 21.6, 21.7 and 24.5°(±0.1°) 2θ using CuKα radiation.

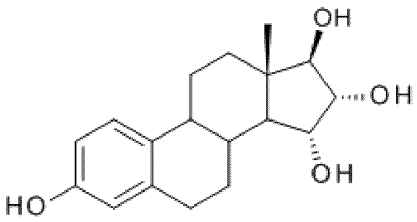
The XRD peaks recited herein should be understood to reflect a precision of ± 0.1 for the 2 theta peaks, and the equivalent precision for d-spacings as per Bragg's law.

Advantageously, the present invention provides that the estetrol monohydrate has a high purity, preferably a purity of at least 95.0%, preferably a purity of at least 96.0%, preferably a purity of at least 97.0%, preferably a purity of at least 97.5%, preferably a purity of at least 98.0%, preferably a purity of at least 99.0%, preferably a purity of at least 99.5% as determined by High-performance liquid chromatography (HPLC) analysis under the conditions disclosed in the Example section of the application.

Contrary to what would be expected from the prior art, the present invention provides (crystalline) estetrol monohydrate with high purity without the need for additional pre-treatment and/or purification steps.

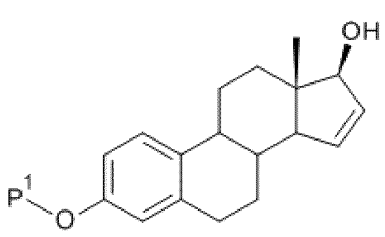
Processes for preparing estetrol from estrone are known in the art and can be found in WO2013050553A1 and WO2012/169096.

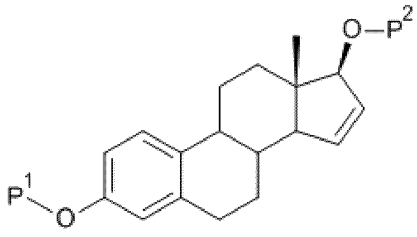
In some embodiments, the process for the preparation of estetrol (formula (I)) from estrone comprises the steps of :



(I)

a) reacting a compound of formula (II), with an acylating or a silylating agent to produce a compound of formula (III),

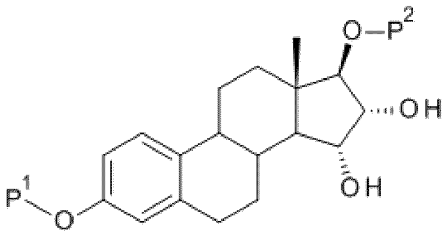




(II) (III)

wherein P^1 is a protecting group selected from R^1CO-, or $R^2Si(R^3)(R^4)$-, P^2 is a protecting group selected from $(R^6R^5R^7)C$-CO-, or $(R^2)Si(R^3)(R^4)$-, wherein R^1 is a group selected from C_{1-6}alkyl or C_{3-6}cycloalkyl, each group being optionally substituted by one or more substituents independently selected from fluoro or C_{1-4}alkyl; R^2, R^3 and R^4 are each independently a group selected from C_{1-6}alkyl or phenyl, each group being optionally substituted by one or more substituents independently selected from fluoro or C_{1-4}alkyl; R^5 is a group selected from C_{1-6}alkyl or phenyl, each group being optionally substituted by one or more substituents independently selected from fluoro or C_{1-4}alkyl; R^6 and R^7 are each independently hydrogen or a group selected from C_{1-6}alkyl or phenyl, each group being optionally substituted by one or more substituents independently selected from fluoro or C_{1-4}alkyl;

b) reacting the compound of formula (III) in the presence of at least one oxidizing agent selected from permanganate salt, osmium oxide, hydrogen peroxide, or iodine and silver acetate to produce compound of formula (IV); and

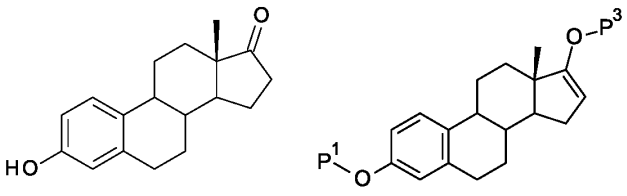


(IV)

c) deprotecting the compound of formula (IV) to produce compound of formula (I).

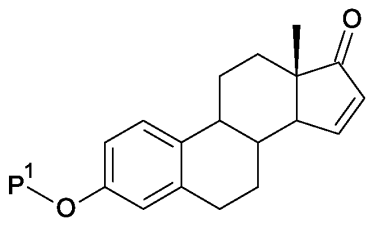
In some embodiments, the process for the preparation of the compound of formula (II) comprises the steps of :

i) reacting a compound of formula (IIA), with an acylating or a silylating agent to produce a compound of formula (IIB), wherein P^1 and P^3 are each independently a protecting group selected from R^2-Si-R^3R^4, or R^1CO-, wherein R^1 is a group selected from C_{1-6}alkyl or C_{3-6}cycloalkyl, each group being optionally substituted by one or more substituents independently selected from fluoro or C_{1-4}alkyl; R^2, R^3 and R^4 are each independently a group selected from C_{1-6}alkyl or phenyl, each group being optionally substituted by one or more substituents independently selected from fluoro or C_{1-4}alkyl;



(IIA) (IIB)

ii) reacting the compound of formula (IIB) in the presence of palladium acetate to produce compound of formula (IIC); and



(IIC)

iii) reacting the compound of formula (IIC) with a reducing agent to produce compound of formula (II).

In some embodiments the reducing agent in step iii) is selected from the group of metal hydride compounds; preferably a metal hydride compound is selected from the group comprising $NaBH_4/CeCl_3$, $LiAlH_4$, $NaBH_4$, $NaBH(OAc)_3$, and $ZnBH_4$.

According to a third aspect, the present invention also encompasses a pharmaceutical composition comprising estetrol monohydrate. The estetrol monohydrate is preferably estetrol monohydrate according to the first aspect as described herein or obtained or obtainable by the process according to the second aspect as described herein.

Preferably, the pharmaceutical composition further comprises at least one pharmaceutically acceptable excipient.

The term “pharmaceutically acceptable" as used herein refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

As used herein, “carrier” or “excipient” includes any and all solvents, diluents, buffers (such as, e.g., neutral buffered saline or phosphate buffered saline), solubilisers, colloids, dispersion media, vehicles, fillers, chelating agents (such as, e.g., EDTA or glutathione), amino acids (such as, e.g., glycine), proteins, disintegrants, binders, lubricants, wetting agents, emulsifiers, sweeteners, colorants, flavourings, aromatisers, thickeners, agents for achieving a depot effect, coatings, antifungal agents, preservatives, antioxidants, tonicity controlling agents, absorption delaying agents, and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active substance, its use in the therapeutic compositions may be contemplated.

Illustrative, non-limiting carriers for use in formulating the pharmaceutical compositions include, for example, oil-in-water or water-in-oil emulsions, aqueous compositions with or without inclusion of organic co-solvents suitable for intravenous (IV) use, liposomes or surfactant-containing vesicles, microspheres, microbeads and microsomes, powders, tablets, capsules, suppositories, aqueous suspensions, aerosols, and other carriers apparent to one of ordinary skill in the art.

Pharmaceutical compositions as intended herein may be formulated for essentially any route of administration, such as without limitation, oral administration (such as, e.g., oral ingestion

or inhalation), intranasal administration (such as, e.g., intranasal inhalation or intranasal mucosal application), parenteral administration (such as, e.g., subcutaneous, intravenous (I.V.), intramuscular, intraperitoneal or intrasternal injection or infusion), transdermal or transmucosal (such as, e.g., oral, sublingual, intranasal) administration, topical administration, rectal, vaginal or intra-tracheal instillation, and the like. In this way, the therapeutic effects attainable by the methods and compositions can be, for example, systemic, local, tissue-specific, etc., depending of the specific needs of a given application.

The present invention further encompasses the use of the pharmaceutical composition according to the third aspect as described herein, preferably for use in a method selected from a method of hormone replacement therapy, a method of treating vaginal dryness, a method of contraception, a method of enhancing libido, of method of treating skin, a method of promoting wound healing, and a method of treating or preventing a disorder selected from the group consisting of autoimmune diseases, breast tumours and colorectal tumours.

The invention is illustrated but not limited by the following examples.

EXAMPLES

METHODOLOGY

The following describes the materials and methods used for all examples unless otherwise stated.

X-Ray Powder Diffraction (XRPD) - To perform XRPD analysis, a PANanalytical Empryean X-ray powder diffractometer was used. The typical XRPD parameters used are listed below. Data Viewer (version 1.4a) from PANanalytic was used for analysis.

Equipment Diffractometer: Stoe Stadi P, G.52.SYS.S072

Evaluation software: WinXPOW by Stoe

Sample preparation and instrumental parameters:

About 40 mg of the sample is placed between two acetate foils with a 0.4 mm thick metal ring as a spacer and then smoothed with two glass slides to obtain a disk of powder. The disk is placed in a Secure Cell (SCell) sample holder.

The x-ray diffraction pattern is recorded with the following instrumental set-up:

Radiation: CuKα1; 40 kV, 40 mA

Collimator : 0.5 x 10 mm

Detector : Mythen1 K

Detector distance: resulting to 0.010° 2theta intrinsic resolution

Monochromator: Ge, curved monochromator

Sample rotation: 1 rps

Scan range: at least 2-40° 2theta

Step size: 0.020° 2theta

Step time: 48 s

Detector step: 1° 2theta

HPLC method:

Eluent: A: Water; B:Methanol; C: Acetonitrile

Gradient of the eluents are indicated in the table below:

Time (in min)	%A	%B	%C
0	80	20	0
6	65	35	0
17	55	45	0
32	25	65	10
48	8	10	82
49	80	20	0
60	80	20	0

Flow rate: 1 mL/min^{-1}

Column: C8, 250 x 4.6 mm, 5 µm

Oven Temperature: 40 °C

Diode Array Detector: 180-800 nm

Wavelength for analysis: 280nm

EXAMPLES

Example 1 – Crude estetrol

In Example 1, crude estetrol was prepared by means of the process disclosed in WO2013050553A1, incorporated herein in its entirety.

Step 1: 3,17-di-tert-butyldimethylsiloxy-estra-1, 3, 5(10)-16-tetraene-17-ol

To a solution of estrone (50g, 0.185 mole) and 2,6-lutidine (62g, 0.58 mole) in dichloromethane

400ml was added drop wise t-butyl-dimethylsilyl-triflate (102.6g, 0.39 mole).The solution was stirred at room temperature for 6 hours. Water (300ml) was added and the organic layer was washed with a diluted solution of sodium carbonate. The dichloromethane solution was partially evaporated and ethyl acetate was added. Diisopropyl ether was added to this solution. The mixture was stirred for 2 hours at 0°C. The precipitate was collected by filtration and dried. 83 g of the title compound were obtained (90% yield).

Step 2: 3-tert-butyldimethylsiloxy-estra-1, 3, 5 (10)-15-tetraene-17-one

To a solution of 3, 17-di-t-butyldimethylsiloxy-estra-1, 3, 5(10)-16-tetraene-17-ol 83 g (0.166 mole) in 400ml of acetonitrile was added $Pd(OAc)_2$ 3.8 g (0.017 mole) in an oxygen atmosphere. The mixture was stirred at 40°C for 12 hours then filtered through a pad of celite. A diluted solution of sodium carbonate was added and the mixture was extracted with ethyl acetate.

After concentration, diisopropyl ether was added and the mixture was stirred at 0°C for one hour. The product (54.7g, 86% yield) was collected by filtration and used in the next step without further purification.

Step 3: 3-tert-butyldimethylsiloxy-estra-1, 3, 5 (10)-15-tetraene-17-ol

The collected material (54.7g, 0.143 mole) was dissolved in THF 300ml and a solution of cerium chloride heptahydrate (53.3g, 0.143 mole) in methanol (300ml) was added. The mixture was cooled to 0°C sodium borohydride (8.12g, 0.213 mole, 1.5eq) was added portion wise keeping the temperature below 9°C. At this end of the addition the mixture was stored for one hour then quenched by addition of a 2N HCl solution (100ml). The solution was partly evaporated in situ and water (4L) was added. The precipitate was collected by filtration and dried. After crystallization from a mixture of ethanol /diisopropyl ether the product was collected by filtration and dried. It weighted 46.6g (85% yield).

Step 4: estra-1,3,5(10),15-tetraene-3,17β-diol bis(dimethyl-tert-butylsilyl) ether

To a solution of 3-t-butyldimethylsiloxy-estra-1,3,5 (10)-15-tetraene-17β-ol (10g, 0.025 mole) in 100 ml of dimethylformamide were added imidazole (4.4g, 0.065 mole) and dimethyl-tert-butylsilyl-chloride (1.5 eq.) and allowed to stand at room temperature for 6 hours. The resulting solution was diluted with ethyl acetate, washed with water and evaporated. The residue was crystallized from methanol to afford (10g) of estra-1,3,5(10),15-tetraene-3,17β-diol bis(dimethyl-tert-butylsilyl) ether.

NMR ($CDCl_3$): 0.08 (6H,s,17-OSi(CH_3)$_2$, 0.18 (6H,3-OSi(CH_3)$_2$, 0.81 (3H,s,18-CH_3), 0.91 (9H,17-OSi-t-Bu), 0.97 (9H,s,3-OSi-t,Bu), 4.33 (1H, broad s, 17 aH), 5.60 (1H,m,15-H, 5.95

(1H,broad,d,16H), 6.45-6.75 (2H,2-and 4H), 7.12 (1H,d,J=8Hz,1H).

mp :89-91°C

Step 5: estra-1,3,5(10), 15α,16α,17β-tetrol

To a stirred solution of estra-1,3,5(10),15-tetraene-3,17β-diol bis(dimethyl-tert-butylsilyl) ether (10 g, 0.02 mole) and formic acid (0.06 mole, 2.3 ml) in acetone (100ml) at 0°C was added gradually a solution of potassium permanganate (3.15g, 0.02 mole) in water (20ml) and acetone (100ml). After completion of the reaction, the reaction was quenched with a 10% aqueous solution of $KHSO_3$. Acetone was partially removed and extracted with ethyl acetate, and washed with water. Ethyl acetate was concentrated under reduced pressure and diluted with heptane. The precipitate was collected by filtration and dissolved in acetone (100ml). To the solution 5N hydrochloric acid (20 ml) was added. After completion of the reaction the resulting solution was diluted with water. The solid was collected by filtration, washed with heptane and crystallized from a mixture of methanol and water to afford crude estetrol.

Example 2 – Form 12

In Example 2, the crude estetrol obtained in Example 1 was used to selectively prepare or isolate estetrol monohydrate (identified as Form 12) according to the process as disclosed herein.

60 kg of crude estetrol was dissolved in 340 L of a mixture of isopropanol and water (53:47) at a temperature of 79.0 °C. The resulting solution was then cooled down to 70.0 °C over a period of 60.0 minutes. The resulting mixture was then stirred at this temperature for 30.0 minutes. The mixture was then cooled down to 20.0 °C over a period of 2.5 hours. The mixture was maintained at 20.0 °C for 30.0 minutes under stirring, and cooled down to 0.0 °C over a period of 1.0 hour , to obtain a suspension. The obtained suspension was stirred at 0.0 °C for a period of at least 1.0 hour before filtering the suspension using a filter dryer. The filter cake was washed with 120 L of pre-cooled water (i.e., with a temperature between 0 °C and 4 °C) and the obtained crystals were dried at 45.0 °C under reduced pressure. The collected estetrol monohydrate was obtained in a yield of 90.0% and a purity of 99.0% as determined by HPLC analysis.

The Form 12 estetrol monohydrate crystals were subsequently analysed by XRPD using CuKα radiation and the resulting diffraction peaks are presented in **FIG. 1**. In particular, X-ray diffraction analysis was performed on a single crystal of estetrol monohydrate Form 12. The single crystal dimensions were 0.50 x 0.10 x 0.06 mm. The structural resolution evidences that estetrol monohydrate Form 12 crystallises as a pure enantiomer in the Triclinic system with a P1 group space. The unit cell dimension comprises 12 estetrol molecules and 13 water

molecules. Unit cell dimensions and properties of the single crystal are summarized in **Table 1** below.

Table 1: Properties of single crystal of estetrol monohydrate (Form 12).

Unit cell dimensions	a = 8.8963 Å	α = 63.931°
	b = 23.6658 Å	β = 89.815°
	c = 26.1027 Å	γ = 84.107°
Volume	4905.0 Å^3	
Crystalline formula	$C_{18}H_{24}O_{5.08}$	
Calculated density	1.307 g/cm^3	

With the set of experimental data obtained from single-crystal analysis, it is possible to derive a characteristic powder diffraction pattern. The characteristic XRPD peaks determined using CuKα radiation of this form (referred to as Form 12) are at 11.9, 12.2, 12.4, 12.8, 13.4, 19.9, 20.7, 20.8, 21.6, 21.7 and 24.5°(±0.1°) 2θ).

Example 3 – Form 12

In Example 3, the crude estetrol obtained in Example 1 is used to selectively prepare or isolate estetrol monohydrate (identified as Form 12) according to the process as disclosed herein.

60 kg of crude estetrol will be dissolved in 340 L of a mixture of isopropanol and water (53:47) at a temperature of 79.0 °C. The mixture will be then cooled down to 20.0 °C over a period of 2.5 hours. The mixture will be maintained at 20.0 °C for 30.0 minutes under stirring, and cooled down to 0.0 °C over a period of 1.0 hour, to obtain a suspension. The obtained suspension will be stirred at 0.0 °C for a period of at least 1.0 hour before filtering the suspension using a filter dryer. The filter cake will be washed with 120 L of pre-cooled water (i.e., with a temperature between 0 °C and 4 °C) and the obtained crystals will be dried at 45.0 °C under reduced pressure.

The Form 12 estetrol monohydrate crystals will be analysed by XRPD using CuKα radiation.

Example 4 – Form 1

A different form of estetrol, identified as estetrol Form 1, was prepared in Example 4 according to the process disclosed in WO2023051937A1.

3.5 g of crude estetrol obtained following the experimental procedures described in Example 1 was suspended under stirring in 63 mL of methanol. The suspension was heated to reflux temperature (72 °C) to complete solution. The solution was then evaporated at reduced

pressure and 14 mL of Isopropyl alcohol (IPA) was added to the residual volume of 28 mL, keeping T > 50 °C. This last step was repeated two more times (final volume 28 mL).

Finally, 17.5 mL of IPA was added and the whole was refluxed until complete dissolution of the solids. The solution was cooled to T = 70 °C and 45.5 mL of water was added keeping T > 60 °C under stirring. Slowly the suspension was distilled keeping T = 55-65 °C under reduced pressure until residual volume of 35 mL. The slurry was slowly cooled at 5 °C, stirred for at least 30 minutes at this temperature and filtered on a buchner filter. The filter cake was washed with water and the solid was dried in vacuum oven at 35 °C for about 18 h.

Estetrol monohydrate (white solid) was obtained (3.50 g) in Form 1.

The obtained estetrol Form 1 crystals were subsequently analysed by XRPD. **FIG. 2** is an XRPD diffractogram of Form 12 estetrol monohydrate of Example 2 and estetrol Form 1 of Example 4 using CuKα radiation. As shown in FIG. 2, each form is characterized by different XRPD peaks. In particular, the peak at 2θ=20.8° of Form 12 estetrol monohydrate shows a distinct split signal. The characteristic XRPD peaks of the Form 1 are thus at 11.9, 12.4, 13.4, 19.9, 20.8, 21.7, 24.5°(±0.1°) 2θ).

FIG. 3 is a zoomed image of the XRPD diffractogram of Form 12 estetrol monohydrate of Example 2 and estetrol Form 1 of Example 4. As shown in FIG. 3, Form 12 estetrol monohydrate further differs from estetrol Form 1 in that additional peaks at 2θ =12.2 and 12.8° can be identified.

Example 5 - Seeding

60 kg of crude estetrol was dissolved in 340 L of a mixture of isopropanol and water (53:47) at a temperature of 79.0 °C. The resulting solution was then cooled down to 70.0 °C over a period of 60.0 minutes. The resulting mixture was then stirred at this temperature for 30.0 minutes. 100 g of seed crystals of estetrol monohydrate (Form 12) were added to the mixture. The mixture was then cooled down to 20.0 °C over a period of 2.5 hours. The mixture was maintained at 20.0 °C for 30.0 minutes, and cooled down to 0.0 °C over a period of 1.0 hour to obtain a suspension. The obtained suspension was stirred at 0.0 °C for a period of at least 1.0 hour before filtering the suspension using a filter dryer. The filter cake was washed with 120 L of pre-cooled water and the obtained crystals were dried at 45.0 °C under reduced pressure. The collected estetrol monohydrate crystals were obtained in a yield of 90.0% and a purity of between 99.0% as determined by HPLC analysis.

The estetrol monohydrate crystals were subsequently analysed by XRPD using CuKα radiation and the resulting diffraction peaks will be corresponding to Form 12 in Example 2.

Example 6 – Different alcohols

A)

100 mg of crude estetrol will be dissolved in 2.0 mL of a mixture of isopropanol and water (1:5) at a temperature of 80.0 °C. The resulting solution will be cooled down to 60.0 °C over a period of 60.0 minutes. The mixture will then be stirred at this temperature for 60.0 minutes. The mixture will then be cooled down to 20.0 °C over a period of 2.5 hours. The mixture will then be stirred at 20.0 °C for 60.0 minutes and cooled down to 0.0 °C over a period of 1.0 hour, to obtain a suspension. The obtained suspension will be stirred at 0.0 °C for a period of at least 1.0 hour before filtering the suspension. The filter cake will be washed with 1 mL of pre-cooled propanol and the obtained crystals dried at 45.0 °C under reduced pressure. The collected estetrol monohydrate crystals will be obtained in a yield of 90.0% and a purity of 98.0% as determined by HPLC analysis. The estetrol monohydrate crystals will be subsequently analysed by XRPD using CuKα radiation and the resulting diffraction peaks will be in line with the results presented for Form 12 in Example 2.

B)

56 mg of crude estetrol was dissolved in 1.0 mL of a mixture of ethanol and water (1:5) at a temperature of 60.0 °C. The solution was then cooled down to 20.0 °C and stirred at said temperature for 6.0 days. The resulting mixture was then cooled down to 0.0 °C over a period of 1.0 hour, to obtain a suspension. The obtained suspension was stirred at 0.0 °C for a period of at least 1.0 hour before filtering the suspension. The filter cake was washed with 2.0 mL of pre-cooled water and the obtained crystals were dried at 45.0°C under reduced pressure. The collected estetrol monohydrate crystals were obtained in a yield of 90.0% and a purity of 99.0% as determined by HPLC analysis. The estetrol monohydrate crystals were subsequently analysed by XRPD using CuKα radiation and the resulting diffraction peaks were identical to the peaks presented for Form 12 in Example 2.

C)

60 mg of crude estetrol will be dissolved in 1.0 mL of a mixture of methanol and water (1:5) at a temperature of 60.0 °C. The resulting solution will then be cooled down to 50.0 °C over a period of 60.0 minutes. The mixture will then be stirred at this temperature for 60.0 minutes. The mixture will then be cooled down to 20.0 °C over a period of 2.5 hours. The mixture will then be stirred at 20.0 °C for 60.0 minutes and cooled down to 0.0 °C over a period of 1.0 hour, to obtain a suspension. The obtained suspension will be stirred at 0.0 °C for a period of at least 1.0 hour before filtering the suspension. The filter cake will be washed with 1.0 mL of pre-cooled water and the obtained crystals was dried at 45.0 °C under reduced pressure. The

collected estetrol monohydrate crystals will be obtained in a yield of 80.0% and a purity of 95.0%. The estetrol monohydrate crystals will be subsequently analysed by XRPD using CuKα radiation and the resulting diffraction peaks will be in line with the results presented for Form 12 in Example 2.

CLAIMS

1. Estetrol monohydrate identified by characteristic XRPD peaks at 11.9, 12.2, 12.4, 12.8 13.4, 19.9, 20.7, 20.8, 21.6, 21.7 and 24.5°(±0.1°) 2θ using CuKα radiation.

2. Process for producing estetrol monohydrate, preferably estetrol monohydrate according to claim 1, comprising the steps of:

   a) dissolving estetrol in a solvent mixture of water and at least one alcohol, preferably at a temperature of from 55.0 °C to 100.0 °C;

   b) cooling the solution to a cooling temperature 2 to obtain a mixture;

   c) maintaining the cooled mixture at the cooling temperature 2;

   d) further cooling the mixture to a cooling temperature 3 to form a suspension;

   e) maintaining the cooled suspension at the cooling temperature 3; and

   f) isolating estetrol monohydrate from the suspension.

3. The process according to claim 2, wherein step b) further comprises the steps of:

   b1) cooling the solution to a cooling temperature 1;

   b2) maintaining the cooled solution at the cooling temperature 1, optionally adding seeding compound;

   b3) cooling the solution to cooling temperature 2 to obtain a mixture.

4. The process according to any one of claims 2 or 3, wherein the cooling temperature 1 is at most 75.0 °C.

5. The process according to any one of claims 2 to 4, wherein cooling of the solution in step b1) is performed over a period of at least 30.0 minutes to at most 6.0 hours.

6. The process according to any one of claims 2 to 5, wherein the solution is stirred in step b2), and preferably wherein stirring is performed over a period of at least 5.0 minutes to at most 10.0 days.

7. The process according to any one of claims 2 to 6, wherein the cooling temperature 2 is at most 40.0 °C.

8. The process according to any one of claims 2 to 7, wherein cooling of the solution to a cooling temperature 2 is performed over a period of at least 30.0 minutes to at most 6.0 hours.

9. The process according to any one of claims 2 to 8, wherein the cooling temperature 3 is at most 5.0 °C.

10. The process according to any one of claims 2 to 9, wherein the at least one alcohol is a water miscible alcohol.
11. The process according to any one of claims 2 to 10, wherein the at least one alcohol is a C_1-C_3 alcohol.
12. The process according to any one of claims 2 to 11, wherein the solvent mixture comprises water and at least one alcohol in a ratio of from 5:1 to 1:5.
13. The process according to any one of claims 2 to 12, wherein step c) further comprises the addition of seeding compound.
14. The process according to any one of claims 2 to 13, wherein step f) further comprises the steps of:

    f1) washing the isolated estetrol monohydrate with a solvent; and/or

    f2) drying the isolated estetrol monohydrate.

15. A pharmaceutical composition comprising estetrol monohydrate according to claim 1, or obtained or obtainable by the process according to any one of claims 2 to 14; and at least one pharmaceutically acceptable excipient.