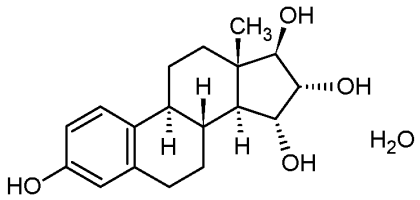
Process for the crystallization of Estetrol monohydrate

Field of invention

The present disclosure relates to a crystallization process of (15α, 16α, 17β)-estra-1, 3,5(10)-triene-3,15,16,17-tetrol monohydrate (Estetrol monohydrate, Compound 1), a potent, and orally active pharmaceutical ingredient. The said process produces Estetrol monohydrate Form 12.

Background of the invention

Estetrol monohydrate ((15α, 16α, 17β)-estra-1,3,5(10)-triene-3,15,16,17-tetrol monohydrate, depicted below, referred as Compound 1 hereinafter) is a steroid with weak estrogenic activity produced endogenously by the fetal liver during human pregnancy.



Compound 1

Estetrol was found to be efficacious in hormone replacement therapy, a method of treating vaginal dryness, a method of treating perimenopausal symptoms (for example hot flashes, night sweats), a method of contraception, a method of enhancing libido, a method of treating skin and promoting wound healing, a method of treating or preventing an autoimmune disorder, breast tumors, prostate cancer and colorectal tumors, and a method of neuroprotection (for example neonatal encephalopathy) (WO 02/094275 A1 , WO 02/094276 A1 , WO 02/094278 A1 , WO 02/094279 A1 , WO 03/041718 A1 , WO 03/103684 A1 , WO 03/103685 A1 , WO 2004/006936 A1 , WO 2004/037269 A1 , WO 2007/081206 A1 , WO 2008/085038 A2, WO 2013/021025 A1 , WO 2013/156329 A1 , WO 2018/024912 A1 , WO 2018/065076 A1 , WO 2019/025031 A1 ; Foidart et al., AJOG Glob Rep. 2023 Nov; 3(4): 100266; Gérard and Foidart, Drugs R D 2023, 23, 77–92; Gaspard et al., Maturitas 124 (2019) p. 153 Abstract P09; Apter et al. Eur J Contracept Reprod HC (2017) 22(4):260-267; Tskitishvili et al., J Endocrinol. (2017) 232(1):85-95; Coelingh Bennick et al., Climacteric (2008) 11 (SuppM):47- 58).

The synthetic method for Compound 1 was first disclosed in Fishman, J and Guzik, H., Tetrahedron Letters, 1967, 30, 2929-2932, after hydrolysis with K_2CO_3 in refluxing methanol gave the desired tetrol. An alternative route for the synthesis was described in Fishmann, Guzik, J.Org. Chem., 1968, 33(8), 3133-3135, wherein after thin layer chromatography the more polar material was crystallized from ethyl acetate. There was

another way to synthetize Estetrol by alkaline hydrolysis and recrystallization from ethyl acetate as described in Suzuki, E., Namba, S., Kuruhara, H., Goto, J., Matsuki, Y., Nambara, T., Steroids, 1995, 60, 277-284.

Estetrol can be used as a monohydrate, as disclosed in WO2015/086643 (Donesta Bioscience B.V.). The said crystalline form (to be referenced as Form 1 hereafter) can be obtained by various crystallization methods as disclosed in various applications, e.g. WO2021/044302 (Richter Gedeon), WO2021/058716 (Industriale Chimica); WO2023/051937 (Industriale Chimica). Furthermore, some isolation process for Estetrol is also disclosed in further applications e.g. WO2004/041839 (Pantarhei), WO2013/012328 (Pantarhei), WO2013/034780 (Crystal Pharma), WO2013/050553 (Estetra), WO2015/040051 (Crystal Pharma).

Polymorphism is the ability of a compound to exist in two or more different crystalline phases that differ in the arrangement of the molecules in crystal lattice (J. Bernstein (2002) Polymorphism in molecular Crystals, Oxford Univ. Press. p. 2-4). Although polymorphs have the same chemical composition, they differ in packing and geometrical arrangement and exhibit different physical properties such as melting point, X-ray diffraction patterns, crystal habit, density, stability, solubility rate, mechanical properties (such as hardness, compatibility, tableting properties, flow, blending), rheological parameters, etc.

Extensive studies are carried out in the pharmaceutical industry for the development of different polymorphs of various drug substances (R. Hilfiker (ed. 2019). Polymorphism in the Pharmaceutical Industry, Wiley-VCH, p. 1-30), to obtain suitable polymorphs or pseudo-polymorphs that possess improved performance characteristics. A general requirement for active ingredients in the development of a pharmaceutical composition is that the active ingredient has the appropriate physical, physico-chemical and chemical parameters. Another important feature is the ability to produce the selected form reproducibly, by means of a robust process with high yield and high purity.

The quality issues and marketing drawbacks of the occurrence of a new, stable polymorph in the commercial phase was demonstrated by the case of Ritonavir (D.K. Bucar, R.W. Lancester, J. Bernstein, Angew. Chem. Int. Ed., 2015, 54, 6972–6993). The more stable form is likely to be formed accidentally during prolonged storage of active ingredient or drug product or during elongated process times of a scale-up or technology transfer. Thus, in general, it is favorable to produce and use the most stable polymorph at commercial scale.

In the prior art Compound 1 is considered the best candidate of Estetrol as disclosed in WO2015/086643 (Donesta Bioscience B.V.). In WO2015/086643 (Donesta Bioscience B.V.) the powder X-ray diffraction pattern of a solid form of Estetrol monohydrate and the pattern of the same sample after heat treatment are disclosed.

In WO2004/041839, Estetrol monohydrate was produced from the reaction mixture in methanol by solvent evaporation and resuspension in water and chloroform. The solid form of the precipitate was not identified by XRPD. The isolation method described in WO2004/041839 is not feasible at commercial scale as the use of chloroform is highly disadvantageous. The same isolation method was described in WO2013/012328.

In WO2013/050553, Estetrol was isolated from the reaction mixture in acetone by the addition of water and the product was recrystallized from methanol-water mixture. Further details of the crystallization process are not disclosed, and the solid form of the precipitate was not identified.

In WO2013/034780, Estetrol was isolated from the reaction mixture in tetrahydrofuran inducing crystallization by the addition of water and evaporation of organic solvent. Solid form of the precipitate was not identified.

In WO2015/040051, Estetrol was isolated from the reaction mixture in methanol inducing crystallization by the addition of water in Example 10. In Example 11, 15 and 16 of WO2015/040051 Estetrol was extracted with tetrahydrofuran but further details of the crystallization process are not disclosed. In Example 17 of WO2015/040051 Estetrol is isolated from methanol and diisopropyl ether by evaporation of the organic solvent. Further details of the crystallization process are not disclosed, and the solid form of the precipitate was not identified.

In WO2021/044302, Estetrol monohydrate was produced via precipitation induced by the addition of water to a clear solution of Estetrol in methanol.

In WO2021/058716, Estetrol monohydrate was produced via precipitation induced by the addition of water to a clear solution of Estetrol in methanol. This process produces Estetrol monohydrate in the same form as disclosed in WO2015/086643. On the other hand, in WO2023/051937, Estetrol monohydrate was produced from isopropyl alcohol solution (after replacement of methanol) by the addition of water. XRPD pattern of the product proves the powder sample to be same polymorph of Estetrol monohydrate as disclosed in WO2015/086643.

Monari et. al. (Crystals 2023, 13, 1211) produced Estetrol monohydrate powder sample via precipitation induced by the addition of water to a clear solution of Estetrol in 2-propanol-water mixture at room temperature. XRPD pattern and detailed solid state characterization of the product proves the powder sample to be the same form of Estetrol monohydrate as disclosed in WO2015/086643.

Detailed crystallization process for the production of Estetrol monohydrate as disclosed in WO2015/086643 is available, e.g. in WO2021/058716 and WO2023/051937.

Said crystalline form is thus obtainable via these processes from a clear solution in methanol or isopropanol by the addition of water as antisolvent.

Although further isolation techniques form various organic solvents are also mentioned in e.g. WO2004/041839, WO2013/050553, WO2013/034780 and WO2015/040051 but the details of the crystallization processes therein are not given to the extent necessary for a reliable reproduction. The solid form of the products is not analyzed either.

Monari et. al published (Crystals 2023, 13, 1211) the single crystal structure of Estetrol monohydrate and disclosed the comparison of the diffraction pattern of the powder sample to the pattern simulated from single crystal data in Figure 2 parts c and d, respectively. Comparison of diffraction pattern simulated from single crystal data (curve d) and the pattern measured for powder sample (curve c) reveals slight but measurable differences of the two patterns: two additional peaks at 12.1 and 12.7° 2θ appear in the pattern simulated from the single data while the diffraction peaks at 13.1, 13.9 and 23.0° 2θ are characteristic in the pattern measured for the powder sample.

Based on the differences above, the X-ray diffraction pattern of Estetrol monohydrate disclosed in the prior art can be identified, it matches curve c. Accordingly, the powder sample in Crystals 2023, 13, 1211 is the same Estetrol monohydrate form as disclosed in WO2015/086643, to be referenced as Form 1 henceforward.

However, the single crystal structure published by Monari et. al is a different crystal form with the same monohydrate composition (12 Estetrol molecules and 12 water molecule per unit cell). This form is a polymorph of Estetrol monohydrate, to be referred to as Form 12 henceforward. Form 1 and Form 12 are two different polymorphs of the monohydrate of Compound 1.

Monari et. al. obtained the single crystal of Estetrol monohydrate by slow evaporation of the solvent from a clear solution in 2-propanol-water mixture. Scientists trained in the art of growing single crystals ensure slow changes, maintaining quasi stationary conditions to produce a few well-developed particles in days or weeks, using dilute solutions with low API content. Scaling up such a process to the laboratory or commercial scale is not feasible due to the increase in process time, high cost of solvents required and lack of proper control of the quasi-stationary conditions in larger volumes. Changing the parameters of such a process may result in a different polymorph. As a consequence, the single crystal growth method is not considered as a crystallization procedure feasible at commercial scale, even though it produced Form 12 single crystals.

In conclusion, Form 1 of Estetrol monohydrate (the same form as disclosed in WO2015/086643) is known in the art and all reliable experimental processes disclosed in the

prior art (WO2021/058716, WO2023/051937, Crystals 2023, 13, 1211) result in this form. Furthermore, Form 12 of Estetrol monohydrate is also known in the art, however, no industrial crystallization method therefor is available in the prior art.

Accordingly, there is an unmet need for providing a reproducible, robust and industrially scalable crystallization method for obtaining Form 12 of Estetrol monohydrate in high yield and high purity.

Summary of the invention

Comparative stability study of Form 1 and Form 12 of Estetrol monohydrate in slurry stirring experiments proved Form 12 to be the stable solid form of Estetrol monohydrate under ambient conditions in various solvents, e.g. the mixture of 2-propanol or ethanol or methanol and water. As proven by the case of Ritonavir, the more stable form is favorable for production at commercial scale in general and the risk of polymorph transformation during prolonged storage of the drug substance or the finished dosage form can mitigated by using the stable polymorph. Thus, in general, Compound 1 Form 12 being the stable polymorph raises a need to provide Compound 1 Form 12 by means of a robust crystallization method feasible for production on commercial scale. Only by such a robust and scalable process can any manufacturer supply Compound 1 Form 12 for pharmaceutical formulation and patient administration purposes.

The present disclosure relates to the crystallization of Compound 1, Form 12.

The process for crystallization produces Form 12 of Estetrol monohydrate reproducibly and is feasible for commercial production.

The main driving force of crystallization of Compound 1 Form 12 in the process described in the invention is cooling: the process starts form the clear solution of Estetrol at elevated temperature and by lowering the temperature a suspension is obtained in the same solvent. The process is carried out in a solvent mixture consisting of a water miscible organic solvent and water. The process of the invention incorporates a controlled cooling profile to ensure the robustness of the process with respect to yield, crystal form and purity. The controlled cooling profile may have a constant cooling rate or may have different stages with different cooling rates or may have different cooling steps with prescribed time limits. The cooling profile may include agitation periods at controlled temperature between different cooling stages. The process may also include additional optional steps to ensure robustness of the process, e.g. seeding. The process may also include additional steps to enhance the yield of the process e.g. dosing water to the suspension of Compound 1 Form 12.

Estetrol monohydrate Form 12 produced by the crystallization process is characterized by XRPD, having diffraction peaks at 12.1, 12.4, 12.7, 13.4, 20.7 and 20.8, (±0.1°) 2θ and devoid of peaks at 13.1, 13.9, 17.9, 18.5 and 23.0°.

Brief description of the drawings

Figure 1 illustrates the X-ray diffraction pattern of Form 1 Estetrol monohydrate.

Figure 2 illustrates the X-ray diffraction pattern of Form 12 Estetrol monohydrate.

Figure 3 illustrates the characteristic differences between the X-ray diffraction patterns of Form 1 and Form 12 Estetrol monohydrate for the 11.5° to 14.0° 2θ region. Arrows indicate the characteristic differences of Form 1 and Form 12.

Figure 4 illustrates the characteristic differences between the X-ray diffraction patterns of Form 1 and Form 12 Estetrol monohydrate for the 19.5° to 25.0° 2θ region. Arrows indicate the characteristic differences of Form 1 and Form 12.

Detailed description of the invention

For analytical studies, the following experimental conditions were used:

Parameters of X-ray powder diffraction measurements:

Instrument:	PANanalytical X'Pert PRO MPD
Radiation:	CuKα
Accelerating voltage:	40 kV
Anode current:	40 mA
Goniometer:	PW3050/60
Scanning speed:	0.0305 s
Increment:	0.0131 °
Sample holder:	PW1818/25 & 40 (transmission, sample between foils)
Sample holder spinner:	PW3064/60 (reflection/transmission spinner)
Spinning speed of sample holder:	1 spin/s
Detector:	PIXcel (PW3018/00)
Uncertainty of the 2θ measurement:	± 0.1 °

Parameters of TGA measurements:

Instrument:	TA Instruments TGA Q5000 or Discovery TGA 5500
Heating rate:	10 °C/min
Sample weight:	~ 2 to 10 mg
Atmosphere:	60 mL/min N_2

Parameters of DSC measurements:

Instrument:	TA Instruments DSC Q1000 or Discovery DSC 2500
Heating rate:	10 °C/min
Sample weight:	~ 1 to 3 mg
Pan type:	open Al pan
Atmosphere:	50 mL/min N_2

Parameters of KF measurements:

The test is performed by volumetric Karl Fischer semi-micro titration according to the prescriptions of the current Ph. Eur (2.5.12.).

Parameters of HPLC related substances measurements:

Sample solution	2 mg/mL (methanol and water (8:2))
Instrument:	Agilent 1260 HPLC
Column	Zorbax SB-C8
Eluents	A: water and formic acid (1000:1.0 V/V)
	B: methanol and formic acid (1000:1.0 V/V)
Flow rate:	1.0 mL/min
Detection wavelength:	280 nm

Gradient profile:

Time (min)	A%	B%
0	70	30
8	53	47
8.1	10	90
11	10	90
11.1	70	30
17	70	30

Method for the identification of Form 12 Estetrol monohydrate

Crystalline solid phases can be identified by X-ray diffraction: the powder X-ray diffraction pattern or the position of one or more diffraction peaks are characteristics of the crystalline material. Therefore, each crystalline form of Compound 1 of the present invention can be identified and distinguished from other crystalline forms by the characteristic XRPD diffraction peaks of the product.

Form 1 and Form 12 of Estetrol monohydrate were prepared as described in reference Example 1 and Example 2, respectively. Powder X-ray diffraction pattern of the two forms were measured and compared to the patterns disclosed in the article by Monari et. al. The experimental peak list of the two forms is summarized in Table 1.

Table 1. The most intensive peaks of Estetrol monohydrate Form 12 and Form 1.

Form 12 reference	Form 1 reference
6.747	
11.914	11.957
12.126	
12.418	12.452
12.739	
	13.127
13.439	13.478
	13.856
14.870	14.888
17.337	17.382
18.625	18.517

Form 12 reference	Form 1 reference
18.841	
19.855	19.806
20.067	20.077
20.655	
20.832	20.757
21.583	21.634
21.666	
22.220	
22.789	
	22.997
23.182	
23.324	
24.485	24.504
25.159	
	25.270
28.756	
30.669	30.658

X-ray diffraction pattern of the two polymorphic forms of Estetrol monohydrate are highly similar. Major diffraction peaks at 12.4 and 13.4°, and further minor peaks at 11.9, 19.9, 21.6 and 24.5° are common for the two forms. The characteristic differences between the two patterns include the splitting of the most intensive diffraction peak at 20.8° for Form 1 to 20.7° and 20.8° for Form 12 (Figures 3-4). Also, the minor peaks at 13.1, 13.9, 17.9, 18.5° and 23.0° are present in Form 1 but absent in Form 12 while the peaks at 12.1 and 12.7° 2θ are only detected in Form 12. As a consequence, the presence of peaks at 12.1 and 12.7° identifies Form 12 unambiguously and the absence of 13.1, 13.9, 17.9°, 18.5° and 23.0° excludes Form 1 in any sample. More specifically, Form 12 can be characterized by XRPD peaks at 12.1, 12.4, 12.7, 13.4, 20.7 and 20.8° (±0.1°) 2θ.

Water content of monohydrate forms can be assessed by various well-known analytical methods e.g. loss on drying (e.g. by TGA) or by Karl-Fisher titration. Stochiometric water content of Estetrol monohydrate is 5.6% thus Estetrol solid phase with about 5.1 to about 6.1% weight loss or water content is considered monohydrate herein.

Comparative stability of Form 1 and Form 12 Estetrol monohydrate

The appearance of Compound 1 Form 12 leads to the necessity to select the more stable polymorph under ambient conditions. According to the rule of phases of thermodynamics only one solid phase can be present in equilibrium in a two-phase system with given temperature, pressure and solvent composition (R. Hilfiker (ed. 2019) Polymorphism in the Pharmaceutical Industry, Wiley-VCH, p. 91-132). Also, in equilibrium, the stable polymorph will be present as the only solid phase. As a consequence, any mixture

of polymorphic forms in contact with a solvent evolving towards equilibrium will end up with the stable polymorph in that solvent system when equilibrium is reached.

Slurry stirring Compound 1 Form 1 or the mixture of the two monohydrate forms in various solvents, e.g. the mixture of 2-propanol or ethanol or methanol and water resulted in pure Compound 1 Form 12 between 0 and 75 °C (Examples 1-3). These experiments prove that the solubility of Form 12 Estetrol monohydrate is lower than that of Form 1 thus Form 12 is more stable than Form 1 in these systems. Both forms being monohydrates also make them two real polymorphs with the same composition, thus the slurry stirring experiments prove Compound 1 Form 12 to be more stable than Form 1 in general. It can be concluded that Form 12 is the stable solid form of Estetrol monohydrate under ambient conditions and also in the range defined by the experiments.

According to Ostwald's rule, the solid first formed on crystallization would usually be the least stable polymorph. By the laws of thermodynamics, in a system reaching equilibrium the most stable polymorph will be present. As a consequence, a crystallization process aiming at the metastable form always carries the risk of conversion to the stable form while a robust process aiming at the stable form must provide control to exclude or convert the metastable form to the desired one. For persons skilled in the art several tools are available for such a control, e.g. prolonged agitation, strictly controlled experimental conditions, seeding etc., but there is no way to predict which combination of the techniques will lead to the process providing the stable form of a given substance. In addition, the process must provide the stable polymorph with high yield and purity to be considered for scale up and the process must be time- and cost-effective at commercial scale.

The use of the stable polymorph in a finished dosage form has the advantage of mitigating the risk of unexpected polymorphic transformation during production. Thus, Compound 1 Form 12 being the stable polymorph raises a need to provide Compound 1 Form 12 by means of a robust crystallization method feasible for production on commercial scale. Only by such a robust and scalable process can any manufacturer supply Compound 1 Form 12 for pharmaceutical formulation and patient administration purposes.

Robust crystallization process for producing Form 12 Estetrol monohydrate

A crystallization process must be reproducible, robust with respect to all critical quality attributes including identity of crystal form. The process should produce the API with high yield, high chemical purity and within reasonable process time.

Estetrol monohydrate Form 12 was obtained by methods suitable for growing single crystals thus the structure could be determined (Monari et. al.). However, said method is not suitable for production of the same crystal form at laboratory or industrial scale.

Form 12 of Compound 1 can be obtained at laboratory scale by prolonged agitation of the slurry of Form 1 of Compound 1 in a suitable solvent at constant temperature (Example 1-3). The said method is suitable to obtain the required crystal form on the gram scale, but such a method is neither cost nor time effective and being such it is not feasible for production scale.

An aspect of the present invention is the crystallization process for Form 12 of Compound 1 from a clear solution at elevated temperature.

In an embodiment the invention is directed to a process for the preparation of Form 12 of Estetrol monohydrate comprising:

a.) dissolving Estetrol in a solvent mixture consisting of a water miscible organic solvent and water having a ratio at an elevated temperature to obtain a solution of Estetrol in said solvent mixture having a volume;

b.) cooling said solution to a final setpoint via at least one cooling stage to obtain a suspension of Form 12 Estetrol monohydrate;

c.) filtering the suspension to obtain a solid and washing said solid;

d.) drying the solid

wherein said Form 12 Estetrol monohydrate has characteristic XRPD peaks using CuKα radiation at 12.1, 12.4, 12.7, 13.4, 20.7 and 20.8° (±0.1°) 2θ.

The starting material, i.e. Estetrol in any form, used for producing the solution is obtained by any suitable preparative method, e.g. the process described in WO2021/044302 (Reference Example 1).

The solution in the process is a solution of Estetrol in a mixture of water and a water miscible solvent.

The water miscible solvent is selected from the group consisting of methanol, ethanol, 2-propanol, 1-propanol, 1-butanol, t-butyl alcohol, acetone, butanone, 2-pentanone, 3-pentanone, methyl butyl ketone, methyl ethyl ketone, methyl isobutyl ketone, methyl acetate, acetonitrile, tetrahydrofuran, 1,4-dioxane, 2-methoxyethanol. Preferably, the water miscible solvent is selected from the group consisting of methanol, ethanol, 2-propanol, acetone and 1-propanol. More preferably, the water miscible solvent is selected from the group consisting of methanol, ethanol and 2-propanol. Most preferably, the water miscible solvent is ethanol.

The amount of the water in the mixture is not more than about 50% by volume. More preferably the amount of water in the mixture is between about 25 to about 50% by volume. Most preferably the amount of water in the mixture is between about 30 and about 35% by volume.

The temperature of the solution, i.e. the elevated temperature, is between the clear point of the mixture and the reflux temperature of the solvent, thus spontaneous precipitation

of any crystalline form of Estetrol cannot occur. Preferably, the temperature of the solution is above room temperature. More preferably, the temperature of the solution is above about 50 °C. Most preferably the temperature of the solution is at or above about 55 °C.

The concentration of Estetrol in the solution above is between about 0.05 and about 0.5 g/mL. Preferably, the concentration of Estetrol in the solution above is between about 0.05 and about 0.25 g/mL. More preferably the concentration of Estetrol in the solution above is between about 0.05 and about 0.2 g/mL.

The present invention provides the process for the preparation of Form 12 of Compound 1 where crystallization is driven by cooling the clear solution of Compound 1.

The cooling of the solution is carried out in a controlled manner, e.g. in a pre-determined time period or with a pre-determined cooling rate. Cooling may be executed in multiple stages with optional additional steps between the stages. Each cooling stage in such multiple stage cooling is controlled by means of pre-determined cooling rate or time period. The final setpoint of the cooling profile can be room temperature or below room temperature e.g. about 0 to about 5 °C.

The present disclosure provides for a crystallization method that is initiated by cooling said clear solution.

In Example 6 of WO2023/051937, Estetrol monohydrate Form 1 is prepared by means of dosing water as an antisolvent to the clear solution of Estetrol in 2-propanol at 60-70 °C. The driving force of the process is the high supersaturation created by the addition of water as antisolvent to the clear solution. This process lacks a cooling step for the initiation of crystallization and results in Form 1 Estetrol monohydrate.

In Example 5 of WO2021/058716, 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 Form 1 Estetrol monohydrate.

In Example 5A of WO2021/044302, Estetrol monohydrate Form 1 is prepared by the addition of water as antisolvent and the removal of methanol as solvent to the mixture containing Estetrol. The driving force of the process is the replacement of solvent (methanol) by an antisolvent (water) at 20-25 °C. The process lacks a cooling step for the initiation of crystallization and results in Form 1 Estetrol monohydrate.

Reference examples above prove that antisolvent addition or solvent removal initiates the crystallization of Form 1 Estetrol monohydrate. In contrast, the present invention initiates

crystallization by cooling the clear solution of Estetrol. Accordingly, the cooling step is an essential element of the present invention to provide Form 12 Estetrol monohydrate.

In another aspect the present invention provides a process for the preparation of Form 12 of Compound 1 where the cooling produces a clear supersaturated solution of Estetrol, which is then seeded with Form 12 Estetrol monohydrate, and the subsequent cooling stage provides a suspension of Form 12 Estetrol monohydrate.

In another embodiment the invention is directed to a process for the preparation of Form 12 of Estetrol monohydrate comprising:

a.) dissolving Estetrol in a solvent mixture consisting of a water miscible organic solvent and water having a ratio at an elevated temperature to obtain a solution of Estetrol in said solvent mixture having a volume;

b.) cooling said solution to a final setpoint via at least one cooling stage to obtain a suspension of Form 12 Estetrol monohydrate;

wherein the cooling produces a supersaturated solution of Estetrol

and wherein the process further comprises

b2.) seeding said supersaturated solution of Estetrol at a seeding temperature with an amount of a seed crystal of Form 12 Estetrol monohydrate to obtain said suspension of Form 12 Estetrol monohydrate

c.) filtering the suspension to obtain a solid and washing said solid;

d.) drying the solid

wherein said Form 12 Estetrol monohydrate has characteristic XRPD peaks using CuKα radiation at 12.1, 12.4, 12.7, 13.4, 20.7 and 20.8° (±0.1°) 2θ.

The seed crystals used in the process are first obtained by slurry stirring Estetrol monohydrate Form 1 in a solvent mixture e.g. as described in Example 2. The seed crystal of Form 12 Estetrol monohydrate is obtained by a process comprising A) suspending Estetrol in a solvent mixture consisting of ethanol and water; B) agitating the suspension; C) optionally cooling the suspension to a temperature and further agitating; D) optionally filtering; and E) optionally drying. Preferably, in step A) the ratio of the mixture is 1:1. Preferably, in step B) the agitation lasts between about one hour to about seven days, and is carried out at a temperature between about 0°C to about 75°C. Preferably, in step C) the temperature to which the suspension is cooled is about 0°C. Preferably, in step E) the optional drying is carried out at reduced pressure optionally under nitrogen atmosphere, and at a temperature between about 25 °C to about 35°C. Optionally, the seed crystals used in the process, especially at commercial scale, are obtained by the process described by the invention, e.g. Examples 4, 5, 6, 7 or 8, 9, 10, or, optionally, by any other method feasible to produce Form 12 Estetrol monohydrate.

The amount of seed crystals used in the process is in the range of about 0.05 to about 5% of the mass of Estetrol in the initial clear solution. Preferably the amount of seed crystals is between about 0.1 and about 2% of the mass of Estetrol in the initial clear solution. More preferably, the amount of seed crystals is between about 0.5 and about 1.5% of the mass of Estetrol in the initial clear solution.

The temperature of the solution at seeding is between room temperature and the elevated temperature of the solution at the start of the cooling process. Preferably, the temperature of the solution at seeding is between about 40 and about 70 °C. More preferably the temperature of the solution at seeding is between about 45 and about 55 °C.

The seeding of the supersaturated solution may induce instant crystallization, or it may initiate a slow crystallization process. Either way, seeding promotes the precipitation of the desired form thus enhancing the robustness of the process. An intermittent isothermal agitation period, e.g. about 30 min or about 1 hour or about 2 hours, may be applied before the next stage of cooling. At high supersaturation around the seeding temperature the formation of undesired Estetrol monohydrate Form 1 crystals may occur, and the faster crystallization of Form 1 may result in a mixture of Form 1 and Form 12 of Compound 1. Limited agitation will promote the formation of the stable form while longer periods would increase process time unnecessarily.

In another embodiment the invention is directed to a process for the preparation of Form 12 of Estetrol monohydrate comprising:

a.) dissolving Estetrol in a solvent mixture consisting of a water miscible organic solvent and water having a ratio at an elevated temperature to obtain a solution of Estetrol in said solvent mixture having a volume;

b.) cooling said solution to a final setpoint via at least one cooling stage to obtain a suspension of Form 12 Estetrol monohydrate;

wherein the cooling produces a supersaturated solution of Estetrol

and wherein the process further comprises

b2.) seeding said supersaturated solution of Estetrol at a seeding temperature with an amount of a seed crystal of Form 12 Estetrol monohydrate to obtain said suspension of Form 12 Estetrol monohydrate

b3.) an agitation carried out after the seeding step b2.) for a period

c.) filtering the suspension to obtain a solid and washing said solid;

d.) drying the solid

wherein said Form 12 Estetrol monohydrate has characteristic XRPD peaks using CuKα radiation at 12.1, 12.4, 12.7, 13.4, 20.7 and 20.8° (±0.1°) 2θ.

The process of the present invention incorporates a controlled cooling step after seeding (with or without an intermittent agitation period). The setpoint of the cooling step is between the final isolation temperature and seeding temperature. Preferably, the setpoint of the cooling step after seeding is between about 10 and about 30 °C. More preferably, the setpoint of the cooling step after seeding is between about 20 and about 30 °C.

The cooling step inducing the formation of crystals, e.g. after seeding or after sponateous nucleation, is essential for ensuring the robustness of the process with respect to producing Form 12 Estetrol monohydrate. The cooling of the solution is carried out in a controlled manner, e.g. in a pre-determined time period or with a pre-determined average cooling rate. Preferably, the pre-determined time period for the cooling is between about 30 and 120 minutes. Preferably, the average cooling rate after seeding is between about 0.5 and about 2 °C/minutes.

In another embodiment the invention is directed to a process for the preparation of Form 12 of Estetrol monohydrate comprising:

a.) dissolving Estetrol in a solvent mixture consisting of a water miscible organic solvent and water having a ratio at an elevated temperature to obtain a solution of Estetrol in said solvent mixture having a volume;

b.) cooling said solution to a final setpoint via at least one cooling stage to obtain a suspension of Form 12 Estetrol monohydrate;

wherein the cooling produces a supersaturated solution of Estetrol

and wherein the process further comprises

b2.) seeding said supersaturated solution of Estetrol at a seeding temperature with an amount of a seed crystal of Form 12 Estetrol monohydrate to obtain said suspension of Form 12 Estetrol monohydrate

b3.) an agitation carried out after the seeding step b2.) for a period

b4.) a further cooling step carried out after step b3.) having an average cooling rate and a further setpoint

c.) filtering the suspension to obtain a solid and washing said solid;

d.) drying the solid

wherein said Form 12 Estetrol monohydrate has characteristic XRPD peaks using CuKα radiation at 12.1, 12.4, 12.7, 13.4, 20.7 and 20.8° (±0.1°) 2θ.

In another aspect of the present invention the yield of the crystallization process is increased by dosing antisolvent to the suspension obtained in previous stages of the cooling profile. The antisolvent of the invention is water. The volume of water added is less than about 200% of the initial volume of the solution. Preferably, the volume of water is less than

about 150% of the initial volume of the solution. More preferably, the volume of water is between about 80 and about 120% of the initial volume of the solution.

In another embodiment the invention is directed to a process for the preparation of Form 12 of Estetrol monohydrate comprising:

a.) dissolving Estetrol in a solvent mixture consisting of a water miscible organic solvent and water having a ratio at an elevated temperature to obtain a solution of Estetrol in said solvent mixture having a volume;

b.) cooling said solution to a final setpoint via at least one cooling stage to obtain a suspension of Form 12 Estetrol monohydrate;

wherein the cooling produces a supersaturated solution of Estetrol

and wherein the process further comprises

b2.) seeding said supersaturated solution of Estetrol at a seeding temperature with an amount of a seed crystal of Form 12 Estetrol monohydrate to obtain said suspension of Form 12 Estetrol monohydrate

b3.) an agitation carried out after the seeding step b2.) for a period

b4.) a further cooling step carried out after step b3.) having an average cooling rate and a further setpoint

b5.) dosing an amount of water as antisolvent to the suspension of Form 12 Estetrol monohydrate

c.) filtering the suspension to obtain a solid and washing said solid;

d.) drying the solid

wherein said Form 12 Estetrol monohydrate has characteristic XRPD peaks using CuKα radiation at 12.1, 12.4, 12.7, 13.4, 20.7 and 20.8° (±0.1°) 2θ.

In another aspect the present invention provides a process for the preparation of Form 12 of Compound 1 where the reproducibility and the yield of the process is enhanced by an isothermal agitation period at certain point of the process, such as after seeding and/or after addition of the antisolvent, and/or at the end of the cooling profile. Such periods are limited in time, e.g. agitation is for about 30 to 120 minutes.

In another embodiment the invention is directed to a process for the preparation of Form 12 of Estetrol monohydrate comprising:

a.) dissolving Estetrol in a solvent mixture consisting of a water miscible organic solvent and water having a ratio at an elevated temperature to obtain a solution of Estetrol in said solvent mixture having a volume;

b.) cooling said solution to a final setpoint via at least one cooling stage to obtain a suspension of Form 12 Estetrol monohydrate;

wherein the cooling produces a supersaturated solution of Estetrol and

wherein the process further comprises

b2.) seeding said supersaturated solution of Estetrol at a seeding temperature with an amount of a seed crystal of Form 12 Estetrol monohydrate to obtain said suspension of Form 12 Estetrol monohydrate

b3.) an agitation carried out after the seeding step b2.) for a period

b4.) a further cooling step carried out after step b3.) having an average cooling rate and a further setpoint

b5.) dosing an amount of water as antisolvent to the suspension of Form 12 Estetrol monohydrate

b6.) optionally agitating the suspension at isothermal conditions for a period after the cooling in step b), after the seeding in step b2.), after the further cooling in step b4.), or after dosing antisolvent in step b5.) or after step b4.)

c.) filtering the suspension to obtain a solid and washing said solid;

d.) drying the solid

wherein said Form 12 Estetrol monohydrate has characteristic XRPD peaks using CuKα radiation at 12.1, 12.4, 12.7, 13.4, 20.7 and 20.8° (±0.1°) 2θ.

In another aspect of the present invention the product, Estetrol monohydrate Form 12, is isolated below room temperature. Preferably, the product, Estetrol monohydrate Form 12 is isolated between about 0 and about 5 °C.

The cooling of the suspension is carried out in a controlled manner e.g. in a pre-determined time period or with a pre-determined average cooling rate. Preferably, the average cooling rate is between about 0.5 and about 2 °C/min. Preferably, the pre-determined time period for the cooling is between about 30 and 120 minutes.

The process of the present invention can be implemented at laboratory on a few gram scale. One of the implementations is demonstrated in Example 4 resulting in Form 12 Estetrol monohydrate. The process was repeated multiple times with different organic solvents and varying critical parameters of the cooling profile including temperature setpoints, cooling rates and isothermal agitation times (e.g. Examples 5 to 8). Each experiment resulted in Form 12 Estetrol monohydrate proving the process to be robust with respect to varying cooling profile parameters in several organic solvents including but not limited to methanol, ethanol and 2-propanol. Water content of the product varied between 5.7 and 6.0% proving each sample to be stochiometric monohydrate. The yield of the experiments was high, above 90% in each experiment and sum of impurities was less than 1% in each experiment.

The process of the present invention was repeated multiple times at 4 L volume, e.g. as described in Example 9. The crystal form of the product is Form 12 Estetrol monohydrate proving the process to be robust with respect to scale up.

The process of the present invention was implemented at commercial scale multiple times, e.g. as described in Example 10. The product was isolated with high yield (above 85%) and purity (total impurities: <1%) and its crystal form is Form 12 Estetrol monohydrate by XRPD proving the process to be applicable for commercial production.

Reference examples

Reference example 1

Preparation of Form 1 Estetrol monohydrate according to WO2021/044302

Preparation: 18.0 g of (15α, 16α, 17β)-3-hydroxyestra-1,3,5(10)-triene-15, 16, 17-triyl-triacetate was suspended in 538 mL of methanol at 20-25 °C under N_2 atmosphere, then 18.0 g potassium carbonate was added portion wise and stirred for 5 hours. The reaction was monitored by TLC.

Work-up: 14.28 mL of cc acetic acid was added to the mixture and stirred after adding 190 mL of water. The methanol was removed by distillation and the product precipitated. The obtained suspension was agitated at 0-5 °C for 1 hour, filtered and washed with water (2 x 20 mL) on the filter. It was dried at 40 °C under vacuum to constant weight. Thus, 12.34 g of white crystal product was obtained as Estetrol monohydrate.

Crystal form by XRPD: Form 1

Reference example 2

Preparation of Form 1 Estetrol monohydrate according to WO2021/058716

8 g of Estetrol was dissolved in 96 mL of methanol and 240 mL of water were dripped into the solution thus prepared. The system was concentrated at reduced pressure until the methanol was completely removed. The suspension was kept under stirring at 15 °C for 30 minutes and the solid filtered on büchner washing with 56 mL of water. The solid was dried at reduced pressure at 45 °C for 6 hours.

Crystal form by XRPD: Form 1, TG: 5.7%

The present invention will be further illustrated by the following Examples without limiting the scope of the present invention to them. From the above description and from the Examples, the skilled person in the art may ascertain the essential features of the invention and without departing from its essence and scope, may make certain changes and modifications in order to adapt the invention to various applications and conditions. As a result, the invention is not limited to the following illustrative examples, but rather to the scope determined by the appended claims.

In general, the crystallization process for obtaining Form 12 of Compound 1 can be carried out according to the common general knowledge of the person skilled in the art based on the methods described for the working examples. Accordingly, suitable solvents, temperatures, and other reaction conditions can be easily selected by the person skilled in

the art. Starting materials are commercially available and/or can be easily prepared by the person skilled in the art according to the literature.

Examples

Example 1

Preparation of Form 12 Estetrol monohydrate

1.004 g of Estetrol monohydrate (Form 1) was suspended in 7 mL of methanol-water 1:1 mixture and agitated at 50-60°C for 5 days. The resulting suspension was filtered and dried at 25°C at reduced pressure to constant weight. 0.67 g (66.7%) of white crystalline product was obtained.

Crystal form by XRPD: Form 12; TG: 5.8%; DSC: m.p. 245.2 °C.

Example 2

Preparation of Form 12 Estetrol monohydrate

20.7 g Estetrol monohydrate (Form 1) was suspended in 100 mL of ethanol-water 1:1 mixture and agitated at 60°C jacket for one day. The suspension was cooled to 0°C and agitated further for 1 hour. Crystals were filtered and dried at 35°C at reduced pressure to constant weight. 12.2 g (58.9%) of white crystalline product was obtained.

Crystal form by XRPD: Form 12, TG: 6.0%; KF: 6.0%; DSC: m.p. 245.0 °C; total impurities by HPLC: 0.05%.

Example 3a

Preparation of Form 12 Estetrol monohydrate

0.14 g Estetrol monohydrate (Form 1 + Form 12) was suspended in 0.4 mL 2-propanol-water 1:1 mixture and agitated at 75°C jacket for one day. The resulting suspension was filtered, and the wet products were measured by XRPD without drying.

Crystal form by XRPD: Form 12.

Example 3b

Preparation of Form 12 Estetrol monohydrate

0.059 g Estetrol monohydrate (Form 1 + Form 12) was suspended in 0.4 mL 2-propanol-water 1:1 mixture and agitated at 0°C jacket for seven days. The resulting suspension was filtered, and the wet products were measured by XRPD without drying.

Crystal form by XRPD: Form 12.

Example 3c

Preparation of Form 12 Estetrol monohydrate

0.04 g Estetrol monohydrate (Form 1 + Form 12) was suspended in 0.4 mL 2-propanol-water 1:1 mixture and agitated at 75°C jacket for seven days. The resulting suspension was filtered, and the wet products were measured by XRPD without drying.

Crystal form by XRPD: Form 12.

Example 4

Preparation of Form 12 Estetrol monohydrate

5.4 g Estetrol monohydrate (Form 1) was dissolved in 40 mL ethanol-water 7:3 mixture at 63-67°C under N_2 atmosphere. The solution was cooled to 55 °C in 10 minutes, seeded with 55 mg of Estetrol monohydrate Form 12. After 30 minutes of agitation at the same temperature the mixture was cooled to 25 °C in 60 minutes. At 25 °C 40 mL water was dosed to the suspension, then it was cooled to 0°C, agitated for half an hour, filtered, and washed with 2 x 5 mL water. The product was dried at 35 °C at reduced pressure to constant weight. 5.1 g of white crystals was obtained (yield: 93.6%).

Crystal form by XRPD: Form 12, TG: 5.9%; DSC: m.p. 245.1°C.

Example 5

Preparation of Form 12 Estetrol monohydrate

3.2 g Estetrol monohydrate (Form 1) was dissolved in 40 mL methanol-water 7:3 mixture at 55-60°C. The solution was cooled to 45 °C in 10-15 minutes, seeded with 32 mg of Estetrol monohydrate Form 12. After 30 minutes of agitation at 45 °C the mixture was cooled to 35 °C in 20 minutes, agitated at 35 °C for 30 minutes, cooled to 25 °C in 20 minutes and agitated at 25 °C for 30 minutes. At 25°C 40 mL water was dosed in 60 minutes and the suspension was agitated for 30 minutes again before cooling to 0°C in 50 minutes. The final suspension was agitated at 0 °C for 60 minutes, the solid was filtered, washed with 2×5 mL water, and dried at 25°C at reduced pressure to constant weight. 2.85 g (89.2%) of white crystalline product was obtained.

Crystalline form by XRPD: Form 12; TG: 6.0%; DSC: m.p. 245.2°C.

Example 6

Preparation of Form 12 Estetrol monohydrate

5.4 g Estetrol monohydrate (Form 1) was dissolved in 40 mL ethanol-water 7:3 mixture at 63-67°C under N_2 atmosphere. The solution was cooled to 51.5 °C in 14 minutes, seeded with 54 mg of Estetrol monohydrate Form 12. After 30 minutes of agitation at the same temperature 40 mL water was dosed to the suspension and it was cooled to 0°C in 103 minutes, agitated for 30 minutes, filtered, washed with 2 x 5 mL water. The product was dried at 35°C at reduced pressure to constant weight. 5.0 g (92.8%) of white crystalline product was obtained.

Crystal form by XRPD: Form 12; TG: 5.8%; GC: 150 ppm ethanol.

Example 7

Preparation of Form 12 Estetrol monohydrate

4.75 g Estetrol monohydrate (Form 1) was dissolved in 25 mL 2-propanol-water 53:47 mixture at 75°C. The solution was cooled to 70°C in 5 minutes, seeded with 48 mg Estetrol

monohydrate Form 12, cooled to 0°C in 70 minutes and the suspension was agitated at 0-5 °C for 60 minutes. The solid was filtered and dried at 25°C at reduced pressure to constant weight. 3.99 g (84.0%) of white crystalline product was obtained.

Crystal form by XRPD: Form 12; TG: 5.8%; DSC: m.p. 245.1°C, total impurities by HPLC: 0.11%.

Example 8

Preparation of Form 12 Estetrol monohydrate

4.80 g Estetrol monohydrate (Form 1) was dissolved in 30 mL isopropanol-water 65:35 mixture at 70-75°C under N_2 atmosphere. The solution was cooled to 63.5°C, seeded with 48 mg Estetrol monohydrate Form 12, agitated at 63.5 °C for 20 minutes, cooled to 25°C in 40 minutes. At 25 °C 35 mL water was dosed in 35 minutes and the suspension was cooled to 0 °C in 25 minutes, agitated there for 60 minutes. The solid was filtered, washed with 2 x 5 mL water, and dried at 35°C at reduced pressure to constant weight. 4.51 g (93.9%) of white crystalline product was obtained.

Crystal form by XRPD: Form 12; TG: 5.7%, total impurities by HPLC: 0.31%.

Example 9

Preparation of Form 12 Estetrol monohydrate

265.4 g Estetrol monohydrate (Form 1) was dissolved in 1963 mL ethanol-water 7:3 mixture at 65-70°C under N_2 atmosphere. The solution was cooled to 51.5°C in 10 minutes, seeded with 2.6 g Estetrol monohydrate Form 12. After 30 minutes of agitation at 52 °C the suspension was cooled to 25°C in 40 minutes. At 25 °C 1963 mL water was dosed to the suspension, then it was cooled to 0°C in 35 minutes, agitated for one hour, filtered, washed with 2×260 mL water, and dried at 35°C at reduced pressure to constant weight. 261.2 g (98.4%) of white crystalline product was obtained.

Crystal form by XRPD: Form 12; KF: 6.0%, total impurities by HPLC: 0.05%.

Example 10

Preparation of Form 12 Estetrol monohydrate (industrial scale)

84.8 kg Estetrol monohydrate (Form 1) was dissolved in the mixture of 327.5 kg ethanol and 188 kg water at 65-70°C under N_2 atmosphere. The solution was cooled to 54°C in 15 minutes, seeded with 0.85 kg Estetrol monohydrate Form 12. After 30 minutes of agitation at 49-54 °C the suspension was cooled to 26°C in 44 minutes. At 23-25 °C 628.1 kg water was dosed to the suspension in 67 minutes, then it was cooled to 0-5°C in 85 minutes, agitated for one hour, filtered, washed with 2× 100 kg water, and dried at 30°C until the water content is between 5.5-6.0% (5.8%) as measured by Karl-Fischer method. 83.7 kg (97.7%) of white crystalline product was obtained.

Crystal form by XRPD: Form 12; total impurities by HPLC: 0.05%

Claims

1. A process for the preparation of Form 12 of Estetrol monohydrate comprising:

a.) dissolving Estetrol in a solvent mixture consisting of a water miscible organic solvent and water having a ratio at an elevated temperature to obtain a solution of Estetrol in said solvent mixture having a volume;

b.) cooling said solution to a final setpoint via at least one cooling stage to obtain a suspension of Form 12 Estetrol monohydrate;

c.) filtering the suspension to obtain a solid and washing said solid;

d.) drying the solid

wherein said Form 12 Estetrol monohydrate has characteristic XRPD peaks using CuKα radiation at 12.1, 12.4, 12.7, 13.4, 20.7 and 20.8° (±0.1°) 2θ.

2. The process according to claim 1 wherein in step a.) the water miscible solvent is selected from the group consisting of methanol, ethanol, 2-propanol, 1-propanol, 1-butanol, t-butyl alcohol, acetone, butanone, 2-pentanone, 3-pentanone, methyl butyl ketone, methyl ethyl ketone, methyl isobutyl ketone, methyl acetate, acetonitrile, tetrahydrofuran, 1,4-dioxane, and 2-methoxyethanol.

3. The process according to claim 2 wherein in step a.) the water miscible alcohol is ethanol.

4. The process according to any one of claims 1 to 3, wherein in step a.) the amount of the water in the solvent mixture is not more than about 50 % by volume.

5. The process according to claim 4, wherein in step a.) the amount of the water in the solvent mixture is between about 30 and about 35 % by volume.

6. The process according to any one of claims 1 to 5, wherein in step a.) the elevated temperature is above room temperature.

7. The process according to claim 6, wherein in step a.) the elevated temperature is at or above about 55 °C.

8. The process according to any one of claims 1 to 7, wherein in step a.) the concentration of Estetrol in the solution is between about 0.05 and about 0.5 g/mL.

9. The process according to claim 8, wherein in step a.) the concentration of Estetrol in the solution is between about 0.05 and about 0.2 g/mL.

10. The process according to any one of claims 1 to 9, wherein in step b.) the final setpoint is at or below room temperature.

11. The process according to claim 10, wherein in step b.) the final setpoint is between about 0°C and about 5°C.

12. The process according to any one of claims 1 to 11, wherein in step b.) the cooling produces a supersaturated solution of Estetrol and wherein the process further comprises step b2.) seeding said supersaturated solution of Estetrol at a seeding temperature with an amount of a seed crystal of Form 12 Estetrol monohydrate to obtain said suspension of Form 12 Estetrol monohydrate.

13. The process according to claim 12, wherein the amount of the seed crystal of Form 12 Estetrol monohydrate is between about 0.5 and about 1.5% of the mass of Estetrol in the solution in step a.).

14. The process according to any one of claims 12 to 13, wherein the seeding temperature is between about 45°C and about 55°C.

15. The process according to any one of claims 12 to 14, wherein the seed crystal of Form 12 Estetrol monohydrate is obtained by the process according to claim 1 to 14 or a process comprising:

A) suspending Estetrol in a solvent mixture consisting of ethanol and water;

B) agitating the suspension;

C) optionally cooling the suspension to a temperature and further agitating;

D) optionally filtering; and

E) optionally drying.

16. The process according to claim 15, wherein

in step A) the ratio of the mixture is 1:1;

in step B) the agitation

- lasts between about one hour to about seven days, and
- is carried out at a temperature between about 0°C to about 75°C;

in step C) the temperature to which the suspension is cooled is about 0°C;

in step E) the optional drying is carried out

- at reduced pressure optionally under nitrogen atmosphere, and
- at a temperature between about 25 °C to about 35°C.

17. The process according to any one of claims 12 to 16, wherein the process further comprises

step b3.) an agitation carried out after the seeding step b2.) for a period.

18. The process according to claim 17, wherein in step b3.) the period lasts about 30 to about 120 minutes.

19. The process according to any one of claims 12 to 18, wherein the process further comprises

step b4.) a further cooling step carried out after step b3.) having an average cooling rate and a further setpoint.

20. The process according to claim 19, wherein in step b4.) the average cooling rate is between about 0.5 °C and about 2 °C/minutes.

21. The process according to any one of claims 19 to 20, wherein in step b4.) the further setpoint is between about 20 °C and about 30 °C.

22. The process according to any one of claims 1 to 21, wherein the process further comprises

step b5.) dosing an amount of water as antisolvent to the suspension of Form 12 Estetrol monohydrate.

23. The process according to claim 22, wherein in step b5.) the amount of water is between about 80 and about 120% of the volume of the solution obtained at step a.).

24. The process according to any one of claims 1 to 23, wherein the process further comprises

step b6.) optionally agitating the suspension at isothermal conditions for a period after the cooling in step b.), after the seeding in step b2.), after the further cooling in step b4.), or after dosing antisolvent in step b5.).

25. The process according to claim 24, wherein in step b6.) the agitating lasts about 30 to about 120 minutes.

26. The process according to any one of claims 1 to 25, wherein step c.) is carried out at a temperature between about 0°C and about 5°C.

27. The process according to any one of claims 1 to 26, wherein step d.) is carried out at a temperature between about 25°C and about 40°C.

28. The process according to any one of claims 1 to 27, wherein step d.) is carried out at reduced pressure.

29. The process according to any one of claims 1 to 28, wherein step d.) is carried out to constant weight or until the water content is between about 5.1 to about 6.1 % as measured by Karl-Fischer method.

30. A pharmaceutical composition comprising Form 12 of Estetrol monohydrate obtained by the process according to any one of the claims 1 to 29.