

### Liquid Cetrorelix composition

The present invention refers to a liquid pharmaceutical Cetrorelix composition. The liquid  
5 pharmaceutical composition comprises and preferably consists of Cetrorelix (salt), a  
stabilizing agent selected from poloxamer having an average molecular weight of 7,000-  
12,000 g/mol, benzyl alcohol, or a mixture thereof, an osmotic agent, and a  
pharmaceutically acceptable solvent comprising an organic acid.

10 Moreover, the present invention refers to a pharmaceutical dosage form comprising the  
liquid pharmaceutical composition of the invention in a container, such as a vial or an  
injection device, e.g., a pre-filled syringe.

In a further aspect, the present invention refers to the liquid pharmaceutical composition or  
15 pharmaceutical dosage form according to the invention for use in inhibiting premature  
luteinizing hormone surges in woman undergoing controlled ovarian stimulation or for use  
in preventing premature ovulation during controlled ovarian stimulation, for example, for use  
in preventing premature ovulation during controlled ovarian stimulation, followed by oocyte  
pick-up and assisted reproductive technique.

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In still a further aspect, the present invention refers to a method for preparing the liquid  
pharmaceutical composition or pharmaceutical dosage form according to the invention, the  
method comprising the steps of providing a first composition comprising a stabilizing agent  
selected from poloxamer having an average molecular weight of 7,000-12,000 g/mol,  
25 benzyl alcohol, or a mixture thereof, and an osmotic agent, preferably D-mannitol; providing  
a second composition comprising or consisting of Cetrorelix (salt), preferably Cetrorelix  
acetate; mixing the first composition and the second composition to obtain a liquid  
composition, and optionally adding an organic acid to the mixture obtained, wherein a  
pharmaceutically acceptable solvent is present in the first composition and/or wherein a  
30 pharmaceutically acceptable solvent is present in the second composition.

Cetrorelix (N-acetyl-3-(2-naphthyl)-D-alanyl-4-chloro-D-phenylalanyl-3-(3-pyridyl)-D-  
alanyl-L-seryl-L-tyrosyl-D-citrullyl-L-leucyl-L-arginyl-L-prolyl-D-alaninamide) is a  
decapeptide with gonadotropin-releasing hormone (GnRH) antagonistic activity. It blocks  
35 the effects of luteinising-hormone releasing hormone (LHRH) in the body. LHRH controls  
the production and release of luteinising hormone (LH), which causes ovulation. During  
fertility treatment, ovarian stimulation is used to make the ovaries produce more egg cells.  
By blocking the effect of LHRH, Cetrorelix stops the production of LH, and therefore

prevents premature ovulation, which can result in the release of egg cells that are immature and unsuitable for use in techniques such as in vitro fertilisation (IVF).

5 A pharmaceutically acceptable Cetorelix salt, Cetorelix acetate, is currently marketed in the form of a freeze-dried powder co-packed with water for injection to reconstitute the powder and to obtain a solution for injection (Cetrotide®). Each powder vial contains 0.25 mg Cetorelix acetate and mannitol. The pH of the reconstituted solution is 4.0-6.0. Due to stability reasons, the reconstituted solution is for immediate use only.

10 There is thus a need to provide a stable liquid composition comprising Cetorelix, which can be provided as a ready-to-use pharmaceutical dosage form without the need of reconstitution prior to administration.

15 US 2013/0303464 A1 discloses a pharmaceutical preparation of Cetorelix acetate, further comprising glacial acetic acid, a tonicity agent, and water. The pH of the preparation is adjusted to about 3.

20 The present inventors surprisingly found that liquid Cetorelix compositions can be stabilized by the incorporation of particular stabilizing agents. It is therefore an object of the present invention to provide an improved liquid pharmaceutical preparation of Cetorelix, having high stability and prolonged shelf life. Further, by providing stable liquid Cetorelix compositions, the administration of Cetorelix is more user friendly and the supply-chain complexity is reduced in comparison to solid Cetorelix compositions.

25 One aspect of the present invention is a liquid pharmaceutical composition, comprising Cetorelix or a pharmaceutically acceptable salt thereof, a stabilizing agent selected from poloxamer having an average molecular weight of 7,000-12,000 g/mol, benzyl alcohol, or a mixture thereof, an osmotic agent, and a pharmaceutically acceptable solvent comprising an organic acid.

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The pharmaceutical composition comprises Cetorelix or a pharmaceutically acceptable salt thereof as an active agent. The pharmaceutically acceptable Cetorelix salt preferably is Cetorelix acetate. The amount of Cetorelix (salt) present in the pharmaceutical composition can be adapted dependent on the pharmaceutical requirements. In one  
35 embodiment, the liquid pharmaceutical composition comprises Cetorelix or a pharmaceutically acceptable salt thereof in an amount of 0.10 – 0.50 mg/ml, such as 0.10 – 0.50 mg/ml Cetorelix or 0.10 – 0.50 mg/ml Cetorelix acetate. In a preferred embodiment,

the liquid pharmaceutical composition comprises an amount of 0.2 – 0.3 mg/ml Cetorelix pure peptide in its acetate salt form. The Cetorelix amount may be 0.25 mg/ml pure peptide in its acetate salt form.

5 The pharmaceutical composition further comprises a stabilizing agent selected from poloxamer having an average molecular weight of 7,000-12,000 g/mol, benzyl alcohol, or a mixture thereof. In one embodiment, the pharmaceutical composition comprises poloxamer having an average molecular weight of 7,000-12,000 g/mol as a stabilizing agent, preferably poloxamer having an average molecular weight of 7,500-10,000 g/mol. Poloxamers are  
10 non-ionic triblock copolymers composed of a central hydrophobic chain of polyoxypropylene (poly(propylene oxide)) flanked by two hydrophilic chains of polyoxyethylene (poly(ethylene oxide)). A suitable poloxamer stabilizing agent according to the present invention is poloxamer 188 (having an average molecular weight of 7680-9510 g/mol). In another embodiment, the pharmaceutical composition comprises benzyl alcohol as a stabilizing  
15 agent.

Additional stabilizing agents are not required in the liquid Cetorelix composition according to the present invention. Particularly, the liquid pharmaceutical composition according to the invention is preferably free of polysorbate and/or cyclodextrin.

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The stabilizing agent may be comprised in the pharmaceutical composition in an amount of 0.5-20.0 mg/ml, preferably 1.0-15.0 mg/ml. The unit mg/ml as used herein defines the amount of a compound in mg relative to the total volume of the pharmaceutical composition in ml. For example, the stabilizing agent may be poloxamer 188 comprised in an amount of  
25 0.5-10.0 mg/ml, preferably 1.0-5.0 mg/ml, or benzyl alcohol comprised in an amount of 5.0-20.0 mg/ml, preferably 7.5-15.0 mg/ml.

The stabilizing agent serves to improve the stability of the liquid pharmaceutical composition according to the invention. It was surprisingly found that the present stabilizing agents allow  
30 for the provision of a particularly stable liquid Cetorelix composition. Specifically, the presence of aggregates, particles and impurities is reduced or even avoided. By this means, relevant pharmaceutical properties such as pH, Cetorelix protein content, and osmolality are maintained during storage. Even when subjected to external stress such as mechanical or thermal impact, the liquid pharmaceutical Cetorelix composition according to the present  
35 invention exhibits desirable pharmaceutical properties and stability.

The liquid pharmaceutical composition further comprises an osmotic agent. Suitable osmotic agents may be selected from the group consisting of mannitol, glycerol, sorbitol, dextrose, sucrose, trehalose, or a combination thereof, preferably D-mannitol, sorbitol, or trehalose. In a particularly preferred embodiment, the osmotic agent is D-mannitol. The osmotic agent may be comprised in the pharmaceutical composition in an amount of 30.0-60.0 mg/ml, preferably 35.0-55.0 mg/ml.

The osmotic agent serves to adapt the osmolality of the liquid pharmaceutical composition according to the invention. The osmolality of the pharmaceutical composition may be 250-375 mOsm/kg, preferably 300-350 mOsm/kg. Specifically, the osmolality may be adapted according to the desired route of administration.

In a preferred embodiment, the pharmaceutical composition is for parenteral administration. Parenteral administration comprises intravenous, intramuscular, subcutaneous, and intradermal administration, such as injection and infusion, preferably subcutaneous injection. A suitable osmolality for parenteral pharmaceutical compositions is 250-375 mOsm/kg, preferably 300-350 mOsm/kg.

The liquid pharmaceutical composition further comprises a pharmaceutically acceptable solvent comprising an organic acid. The organic acid may be any suitable organic acid and is preferably selected from acetic acid, lactic acid, glutamic acid, gluconic acid, succinic acid, and a mixture thereof, more preferably acetic acid. The pharmaceutically acceptable solvent may comprise the organic acid in the form of an acidic buffer or a free organic acid. Additionally, the solvent may comprise water, such as water for injection. In a preferred embodiment, the pharmaceutically acceptable solvent is an aqueous organic acid or an aqueous acidic buffer, preferably an aqueous organic acid. In one embodiment, the concentration of the organic acid (e.g. the concentration of the organic acid) in the liquid pharmaceutical composition is from about 1mM to about 20 mM, such as of from about 1 mM to about 10 mM, for example of from about 1 mM to about 5 mM, such as about 2 mM. In one embodiment, the concentration of the organic acid (e.g. the concentration of acetic acid) in the liquid pharmaceutical composition is about 2 mM.

An acidic buffer according to the present invention comprises the organic acid and a salt thereof (in dissolved form). Suitable salts are metal salts, such as alkali metal salts, e.g., sodium salts and potassium salts, or alkaline earth metal salts, e.g., calcium salts, ammonium or amine salts. For example, the pharmaceutically acceptable solvent may comprise an acetate buffer, such as sodium acetate/acetic acid buffer or ammonium

acetate/acetic acid buffer. The concentration of the acidic buffer, preferably acetate buffer, in the liquid pharmaceutical composition may be 20 mM. In one embodiment, the concentration of acidic buffer (e.g. the concentration of acetate buffer) in the liquid pharmaceutical composition is from about 1 mM to about 20 mM, such as of from about 1 mM to about 10 mM, for example of from about 1mM to about 5 mM, such as about 2mM. In one embodiment, the concentration of acidic buffer (e.g. the concentration of acetate buffer) in the liquid pharmaceutical composition is about 2 mM.

In a preferred embodiment, the pharmaceutically acceptable solvent comprises the organic acid in the form of a free organic acid. The term "free organic acid" according to the present invention refers to an organic acid in the absence of a salt thereof. In a preferred embodiment, the pharmaceutically acceptable solvent of the invention is an aqueous free organic acid, preferably aqueous acetic acid. In the presence of water, the free organic acid is present in its protonated and deprotonated form.

The free organic acid may be selected from acetic acid, lactic acid, glutamic acid, gluconic acid, succinic acid, and a mixture thereof, preferably acetic acid, gluconic acid and glutamic acid. In a particularly preferred embodiment, the free organic acid is acetic acid. The present inventors surprisingly found that the stability of the liquid pharmaceutical composition can be further improved by the presence of free acetic acid comprised in the pharmaceutically acceptable solvent. In a preferred embodiment, the pharmaceutically acceptable solvent is aqueous acetic acid, e.g., comprised in a concentration of 0.05-0.50 mg/ml, preferably 0.10-0.30 mg/ml.

The organic acid may serve to adjust the pH of the liquid pharmaceutical composition. The pH of the pharmaceutical composition may be 4.0-6.0, preferably 4.3-5.1, and more preferably 4.5-5.0. The organic acid, preferably acetic acid, may thus be comprised in an amount to adjust the pH of the pharmaceutical composition to a value of 4.0-6.0, preferably 4.3-5.1, and more preferably 4.5-5.0. The pH of the pharmaceutical composition may be of from about 4.0 to about 6.0, such as of from about 4.3 to about 5.1, for example of from about 4.5 to about 5.0, such as about 4.7. The organic acid, for example acetic acid, may thus be comprised in an amount to adjust the pH of the pharmaceutical composition to a value of from about 4.0 to about 6.0, such as of from about 4.3 to about 5.1, for example of from about 4.5 to about 5.0, such as about 4.7.

The liquid pharmaceutical composition according to the invention may have any suitable form and is particularly in the form of a solution. In one embodiment, the liquid pharmaceutical composition is a single-dose formulation. A single-dose formulation particularly consists of Cetorelix or a pharmaceutically acceptable salt thereof, a stabilizing agent selected from poloxamer having an average molecular weight of 7,000-12,000 g/mol, benzyl alcohol, or a mixture thereof, an osmotic agent, and a pharmaceutically acceptable solvent comprising an organic acid.

In another embodiment, the liquid pharmaceutical composition is a multi-dose formulation optionally comprising further excipients such as a pharmaceutically acceptable preservative in an effective amount. Pharmaceutically acceptable preservatives are known in the art and comprise m-cresol, phenol, benzylalcohol and benzalkonium chloride, for example. An "effective amount" of preservative means, that the amount is sufficient to prevent microbial growth in the liquid pharmaceutical composition. A multi-dose formulation particularly consists of Cetorelix or a pharmaceutically acceptable salt thereof, a stabilizing agent selected from poloxamer having an average molecular weight of 7,000-12,000 g/mol, benzyl alcohol, or a mixture thereof, an osmotic agent, a pharmaceutically acceptable preservative in an effective amount, and a pharmaceutically acceptable solvent comprising an organic acid.

In a preferred embodiment, the present invention is directed to a liquid pharmaceutical composition comprising and preferably consisting of Cetorelix acetate in a concentration of 0.10-0.50 mg/ml, poloxamer 188 in a concentration of 0.5-10.0 mg/ml, D-mannitol in a concentration of 35.0-60.0 mg/ml, acetic acid in a concentration to adjust the pH of the pharmaceutical composition to a value of 4.0-6.0 and water for injection. In a particularly preferred embodiment, the present invention is directed to a liquid pharmaceutical composition comprising and preferably consisting of Cetorelix acetate in a concentration of 0.2 – 0.3 mg/ml Cetorelix pure peptide in its acetate salt form, poloxamer 188 in a concentration of 1.5 mg/ml, D-mannitol in a concentration of 54.8 mg/ml, acetic acid in a concentration of 0.1 mg/ml and water for injection.

In a further preferred embodiment, the present invention is directed to a liquid pharmaceutical composition comprising and preferably consisting of Cetorelix acetate in a concentration of 0.10-0.50 mg/ml, benzyl alcohol in a concentration of 5.0-20.0 mg/ml, D-mannitol in a concentration of 30.0-55.0 mg/ml, acetic acid in a concentration to adjust the pH of the pharmaceutical composition to a value of 4.0-6.0 and water for injection. In a particularly preferred embodiment, the present invention is directed to a liquid

pharmaceutical composition comprising and preferably consisting of Cetrorelix acetate in a concentration of 0.2 – 0.3 mg/ml Cetrorelix pure peptide in its acetate salt form, benzyl alcohol in a concentration of 10 mg/ml, D-mannitol in a concentration of 37.8 mg/ml, acetic acid in a concentration of 0.1 mg/ml and water for injection.

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In a further aspect, the present invention refers to a pharmaceutical dosage form comprising the liquid pharmaceutical composition according to the present invention as described herein in a container. Suitable containers for liquid pharmaceutical compositions are known to a person skilled in the art. The selection of a suitable container may depend on the desired route of administration. In a preferred embodiment, the pharmaceutical dosage form of the present invention is a parenteral dosage form, preferably for injection. A suitable container, particularly for a parenteral pharmaceutical dosage form, may be selected from an ampoule or vial such as a sterile vial, or an injection device such as a pre-filled syringe or an autoinjector.

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The pharmaceutical dosage form may be a single-dose or multiple-dose dosage form. A single-dose dosage form may comprise the liquid pharmaceutical composition in a pre-filled syringe or an autoinjector, for example. A multiple-dose dosage form may comprise the liquid pharmaceutical composition in a sterile vial or a pre-filled cartridge, for example.

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In a further aspect, the present invention refers to a liquid pharmaceutical composition or a pharmaceutical dosage form according to the present invention as described herein for use in inhibiting premature luteinizing hormone surges in woman undergoing controlled ovarian stimulation. In a further aspect, the present invention refers to a liquid pharmaceutical composition or a pharmaceutical dosage form according to the present invention, as described herein, for use in preventing premature ovulation during controlled ovarian stimulation, for example, for use in preventing premature ovulation during controlled ovarian stimulation, followed by oocyte pick-up and assisted reproductive technique.

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In a further aspect, the present invention refers to a method for inhibiting premature luteinizing hormone surges in woman undergoing controlled ovarian stimulation, comprising administering to the woman a pharmaceutically effective amount of a liquid pharmaceutical composition according to the present invention as described herein. The method may include the use of a pharmaceutical dosage form according to the present invention as described herein. In further aspects, a pharmaceutically effective amount of a liquid pharmaceutical composition according to the present invention may be administered to

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- suppress serum estradiol levels in woman at risk of OHSS, post ovarian stimulation with gonadotropins and oocyte retrieval; or to down-regulate the pituitary pre-ovarian stimulation in post-pubertal/premenopausal females attempting to preserve their fertility options with medically assisted reproduction and oocytes/embryos cryopreservation prior to potential
- 5 sterilizing effects of chemotherapeutic agents and pelvic radiation, or the need to surgically remove the gonads; or to random start controlled ovarian stimulation using an antagonist protocol, regardless of the phase of the menstrual cycle in post-pubertal/premenopausal females to preserve fertility options with medically assisted reproduction and cryopreservation oocytes or embryos prior to potential sterilizing effects of
- 10 chemotherapeutic agents and pelvic radiation, or the need to surgically remove the gonads; or to provide protective effect on ovarian function, when used during chemotherapy in post-pubertal/premenopausal females; or to effect pituitary down-regulation in hormone controlled cryopreserved embryo/blastocyst replacement treatment cycle.
- 15 An effective amount of the liquid pharmaceutical composition can vary depending on the route of administration, the age and weight of the patient, the nature and severity of the diseases to be treated, and similar factors. The daily dose can be given as a single dose, which is to be administered once, or be subdivided into two or more daily doses.
- 20 The liquid pharmaceutical composition of the invention may be administered alone or as a combination therapy with further active agents.

In a still further aspect, the present invention refers to a method for preparing a liquid pharmaceutical composition or a pharmaceutical dosage form according to the present

25 invention as described herein, the method comprising the steps of:

- a) providing a first composition comprising a stabilizing agent selected from poloxamer having an average molecular weight of 7,000-12,000 g/mol, benzyl alcohol, or a mixture thereof, and an osmotic agent,
  - b) providing a second composition comprising or consisting of Cetorelix or a
  - 30 pharmaceutically acceptable salt thereof,
  - c) mixing the first composition of step a) and the second composition of step b) to obtain a liquid composition, and
  - d) optionally adding an organic acid and/or a base such as sodium hydroxide to the mixture obtained in step c),
- 35 wherein a pharmaceutically acceptable solvent is present in the first composition and/or wherein a pharmaceutically acceptable solvent is present in the second composition.

In one embodiment, a pharmaceutically acceptable solvent is present in the first composition. In another embodiment, a pharmaceutically acceptable solvent is present in the second composition. In a preferred embodiment, a pharmaceutically acceptable solvent is present in the first composition and second composition. The solvent of the first and second composition may be the same or different. In one embodiment, the solvent of the first and second composition is the same and is preferably an aqueous organic acid, preferably acetic acid. In another embodiment, the solvent of the first and second composition is different, wherein preferably one of the solvents is water for injection and the other solvent is an aqueous organic acid, preferably aqueous acetic acid.

The first composition comprises the stabilizing agent, the osmotic agent and optionally a pharmaceutically acceptable solvent, such as aqueous acetic acid or water for injection. To obtain the first composition, step a) may comprise mixing the stabilizing agent and the osmotic agent. Optionally, step a) may comprise mixing the stabilizing agent, the osmotic agent and a pharmaceutically acceptable solvent, preferably aqueous acetic acid, to obtain the first composition.

The second composition comprises or consists of Cetorelix or a pharmaceutically acceptable salt thereof, preferably Cetorelix acetate, and optionally a pharmaceutically acceptable solvent, such as aqueous acetic acid or water for injection. To obtain the second composition, step b) may comprise mixing Cetorelix or a pharmaceutically acceptable salt thereof, preferably Cetorelix acetate, with a pharmaceutically acceptable solvent, preferably aqueous acetic acid. In one embodiment, the second composition is an aqueous solution of Cetorelix or a pharmaceutically acceptable salt thereof, preferably Cetorelix acetate. The aqueous Cetorelix solution may further comprise an organic acid as described herein, preferably acetic acid.

In step c), the first composition and the second composition are mixed to obtain a liquid composition. Mixing may comprise adding the first composition and the second composition in a vessel and stirring. Further, step c) may comprises adding a pharmaceutically acceptable solvent, preferably water for injection, to the first and second composition. Thus, mixing of step c) may comprise adding the first composition, the second composition and the pharmaceutically acceptable solvent in a vessel and stirring.

In some embodiments, the method comprises a step d) of adding an organic acid and/or a base such as sodium hydroxide to the mixture obtained in step c). The organic acid is as described herein. Step d) is particularly performed in case at least one of the solvents of

the first and second composition and specifically both solvents do not contain an organic acid.

5 If required, the method can further comprise a step of adjusting the pH of the liquid composition obtained in step c) or d) to a predetermined value by adding a pharmaceutically acceptable acid or base such as sodium hydroxide. The predetermined pH value can depend on the desired route of administration and may be 4.0-6.0, preferably 4.3-5.1, and more preferably 4.5-5.0. An adjustment of the pH may be performed by any pharmaceutically acceptable acid or base, such as acetic acid or sodium hydroxide.

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The method of the invention may further comprise a step e) of sterile filtration of the liquid composition obtained in step c) or d) to obtain a sterile liquid composition. The liquid composition obtained in steps c) or d) or the sterile filtered, liquid composition obtained in 15 step e) may then be filled in a container, such as an ampoule or vial e.g. a sterile vial, or an injection device, e.g. a syringe or an autoinjector, to obtain a pharmaceutical dosage form according to the present invention.

The term "poloxamer", as used herein, refers, for example, to a block copolymer (e.g. di- or 20 tri-copolymer block) made of a chain of polyoxypropylene (the term "propylene oxide" may be used interchangeably herein) flanked by two chains of polyoxyethylene (the term "ethylene oxide" may be used interchangeably herein). The term "poloxamer" may encompass distinct compounds because different lengths for the polyoxypropylene and polyoxyethylene chains may be used in combination. The particular combination of 25 polyoxypropylene and polyoxyethylene chains present in a poloxamer may give rise to particular chemical and/or biophysical properties. In some embodiments, the poloxamer has the chemical formula of  $\text{HO}(\text{C}_2\text{H}_4\text{O})_n(\text{C}_3\text{H}_6\text{O})_m(\text{C}_2\text{H}_4\text{O})_n\text{H}$ . In some embodiments,  $n$  (i.e., the polyoxyethylene chain length) has a value from about 60 to about 150. In some embodiments,  $m$  (i.e., the polyoxypropylene chain length) has a value from about 25 to 30 about 60. Poloxamers are often described by a numbering system that designates their approximate molecular weight and percentage of polyoxyethylene content. These values may refer to an average value in a poloxamer composition, rather than an absolute value of each poloxamer molecule in the composition. Under this system, the first two digits are multiplied by 100 to give the approximate molecular weight of the polyoxypropylene block, 35 and the third digit is multiplied by 10 to give the percentage by weight of the polyoxyethylene block. For example, poloxamer 188 (CAS No. 9003-11-6) may refer to a poloxamer with  $n$  having a value of about 80 and with  $m$  having a value of about 27 as in the formula depicted

above. Poloxamer 237 may refer to a poloxamer with n having a value of about 64 and with m having a value of about 37. Poloxamer 338 may refer to a poloxamer with n having a value of about 141 and with m having a value of about 44. Poloxamer 407 may refer to a poloxamer with n having a value of about 101 and with m having a value of about 56. In  
5 some embodiments, poloxamer 188 may refer to a poloxamer with n having a value of about 80, with m having a value of about 27 as in the formula depicted above, and with the poloxamer having an average molecular weight of from about 7680 to about 9510 g/mol.

The term "stabilizing agent", as used herein, refers, for example, to substances, or a mixture  
10 of substances, that are able to stabilize a polypeptide during storage and/or production of a composition comprising the polypeptide, for example, to minimize the formation of aggregates (e.g. insoluble and/or soluble) and/or chemical degradation. In another embodiment, refers, for example, to substances, or a mixture of substances, that are able to stabilize the polypeptide during storage and/or production of the compositions so that  
15 substantial retention of biological activity is maintained.

The term "osmotic agent", as used herein, refers, for example, to an excipient suitable for inducing osmosis, for example it creates an osmotic pressure within the liquid formulation or creates an osmotic gradient, for example, to generate a driving force for the uptake of  
20 fluid. Osmotic agents are generally ionic compounds which include, but are not limited, to water soluble salts, such as water soluble salts of organic acids (e.g. sodium acetate, potassium acetate, magnesium succinate, sodium benzoate, sodium citrate, and sodium ascorbate), water soluble salts of inorganic acids (e.g. magnesium chloride or magnesium sulfate, lithium chloride, sodium chloride, potassium chloride, lithium hydrogen phosphate,  
25 sodium hydrogen phosphate, potassium hydrogen phosphate, lithium dihydrogen phosphate, sodium dihydrogen phosphate, or potassium dihydrogen phosphate), non ionic organic compounds with water solubility (e.g., carbohydrates such as mannitol, sorbitol, arabinose, ribose, xylose, glucose, fructose, mannose, galactose, sucrose, maltose, lactose, and raffinose), water- soluble amino acids (e.g. glycine, leucine, alanine, or  
30 methionine), hydrophilic polymers, urea or urea derivatives.

The term "osmolality", as used herein, refers to the osmotic concentration expressed in terms of milliosmoles of solutes per one kg of solvent [mOsm/kg]. This concentration can be measured by use of an osmometer.  
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The term "pharmaceutically acceptable solvent", as used herein, refers, for example, to water, water for injection, aqueous buffer solutions that are physiologically compatible, or

- aqueous solutions containing organic solvents that are physiologically compatible, for example, any liquid medium used for dilution or dissolution of parenteral, oral or peroral formulations, such as water, aqueous organic solvent, non-aqueous organic solvent and other liquids used in the pharmaceutical industry. For example, it may be selected from the
- 5 group consisting of water (e.g. water for injection), saline solution (e.g. physiological saline, isotonic sodium chloride), Ringer's solution, glycerin, diglycerides, alcohols, propylene glycol, polyethylene glycol, propylene glycol, benzyl benzoate, glycerol, ethyl acetate, oils (e.g. corn oil, castor oil, squalene, mineral oil) and mixtures thereof. In one embodiment, the pharmaceutically acceptable solvent is purified water. In another embodiment, the
- 10 pharmaceutically acceptable solvent is an aqueous organic acid (e.g. aqueous acetic acid). A non-comprehensive list of pharmaceutically acceptable solvents is, for example, provided in U.S. Department of Health & Human Services, Food & Drug Administration, "Guidance for Industry: Q3C Impurities: Residual Solvents," December 1997 or its current issue.
- 15 The term "water" or "water for injection" refers to water that meets the requirements of, for example, U.S.P Water for Injection (or foreign quality standard equivalent). Examples include water labeled or marketed as "Low Endotoxin U.S.P. Purified Water" and "WFI Quality Water."
- 20 The term "solution", as used herein, refers, for example, to a homogeneous liquid preparation of one or more solutes in one or more solvents.

The term "aqueous organic acid", as used herein, for example, in the context of pharmaceutically acceptable solvent, refers to any solution comprising an organic acid, as

25 defined herein, in which water is either the only solvent or one of the main solvents (e.g. equal or above 50% V/V).

The term "glacial acetic acid", as used herein, refers, for example, to acetic acid having a purity of at least 99.8 percent per weight.

30 The term "buffer agent", as used herein, refers, for example, to an agent that imparts suitable pH characteristics to the liquid pharmaceutical composition provided herein (e.g. acetate buffer, citrate buffer, phosphate buffer, borate buffer, or a combination thereof). Said buffer agents are used for adjusting the pH of the compositions of the

35 invention to a pH, as defined herein. The pH values mentioned in the present application are, for example, measured at room temperature (e.g. about 23°C ±0.5°C) with a pHmeter.

The term “aqueous acidic buffer”, as used herein, for example, in the context of pharmaceutically acceptable solvent, refers to any solution comprising an acidic buffer, as defined herein, in which water is either the only solvent or one of the main solvents (e.g. equal or above 50% V/V).

The term “aqueous free organic acid”, as used herein, for example, in the context of pharmaceutically acceptable solvent, refers to any solution comprising a free organic acid, as defined herein, in which water is either the only solvent or one of the main solvents (e.g. equal or above 50% V/V).

The term “aqueous acetic acid”, as used herein, for example, in the context of pharmaceutically acceptable solvent, refers to any solution comprising acetic acid, as defined herein, in which water is either the only solvent or one of the main solvents (e.g. equal or above 50% V/V).

The term “organic acid”, as used herein, means an organic compound with acidic properties and includes carboxylic acids, whose acidity is associated with their carboxyl group. Non-limiting examples of organic acids include acetic acid (e.g. glacial acetic acid), butyric acid, capric acid, caproic acid, caprylic acid, citric acid, formic acid, fumaric acid, gallic acid, lactic acid, maleic acid, malic acid, propionic acid, sorbic acid, succinic acid, tannic acid and tartaric acid. In one embodiment, the term “organic acid” is intended to include compounds, such as salts, which will form such an organic acid in use upon contact with water.

As used herein the term “free organic acid” refers to an organic acid compound that is in its protonated state (i.e. not salt form) when in solution (i.e., at or less than its pKa value).

As used herein, “acid buffer” or “acidic buffer” refers to a chemical compound or compounds in a composition which act to maintain the pH in a desired acidic range. Typically, such acid buffers can include a weak acid and its salt or a weak base and its salt. Upon addition of an acid or a base, properly selected, acid buffers will allow only minor changes in pH.

As used herein, the term “excipient” or “pharmaceutical excipient” or “pharmaceutically acceptable excipient” refers to any compound that is part of a formulation that is not an active ingredient, i.e., one that has no relevant biological activity, and which is added to the formulation, for example, to provide specific characteristics (e.g. providing protection to the active ingredient from chemical degradation).

- As used herein, reference to an amount (e.g. mg, mg/ml, percentage) of Cetrorelix, or a pharmaceutical acceptable salt thereof, is to be understood to refer the amount of Cetrorelix in the free form, which will be adapted accordingly for a pharmaceutically acceptable salt thereof (e.g. adapted accordingly for its acetate salt form). For example, about 0.25 mg cetrorelix is equivalent to about 0.26 – 0.27 mg cetrorelix acetate, accordingly, a concentration of cetrorelix acetate that ranges from about 0.26 - 0.27 mg/ml, would be equivalent to about 0.25 mg/ml of cetrorelix (i.e. base).
- As used herein, the term “Cetrorelix pure peptide”, is to be understood to refer the amount of Cetrorelix free form (i.e. base a.k.a free base), which will be adapted accordingly for a pharmaceutically acceptable salt thereof (e.g. adapted accordingly for its acetate salt form). For example, about 0.25 mg cetrorelix is equivalent to about 0.26 – 0.27 mg cetrorelix acetate, accordingly, a concentration of cetrorelix acetate that ranges from about 0.26 - 0.27 mg/ml, would be equivalent to about 0.25 mg/ml of Cetrorelix (i.e. base).

- As used herein, the term “Cetrorelix pure peptide in its acetate salt form” is to be understood to refer the amount of Cetrorelix (i.e. free form i.e. base a.k.a free base), which is thus adapted accordingly for the Cetrorelix acetate salt. Accordingly, for example, the expression “Cetrorelix acetate in a concentration of about 0.2 to about 0.3 mg/ml Cetrorelix pure peptide in its acetate salt form” means that the acetate salt of Cetrorelix (i.e. Cetrorelix acetate) is present with a corresponding concentration of Cetrorelix (i.e. free form i.e. base a.k.a free base) in an amount of from about 0.2 to about 0.3 mg/ml, thus the concentration of a Cetrorelix acetate is adapted accordingly for the acetate salt form. Similarly, the expression “Cetrorelix acetate in a concentration of from about 0.10 to about 0.60 mg/ml of Cetrorelix” means that the acetate salt of Cetrorelix (i.e. Cetrorelix acetate) is present with a corresponding concentration of Cetrorelix (i.e. free form i.e. base a.k.a free base) in an amount of from about 0.10 to about 0.60 mg/ml, therefore the concentration of Cetrorelix acetate is adapted accordingly for the acetate salt form. By analogy, the expression “Cetrorelix, or a pharmaceutically acceptable salt thereof, in an amount of from about 0.10 to about 0.50 mg/ml of Cetrorelix” means that either Cetrorelix is present in an amount of from about 0.10 to about 0.50 mg/ml of Cetrorelix or a pharmaceutically acceptable salt of Cetrorelix is present with a corresponding concentration of from about 0.10 to about 0.50 mg/ml of Cetrorelix (i.e. free form i.e. base a.k.a free base), therefore the concentration of said pharmaceutically acceptable salt of Cetrorelix is adapted accordingly for such a salt form. For example, about 0.25 mg

cetrorelix is equivalent to about 0.26 – 0.27 mg cetrorelix acetate, accordingly, a concentration of cetrorelix acetate that ranges from about 0.26 - 0.27 mg/ml, would be equivalent to about 0.25 mg/ml of Cetrorelix (i.e. base).

- 5 As used herein, the terms "free form" or "free forms" or "in free form" or "in the free form" refers to the compound in non-salt form, such as the base free form.

The term "cyclodextrin free" or "free of cyclodextrin" as used herein refers to a pharmaceutical composition, for example as described herein, with no added cyclodextrin.

- 10 In one embodiment, it means that no cyclodextrin is intentionally added to (or present) in the pharmaceutical composition.

The term "polysorbate free" or "free of polysorbate" as used herein refers to a pharmaceutical composition, for example as described herein, with no added polysorbate.

- 15 In one embodiment, it means that no polysorbate is intentionally added to (or present) in the pharmaceutical composition.

The term "composition" or "pharmaceutical composition" is defined herein to refer, for example, to a mixture containing at least one active ingredient or therapeutic agent to be administered to a subject, in order to treat a subject, for example as herein defined.

20

The term "liquid pharmaceutical composition", as used herein, refers, for example, to a pharmaceutical composition comprising one or more pharmaceutically active ingredient, or a pharmaceutically acceptable salt thereof, one or more pharmaceutically acceptable solvent (e.g. as defined herein, such as water) and optionally one or more pharmaceutically acceptable excipients (e.g. as defined herein, such as preservatives). In one embodiment, the liquid pharmaceutical composition is a solution.

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- 30 The term "pharmaceutical dosage form" or "pharmaceutical preparation", as used herein, refers, for example, to a finished dosage form, e.g. a solution, that contains a pharmaceutically active ingredient in the form in which it will be administered to a patient.

The terms "drug", "drug substance", "active substance", "active ingredient", "pharmaceutically active ingredient", "pharmaceutical active ingredient", "active agent" or "therapeutic agent" are to be understood as meaning a compound in free form, or in the

35

form of a pharmaceutically acceptable salt, intended to furnish pharmacological activity (e.g. for the treatment or prevention of a disease).

5 As used herein, the terms "salt", "salts" or "salt form" refers to an acid addition or base addition salt of a compound. "Salts" include in particular "pharmaceutically acceptable salts". The term "pharmaceutically acceptable salts" refers to salts that retain the biological effectiveness and properties of the compounds and, which typically are not biologically or otherwise undesirable.

10 The term "one or more" refers to either one or a number above one (e.g. 2, 3, 4, 5, etc.).

The term "at least one", as used herein, refers to a minimum of one, for example 1, 2, 3, 4 or 5, such as 1 or 2, for example 1.

15 The term "optionally", as used herein, means that the referenced "element" (e.g. excipient) may be present or not.

20 As used herein, a "multi-dose formulation" or "multidose formulation" refers, for example, to a formulation that contains multiple doses of a therapeutic agent and that can be directly administered to provide several single doses of the therapeutic agent. The doses can be administered over the course of, for example, minutes, hours, weeks, days or months. Multi-dose formulations can allow, for example, dose adjustment or dose-splitting.

25 As used herein, a "single-dose formulation" or "singledose formulation" refers, for example, to a formulation designed for use with a single patient as a single injection or infusion.

The term "parenteral administration", as used herein, means, for example, that the administration is not through the gastrointestinal tract, but rather through some other route, such as via the subcutaneous, intramuscular, intravenous or intradermal route.

30 The term "parenteral dosage form", as used herein, refers, for example, to a pharmaceutical preparation that is administered parenterally (e.g. via the subcutaneous, intramuscular, intravenous or intradermal route).

35 As used herein, the term "preservative" or "pharmaceutically acceptable preservative" refers, for example, to a substance or excipient having a preservative activity, accordingly it is capable of preventing deterioration of a product (e.g. for a long period of time) and maintaining the original state of the product by preventing microbial growth. In one

embodiment, the preservative is an "antimicrobial preservative" or "antimicrobial agent", for example, a substance that has antimicrobial activity or antimicrobial effect, for example, it provides defense effect against deterioration caused by contamination by microorganisms (e.g. bacteria, mold, or yeasts) or it prevents microbial growth. In one  
5 embodiment, the "antimicrobial preservative" or "antimicrobial agent" is a bacteriostatic agent.

The terms "treating", "treatment", or the like as used herein, are to be construed as generally understood in the art, for example, as meaning reducing, hindering the development of, controlling, delaying, alleviating and/or reversing symptoms of a  
10 condition or disease in a subject or relieving or attenuating the disorder or disease, i.e., causing regression of the disorder or disease. In one embodiment, the term "treat" "treating" "treatment" or "therapy", as used herein, means obtaining beneficial or desired results, for example, clinical results. One aspect of the treatment is, for example, that said treatment should have a minimal adverse effect on the patient. In  
15 one embodiment, the term "method for the treatment", as used herein, refers to "method to treat".

The term "about", as used herein, for example, when used to modify a numerically defined parameter means that the parameter may vary by as much as 10% below or above the  
20 stated numerical value for that parameter (e.g. a numerical value X means, for example,  $X \pm 10\%$ , including all the values within this range).

As used herein, the term "patient" refers to a subject who is diseased and would benefit from the treatment.

25 As used herein, the term "subject" refers to a mammalian organism, preferably a human being, such as a woman.

As used herein, a subject is "in need of" a treatment, if such a subject (patient) would benefit biologically, medically or in quality of life from such a treatment.

30 The term "a therapeutically effective amount" or "an effective amount" of a compound of the present invention refers, for example, to an amount of a compound of the present invention that elicits the biological or medical response of a subject. In another embodiment, the term refers to the amount of the compound of the present invention that, when administered to a subject, is  
35 effective to at least partially ameliorate a condition, or a disorder or a disease.

The term "compound of the present invention", as used herein, refers to Cetrorelix (i.e. free form/free base) or a pharmaceutically acceptable salt thereof (e.g. cetrorelix acetate). Cetrorelix is also known by the name D-alaninamide, *N*-Acetyl-3-(2-naphthalenyl)-D-alanyl-4-chloro-D-phenylalanyl-3-(3-pyridinyl)-D-alanyl-L-seryl-L-tyrosyl-*N*5-(aminocarbonyl)-D-ornithyl-L-leucyl-L-arginyl-L-prolyl-, alternatively by the name *N*-Acetyl-3-(2-naphthalenyl)-D-alanyl-4-chloro-D-phenylalanyl-3-(3-pyridinyl)-D-alanyl-L-seryl-L-tyrosyl-*N*5-(aminocarbonyl)-D-ornithyl-L-leucyl-L-arginyl-L-prolyl-D-alaninamide, and also by the name *N*-acetyl-3-(2-naphthyl)-D-alanyl-4-chloro-D-phenylalanyl-3-(3-pyridyl)-D-alanyl-L-seryl-L-tyrosyl-D-citrullyl-L-leucyl-L-arginyl-L-prolyl-D-alaninamide. Cetrorelix acetate is also known by the name *N*-acetyl-3-(2-naphthalenyl)-D-alanyl-4-chloro-D-phenylalanyl-3-(3-pyridinyl)-D-alanyl-L-seryl-L-tyrosyl-*N*5-(aminocarbonyl)-D-ornithyl-L-leucyl-L-arginyl-L-prolyl-D-alaninamide acetic acid salt, and alternatively by the name *N*-acetyl-3-(2-naphthyl)-D-alanyl-4-chloro-D-phenylalanyl-3-(3-pyridyl)-D-alanyl-L-seryl-L-tyrosyl-D-citrullyl-L-leucyl-L-arginyl-L-prolyl-D-alaninamide, acetate salt. The corresponding CAS registry numbers are 120287-85-6 (free base) and 145672-81-7 (Cetrorelix acetate).

The invention is further defined by, but not limited to, the following items.

1. A liquid pharmaceutical composition, comprising or consisting of
  - Cetrorelix or a pharmaceutically acceptable salt thereof,
  - a stabilizing agent selected from poloxamer having an average molecular weight of 7,000-12,000 g/mol, benzyl alcohol, or a mixture thereof,
  - an osmotic agent, and
  - a pharmaceutically acceptable solvent comprising an organic acid.
2. The liquid pharmaceutical composition of item 1, wherein the pharmaceutically acceptable Cetrorelix salt is Cetrorelix acetate.
3. The liquid pharmaceutical composition according to any of the preceding items, comprising Cetrorelix or a pharmaceutically acceptable salt thereof in an amount of 0.10 – 0.50 mg/ml, preferably in an amount of 0.2 – 0.3 mg/ml Cetrorelix pure peptide in its acetate salt form.
4. The liquid pharmaceutical composition according to any of the preceding items, wherein the stabilizing agent comprises poloxamer having an average molecular weight of 7,500-10,000 g/mol.

5. The liquid pharmaceutical composition according to any of the preceding items, wherein the stabilizing agent is comprised in an amount of 0.5-20.0 mg/ml, preferably 1.0-15.0 mg/ml.
- 5
6. The liquid pharmaceutical composition according to any of the preceding items, wherein the stabilizing agent is poloxamer 188 comprised in an amount of 0.5-10.0 mg/ml, preferably 1.0-5.0 mg/ml.
- 10
7. The liquid pharmaceutical composition according to any of the preceding items, wherein the stabilizing agent is benzyl alcohol comprised in an amount of 5.0-20.0 mg/ml, preferably 7.5-15.0 mg/ml.
8. The liquid pharmaceutical composition according to any of the preceding items, wherein the osmotic agent is selected from the group consisting of mannitol, glycerol, sorbitol, sodium chloride, potassium chloride, dextrose, sucrose, trehalose, or a combination thereof, preferably D-mannitol.
- 15
9. The liquid pharmaceutical composition according to any of the preceding items, wherein the osmotic agent is comprised in an amount of 30.0-60.0 mg/ml, preferably 35.0-55.0 mg/ml.
- 20
10. The liquid pharmaceutical composition according to any of the preceding items, wherein the pharmaceutical composition has an osmolality of 250-375 mOsm/kg, preferably 300-350 mOsm/kg (e.g. 318 mOsm/kg).
- 25
11. The liquid pharmaceutical composition according to any of the preceding items, wherein the pharmaceutically acceptable solvent comprises the organic acid in the form of an acidic buffer or a free organic acid, preferably wherein the pharmaceutically acceptable solvent is an aqueous organic acid or an aqueous acidic buffer.
- 30
12. The liquid pharmaceutical composition according to any of the preceding items, wherein the organic acid is acetic acid, lactic acid, glutamic acid, gluconic acid, succinic acid, or a mixture thereof, preferably acetic acid.
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13. The liquid pharmaceutical composition according to any of the preceding items, wherein the acidic buffer is an acetate buffer, preferably comprised in a concentration of 20 mM or 2 mM.
- 5 14. The liquid pharmaceutical composition according to any of the preceding items, wherein the pharmaceutically acceptable solvent is an aqueous free organic acid or water, preferably aqueous acetic acid.
- 10 15. The liquid pharmaceutical composition according to any of the preceding items, wherein the pharmaceutically acceptable solvent is aqueous acetic acid comprised in an acetic acid concentration of 0.05-0.50 mg/ml, preferably 0.10-0.30 mg/ml (e.g. 0.1 mg/mL).
- 15 16. The liquid pharmaceutical composition according to any of the preceding items, wherein the pH of the pharmaceutical composition is 4.0-6.0, preferably 4.3-5.1, and more preferably 4.5-5.0.
17. The liquid pharmaceutical composition according to any of the preceding items, wherein the liquid pharmaceutical composition is in the form of a solution.
- 20 18. The liquid pharmaceutical composition according to any of the preceding items, wherein the organic acid, preferably acetic acid, is comprised in an amount to adjust the pH of the pharmaceutical composition to a value of 4.0-6.0, preferably 4.3-5.1, and more preferably 4.5-5.0.
- 25 19. The liquid pharmaceutical composition according to any of the preceding items, wherein the pharmaceutical composition is for parenteral administration.
20. The liquid pharmaceutical composition according to any of the preceding items, wherein the pharmaceutical composition is free of polysorbate.
- 30 21. The liquid pharmaceutical composition according to any of the preceding items, wherein the pharmaceutical composition is free of cyclodextrin.
22. The liquid pharmaceutical composition according to any of the preceding items, wherein the pharmaceutical composition comprises and preferably consists of Cetorelix acetate in a concentration of 0.2 – 0.3 mg/ml Cetorelix pure peptide in its acetate salt form, poloxamer
- 35

188 in a concentration of 1.5 mg/ml, D-mannitol in a concentration of 54.8 mg/ml, acetic acid in a concentration of 0.1 mg/ml and water for injection.

23. The liquid pharmaceutical composition according to any of the preceding items, wherein  
5 the pharmaceutical composition comprises and preferably consists of Cetorelix acetate in a concentration of 0.2 – 0.3 mg/ml Cetorelix pure peptide in its acetate salt form, benzyl alcohol in a concentration of 10 mg/ml, D-mannitol in a concentration of 37.8 mg/ml, acetic acid in a concentration of 0.1 mg/ml and water for injection.
- 10 24. The liquid pharmaceutical composition according to any of the preceding items, which is a single-dose formulation.
24. The liquid pharmaceutical composition according to any of items 1-23, which is a multi-dose formulation comprising a pharmaceutically acceptable preservative in an effective  
15 amount.
25. A pharmaceutical dosage form comprising the liquid pharmaceutical composition according to any of items 1-24 in a container.
- 20 26. The pharmaceutical dosage form according to item 25, wherein the container is an ampoule or vial such as a sterile vial, an injection device such as a pre-filled syringe, or an autoinjector.
27. The pharmaceutical dosage form according to any of items 25-26, which is a parenteral  
25 dosage form, preferably for injection.
28. The pharmaceutical dosage form according to any of items 25-27, which is a single-dose or multiple-dose dosage form.
- 30 29. A liquid pharmaceutical composition according to any of items 1-24 or a pharmaceutical dosage form according to any of items 25-28 for use in inhibiting premature luteinizing hormone surges in woman undergoing controlled ovarian stimulation or for use in preventing premature ovulation during controlled ovarian stimulation, for example, for use in preventing premature ovulation during controlled ovarian stimulation, followed by oocyte  
35 pick-up and assisted reproductive technique.

30. A method for preparing a liquid pharmaceutical composition according to any of items 1-24 or a pharmaceutical dosage form according to any of items 25-28, comprising the steps of:
- a) providing a first composition comprising a stabilizing agent selected from poloxamer having an average molecular weight of 7,000-12,000 g/mol, benzyl alcohol, or a mixture thereof, and an osmotic agent;
  - b) providing a second composition comprising or consisting of Cetorelix or a pharmaceutically acceptable salt thereof;
  - c) mixing the first composition of step a) and the second composition of step b) to obtain a liquid composition; and
  - d) optionally adding an organic acid to the mixture obtained in step c),
- wherein a pharmaceutically acceptable solvent is present in the first composition and/or wherein a pharmaceutically acceptable solvent is present in the second composition.
31. The method of item 30, wherein a pharmaceutically acceptable solvent is present in the first and second composition, wherein the solvent of the first and second composition is the same or different, preferably wherein one of the solvents is water for injection and the other solvent is an aqueous organic acid, preferably aqueous acetic acid.
32. The method of any of items 30-31, wherein step a) comprises mixing the stabilizing agent and the osmotic agent and optionally a pharmaceutically acceptable solvent, preferably aqueous acetic acid, to obtain the first composition.
32. The method of any of items 30-31, wherein step b) comprises mixing Cetorelix or a pharmaceutically acceptable salt thereof with a pharmaceutically acceptable solvent, preferably aqueous acetic acid, to obtain the second composition.
33. The method of any of items 30-32, wherein the second composition is an aqueous solution of Cetorelix or a pharmaceutically acceptable salt thereof.
34. The method of any of items 30-33, further comprising e) sterile filtration of the liquid composition obtained in step c) or d) to obtain a sterile liquid composition.
35. The method of any of items 30-34, wherein step c) further comprises adding a pharmaceutically acceptable solvent, preferably water for injection, to the first and second composition.

36. The method of any of items 30-35, further comprising the step of adjusting the pH of the liquid composition obtained in step c) or d) to a predetermined value by adding a pharmaceutically acceptable acid, preferably acetic acid, or a pharmaceutically acceptable base, preferably sodium hydroxide.

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37. The method of item 36, wherein the predetermined pH value is 4.0-6.0, preferably 4.3-5.1, and more preferably 4.5-5.0.

38. The method of any of items 30-37, further comprising filling the liquid composition obtained in step c), d), or e) in a container, such as an ampoule, vial or an injection device, such as a syringe or an autoinjector.

39. A liquid pharmaceutical composition according to any of items 1-24 or a pharmaceutical dosage form according to any of items 25-28 for use in suppressing serum estradiol levels in woman at risk of OHSS, post ovarian stimulation with gonadotropins and oocyte retrieval; or for use to down-regulate the pituitary pre-ovarian stimulation in post-pubertal/premenopausal females attempting to preserve their fertility options with medically assisted reproduction and oocytes/embryos cryopreservation prior to potential sterilizing effects of chemotherapeutic agents and pelvic radiation, or the need to surgically remove the gonads; or for use to random start controlled ovarian stimulation using an antagonist protocol, regardless of the phase of the menstrual cycle in post-pubertal/premenopausal females to preserve fertility options with medically assisted reproduction and cryopreservation oocytes or embryos prior to potential sterilizing effects of chemotherapeutic agents and pelvic radiation, or the need to surgically remove the gonads; or for use to provide protective effect on ovarian function, when used during chemotherapy in post-pubertal/premenopausal females; or for use to effect pituitary down-regulation in hormone controlled cryopreserved embryo/blastocyst replacement treatment cycle.

40. A liquid pharmaceutical composition according to any of items 1-24 or a pharmaceutical dosage form according to any of items 25-28, which is to be administered as a combination therapy with further active agents.

#### **(A) EMBODIMENTS**

Item 1a: A liquid pharmaceutical composition, comprising, or consisting of:  
- Cetorelix or a pharmaceutically acceptable salt thereof,

- a stabilizing agent selected from poloxamer having an average molecular weight of from about 7,000 to about 12,000 g/mol, benzyl alcohol, or a mixture thereof,
- an osmotic agent, and
- a pharmaceutically acceptable solvent comprising an organic acid.

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Item 2a: The liquid pharmaceutical composition of Item 1a, wherein the pharmaceutically acceptable Cetorelix salt is Cetorelix acetate.

Item 3a: The liquid pharmaceutical composition according to Items 1a or 2a, comprising  
10 Cetorelix, or a pharmaceutically acceptable salt thereof, in an amount of from about 0.10 to about 0.50 mg/ml of Cetorelix, such as in an amount of from about 0.2 to about 0.3 mg/ml of Cetorelix; or

the liquid pharmaceutical composition according to Items 1a or 2a, comprising Cetorelix, or a pharmaceutically acceptable salt thereof, in an amount of from about 0.21 to about 0.26  
15 mg/ml of Cetorelix, for example in an amount of from about 0.22 to about 0.25 mg/ml of Cetorelix, such as about 0.21, 0.22, 0.23, 0.24 or 0.25 mg/ml of Cetorelix, for example about 0.22 or 0.25 mg/ml of Cetorelix.

Item 4a: The liquid pharmaceutical composition according to any of the preceding Items 1a-  
20 3a, wherein the stabilizing agent comprises poloxamer having an average molecular weight of from about 7,500 to about 10,000 g/mol.

Item 5a: The liquid pharmaceutical composition according to any of the preceding Items 1a-  
4a, wherein the stabilizing agent is comprised in an amount of from about 0.5 to about 20.0  
25 mg/ml, for example of from about 1.0 to about 15.0 mg/ml, such as of from about 1.0 to about 15.0 mg/ml, for example as of from about 1.0 to about 10.0 mg/ml, such as about 1.5 mg/ml.

Item 6a: The liquid pharmaceutical composition according to any of the preceding Items 1a-  
30 5a, wherein the stabilizing agent is poloxamer 188 comprised in an amount of from about 0.5 to about 10.0 mg/ml, for example of from about 1.0 to about 5.0 mg/ml, such as of from about 1.0 to about 3.0 mg/ml, for example about 1.5 mg/ml.

Item 7a: The liquid pharmaceutical composition according to any of the preceding Items 1a-  
35 6a, wherein the stabilizing agent is benzyl alcohol comprised in an amount of from about 5.0 to about 20.0 mg/ml, for example of from about 7.5 to about 15.0 mg/ml, such as of from about 8.0 to about 12.0 mg/ml, for example about 10.0 mg/ml.

Item 8a: The liquid pharmaceutical composition according to any of the preceding Items 1a-7a, wherein the osmotic agent is selected from the group consisting of mannitol, glycerol, sorbitol, sodium chloride, potassium chloride, dextrose, sucrose, trehalose, or a combination thereof, for example mannitol (e.g. D-mannitol).

Item 9a: The liquid pharmaceutical composition according to any of the preceding Items 1a-8a, wherein the osmotic agent is comprised in an amount of from about 30.0 to about 60.0 mg/ml, for example of from about 35.0 to about 55.0 mg/ml, such as of from about 36.0 to about 55.0 mg/ml, for example about 37.8 mg/ml or about 54.8 mg/ml.

Item 10a: The liquid pharmaceutical composition according to any of the preceding Items 1a-9a, wherein the pharmaceutical composition has an osmolality of from about 250 to about 375 mOsm/kg, for example of from about 300 to about 350 mOsm/kg, such as 318 mOsm/kg.

Item 11a: The liquid pharmaceutical composition according to any of the preceding Items 1a-10a, wherein the pharmaceutically acceptable solvent comprises the organic acid in the form of an acidic buffer or a free organic acid, for example wherein the pharmaceutically acceptable solvent is an aqueous organic acid or an aqueous acidic buffer.

Item 12a: The liquid pharmaceutical composition according to any of the preceding Items 1a-11a, wherein the organic acid is acetic acid, lactic acid, glutamic acid, gluconic acid, succinic acid, tartaric acid, formic acid, or a mixture thereof; such as acetic acid, lactic acid, glutamic acid, gluconic acid, succinic acid, or a mixture thereof; for example acetic acid.

Item 13a: The liquid pharmaceutical composition according to any of the preceding Items 1a-12a, wherein the acidic buffer is an acetate buffer, for example comprised in a concentration of about 20 mM or 2 mM.

Item 14a: The liquid pharmaceutical composition according to any of the preceding Items 1a-13a, wherein the pharmaceutically acceptable solvent is an aqueous free organic acid, for example aqueous acetic acid.

Item 15a: The liquid pharmaceutical composition according to any of the preceding Items 1a-14a, wherein

- the pharmaceutically acceptable solvent is aqueous acetic acid, and

- acetic acid is comprised in the liquid pharmaceutical composition in a concentration of from about 0.05 to about 0.50 mg/ml, for example of from 0.10 to about 0.30 mg/ml, such as of from about 0.10 to about 0.20 mg/ml, for example about 0.10 mg/ml.

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Item 16a: The liquid pharmaceutical composition according to any of the preceding Items 1a-15a, wherein the pH of the pharmaceutical composition is of from about 4.0 to about 6.0, for example of from about 4.3 to about 5.1, such as of from about 4.5 to about 5.0, for example about 4.5, about 4.7 or about 5.0, such as about 4.7.

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Item 17a: The liquid pharmaceutical composition according to any of the preceding Items 1a-16a, wherein the liquid pharmaceutical composition is in the form of a solution.

Item 18a: The liquid pharmaceutical composition according to any of the preceding Items 1a-17a, wherein the organic acid, for example acetic acid, is comprised in an amount to adjust the pH of the pharmaceutical composition to a value of from about 4.0 to about 6.0, for example of from about 4.3 to about 5.1, such as of from about 4.5 to about 5.0, for example about 4.5, about 4.7 or about 5.0.

Item 19a: The liquid pharmaceutical composition according to any of the preceding Items 1a-18a, wherein the pharmaceutical composition is for parenteral administration.

Item 20a: The liquid pharmaceutical composition according to any of the preceding Items 1a-19a, wherein the pharmaceutical composition is free of polysorbate.

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Item 21a: The liquid pharmaceutical composition according to any of the preceding Items 1a-20a, wherein the pharmaceutical composition is free of cyclodextrin.

Item 22a: The liquid pharmaceutical composition according to any of the preceding Items 1a-21a, wherein the pharmaceutical composition comprises (e.g. consists of) Cetorelix acetate in a concentration of from about 0.2 to about 0.3 mg/ml of Cetorelix, poloxamer 188 in a concentration of about 1.5 mg/ml, D-mannitol in a concentration of about 54.8 mg/ml, acetic acid in a concentration of about 0.1 mg/ml and water for injection; or the liquid pharmaceutical composition according to any of the preceding Items 1a-21a, wherein the pharmaceutical composition comprises (e.g. consists of) Cetorelix acetate in a concentration of from about 0.10 to about 0.50 mg/ml of Cetorelix, poloxamer 188 in a concentration of from about 0.5 to about 10.0 mg/ml, D-mannitol in a concentration of from

about 35.0 to about 60.0 mg/ml, acetic acid in a concentration to adjust the pH of the pharmaceutical composition to a value of from about 4.0 to about 6.0 and water for injection.

5 Item 23a: The liquid pharmaceutical composition according to any of the preceding Items 1a-22a, wherein the pharmaceutical composition comprises (e.g. consists of) Cetorelix acetate in a concentration of from about 0.2 to about 0.3 mg/ml of Cetorelix, benzyl alcohol in a concentration of about 10 mg/ml, D-mannitol in a concentration of about 37.8 mg/ml, acetic acid in a concentration of about 0.1 mg/ml and water for injection; or  
10 the liquid pharmaceutical composition according to any of the preceding Items 1a-22a, wherein the pharmaceutical composition comprises (e.g. consists of) Cetorelix acetate in a concentration of from about 0.10 to about 0.50 mg/ml, benzyl alcohol in a concentration of from about 5.0 to about 20.0 mg/ml, D-mannitol in a concentration of from about 30.0 to about 55.0 mg/ml, acetic acid in a concentration to adjust the pH of the pharmaceutical composition to a value of from about 4.0 to about 6.0 and water for injection.

15 Item 24a: The liquid pharmaceutical composition according to any of the preceding Items 1a-23a, which is a single-dose formulation.

20 Item 25a: The liquid pharmaceutical composition according to any of Items 1a-23a, which is a multi-dose formulation comprising a pharmaceutically acceptable preservative in an effective amount.

25 Item 26a: A pharmaceutical dosage form comprising the liquid pharmaceutical composition according to any of Items 1a-25a in a container.

Item 27a: The pharmaceutical dosage form according to Item 26a, wherein the container is an ampoule, a vial (e.g. a sterile vial), an injection device (e.g. a pre-filled syringe), or an autoinjector.

30 Item 28a: The pharmaceutical dosage form according to any of Items 26a-27a, which is a parenteral dosage form (e.g. a parenteral dosage form for injection).

Item 29a: The pharmaceutical dosage form according to any of Items 26a-28a, which is a single-dose or multiple-dose dosage form.

35 Item 30a: A liquid pharmaceutical composition according to any of Items 1a-25a or a pharmaceutical dosage form according to any of Items 26a-29a for use in inhibiting

premature luteinizing hormone surges in woman undergoing controlled ovarian stimulation or for use in preventing premature ovulation during controlled ovarian stimulation, for example, for use in preventing premature ovulation during controlled ovarian stimulation, followed by oocyte pick-up and assisted reproductive technique.

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Item 31a: A method for preparing a liquid pharmaceutical composition according to any of Items 1a-25a or a pharmaceutical dosage form according to any of Items 26a-29a, comprising the steps of:

10 a) providing a first composition comprising an osmotic agent and a stabilizing agent selected from poloxamer having an average molecular weight of from about 7,000 to about 12,000 g/mol, benzyl alcohol and a mixture thereof;

b) providing a second composition comprising, or consisting of, Cetorelix or a pharmaceutically acceptable salt thereof;

15 c) mixing the first composition of step a) and the second composition of step b) to obtain a liquid composition; and

d) optionally adding an organic acid to the mixture obtained in step c), wherein a pharmaceutically acceptable solvent is present in the first composition, and optionally wherein a pharmaceutically acceptable solvent is present in the second composition.

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Item 32a: The method of Item 31a, wherein a pharmaceutically acceptable solvent is present in the first and second composition, wherein the solvent of the first and second composition is the same or different, for example wherein one of the solvents is water for injection and the other solvent is an aqueous organic acid (e.g. aqueous acetic acid).

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Item 33a: The method of Items 31a or 32a, wherein step a) comprises mixing the stabilizing agent and the osmotic agent and optionally a pharmaceutically acceptable solvent, for example aqueous acetic acid, to obtain the first composition.

30 Item 34a: The method of any of Items 31a-33a, wherein step b) comprises mixing Cetorelix or a pharmaceutically acceptable salt thereof with a pharmaceutically acceptable solvent, for example aqueous acetic acid, to obtain the second composition.

Item 35a: The method of any of Items 31a-34a, wherein the second composition is an aqueous solution of Cetorelix or a pharmaceutically acceptable salt thereof.

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Item 36a: The method of any of Items 31a-35a, further comprising e) sterile filtration of the liquid composition obtained in step c) or d) to obtain a sterile liquid composition.

5 Item 37a: The method of any of Items 31a-36a, wherein step c) further comprises adding a pharmaceutically acceptable solvent, for example water for injection, to the first and second composition.

10 Item 38a: The method of any of Items 31a-37a, further comprising the step of adjusting the pH of the liquid composition obtained in step c) or d) to a predetermined value by adding a pharmaceutically acceptable acid, for example acetic acid, or a pharmaceutically acceptable base, for example sodium hydroxide.

15 Item 39a: The method of Item 38a, wherein the predetermined pH value is of from about 4.0 to about 6.0, for example of from about 4.3 to about 5.1, such as of from about 4.5 to about 5.0.

Item 40a: A method for preparing a liquid pharmaceutical composition according to any of items 1a to 25a or a pharmaceutical dosage form according to any of items 26a to 29a, comprising the steps of:

20 a) providing a first composition comprising an osmotic agent and a stabilizing agent selected from the group consisting of poloxamer having an average molecular weight of from about 7,000 to about 12,000 g/mol;

b) providing a second composition comprising Cetorelix, or a pharmaceutically acceptable salt thereof, and an organic (e.g. acetic acid);

25 c) mixing the first composition of step a) and the second composition of step b) to obtain a liquid composition; and

d) adjusting the pH by adding a pharmaceutically acceptable acid (e.g. acetic acid) or a pharmaceutically acceptable base (e.g. sodium hydroxide),

30 wherein a pharmaceutically acceptable solvent is present in the first composition and in the second composition.

Item 41a: The method of item 40a, wherein the first and second composition comprise a pharmaceutically acceptable solvent, which is the same or different for each, for example wherein the solvent is the same and it is water for injection.

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Item 42a: The method of items 40a or 41a, wherein step a) comprises mixing the stabilizing agent, the osmotic agent and the pharmaceutically acceptable solvent (e.g. water) to obtain the first composition.

- 5    Item 43a: The method of any of items 40a to 42a, wherein step b) comprises mixing Cetorelix, or a pharmaceutically acceptable salt thereof, with a pharmaceutically acceptable solvent, for example water, and an organic acid to obtain the second composition.

- 10   Item 44a: The method of any of items 40a to 43a, wherein the second composition comprises an aqueous solution of Cetorelix or a pharmaceutically acceptable salt thereof (e.g. Cetorelix acetate).

- 15   Item 45a: The method of any of items 40a to 44a, wherein in step d) the pH is adjusted to a value of from about 3.5 to about 6.0, such as of from about 3.5 to about 5.0, for example about 3.5, 4.5 or 5.0, for example of from about 4.0 to about 5.0, for example about 4.5.

- 20   Item 46a: The method of any of items 40a to 45a, wherein step d) further comprises adding a pharmaceutically acceptable solvent, for example water, to achieve the final weight of the liquid pharmaceutical composition.

Item 47a: The method of any of items 40a to 46a, further comprising a step f) of sterile filtration of the liquid composition obtained in step e) to obtain a sterile liquid composition.

- 25   Item 48a: The method of any of Items 31a-47a, further comprising filling the liquid composition obtained in step c), d), or e) in a container, such as an ampoule, vial or an injection device (e.g. a syringe or an autoinjector).

- 30   Item 49a: A liquid pharmaceutical composition according to any of Items 1a-25a or a pharmaceutical dosage form according to any of Items 26a-29a for use in suppressing serum estradiol levels in woman at risk of OHSS, post ovarian stimulation with gonadotropins and oocyte retrieval; or for use to down-regulate the pituitary pre-ovarian stimulation in post-pubertal/premenopausal females attempting to preserve their fertility options with medically assisted reproduction and oocytes/embryos cryopreservation prior  
35   to potential sterilizing effects of chemotherapeutic agents and pelvic radiation, or the need to surgically remove the gonads; or for use to random start controlled ovarian stimulation using an antagonist protocol, regardless of the phase of the menstrual cycle in post-

- pubertal/premenopausal females to preserve fertility options with medically assisted reproduction and cryopreservation oocytes or embryos prior to potential sterilizing effects of chemotherapeutic agents and pelvic radiation, or the need to surgically remove the gonads; or for use to provide protective effect on ovarian function, when used during
- 5 chemotherapy in post-pubertal/premenopausal females; or for use to effect pituitary down-regulation in hormone controlled cryopreserved embryo/blastocyst replacement treatment cycle.

- Item 50a: A liquid pharmaceutical composition according to any of Items 1a-25a or a
- 10 pharmaceutical dosage form according to any of Items 26a-29a, which is to be administered as a combination therapy with further active agents.

### **(B) EMBODIMENTS**

- 15 Further embodiments (multi-dose formulation)

Item 1b: A liquid pharmaceutical composition, comprising or consisting of

- Cetorelix or a pharmaceutically acceptable salt thereof,
- a stabilizing agent selected from a poloxamer having an average molecular weight

20 of from about 7,000 to about 12,000 g/mol,

- an osmotic agent,
- a pharmaceutically acceptable solvent comprising an organic acid, and
- a pharmaceutically acceptable preservative.

- 25 Item 2b: The liquid pharmaceutical composition of item 1b, wherein the pharmaceutically acceptable Cetorelix salt is Cetorelix acetate.

- Item 3b: The liquid pharmaceutical composition according to items 1b or 2b, comprising Cetorelix, or a pharmaceutically acceptable salt thereof, in an amount of from about 0.10
- 30 to about 0.80 mg/ml Cetorelix, such as in an amount of from about 0.4 to about 0.6 mg/ml Cetorelix, for example in an amount of about 0.5 to about 0.6 mg/ml Cetorelix, such as in an amount of about 0.49 mg/ml Cetorelix.

- Item 4b: The liquid pharmaceutical composition according to any of the preceding items 1b-
- 35 3b, wherein the stabilizing agent comprises a poloxamer having an average molecular weight of from about 7,000 to about 10,000 g/mol.

Item 5b: The liquid pharmaceutical composition according to any of the preceding items 1b-4b, wherein the stabilizing agent is comprised in an amount of from about 0.5 to about 20.0 mg/ml, such as of from about 1.0 to about 15.0 mg/ml, for example of from about 1.0 to about 5.0 mg/ml.

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Item 6b: The liquid pharmaceutical composition according to any of the preceding items 1b-5b, wherein the stabilizing agent is poloxamer 188 comprised in an amount of from about 0.5 to about 10.0 mg/ml, for example of from about 1.0 to about 5.0 mg/ml, such as about 1.5 mg/ml.

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Item 7b: The liquid pharmaceutical composition according to any of the preceding items 1b-6b, wherein the osmotic agent is selected from the group consisting of mannitol, glycerol, sorbitol, sodium chloride, potassium chloride, dextrose, sucrose, trehalose, and a combination thereof, such as mannitol (e.g. D-mannitol).

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Item 8b: The liquid pharmaceutical composition according to any of the preceding items 1b-7b, wherein the osmotic agent is comprised in an amount of from about 30.0 to about 60.0 mg/ml, such as of from about 35.0 to about 55.0 mg/ml, for example about 54.8 mg/ml.

20 Item 9b: The liquid pharmaceutical composition according to any of the preceding items 1b-8b, wherein the pharmaceutical composition has an osmolality of from about 300 to about 500 mOsm/kg, for example of from 330 to about 490 mOsm/kg (e.g. 330, 349, 351, 353, 357, 361, 366, 385, 387, 388, 413, 469, 472 or 485).

25 Item 10b: The liquid pharmaceutical composition according to any of the preceding items 1b-9b, wherein the pharmaceutically acceptable solvent comprises the organic acid in the form of an acidic buffer or a free organic acid, for example wherein the pharmaceutically acceptable solvent is an aqueous organic acid or an aqueous acidic buffer.

30 Item 11b: The liquid pharmaceutical composition according to any of the preceding items 1b-10b, wherein the organic acid is acetic acid, lactic acid, glutamic acid, gluconic acid, succinic acid, tartaric acid, formic acid, or a mixture thereof, such as acetic acid, lactic acid, glutamic acid, gluconic acid, succinic acid, or a mixture thereof; for example the organic acid is acetic acid.

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Item 12b: The liquid pharmaceutical composition according to any of the preceding items 1b-11b, wherein the acidic buffer is an acetate buffer, for example wherein the acidic buffer is an acetate buffer comprised in a concentration of 20 mM or 2 mM.

- 5    Item 13b: The liquid pharmaceutical composition according to any of the preceding items 1b-12b, wherein the pharmaceutically acceptable solvent is an aqueous free organic acid, such as aqueous acetic acid.

- 10   Item 14b: The liquid pharmaceutical composition according to any of the preceding items 1b-13b, wherein
- the pharmaceutically acceptable solvent is aqueous acetic acid, and
  - acetic acid is comprised in the liquid pharmaceutical composition in a concentration of from about 0.05 to about 0.50 mg/ml, such as of from about 0.10 to about 0.30 mg/ml, for example about 0.1 mg/ml.

- 15   Item 15b: The liquid pharmaceutical composition according to any of the preceding items 1b-14b, wherein the pH of the pharmaceutical composition is of from about 3.5 to about 6.0, such as of from about 3.5 to about 5.0, for example about 3.5, 4.5 or 5.0, for example of from about 4.0 to about 5.0, for example about 4.5.

- 20   Item 16b: The liquid pharmaceutical composition according to any of the preceding items 1b-15b, wherein the liquid pharmaceutical composition is in the form of a solution.

- 25   Item 17b: The liquid pharmaceutical composition according to any of the preceding items 1b-16b, wherein the organic acid, for example acetic acid, is comprised in an amount to adjust the pH of the pharmaceutical composition to a value of from about 3.5 to about 6.0, such as of from about 3.5 to about 5.0, for example about 3.5, 4.5 or 5.0, for example of from about 4.0 to about 5.0, for example about 4.5.

- 30   Item 18b: The liquid pharmaceutical composition according to any of the preceding items 1b-17b, wherein the pharmaceutical composition is for parenteral administration.

- 35   Item 19b: The liquid pharmaceutical composition according to any of the preceding items 1b-18b, wherein the pharmaceutical composition is free of polysorbate.

- Item 20b: The liquid pharmaceutical composition according to any of the preceding items 1b-19b, wherein the pharmaceutical composition is free of cyclodextrin.

Item 21b: The liquid pharmaceutical composition according to any of the preceding items 1b-21b, wherein the pharmaceutically acceptable preservative is selected from the group consisting of phenol, chlorobutanol, methylparaben, propylparaben, phenoxyethanol, benzalkonium chloride, m-cresol, benzyl alcohol, such as phenol, phenoxyethanol, benzalkonium chloride, m-cresol, benzyl alcohol.

Item 22b: The liquid pharmaceutical composition according to any of the preceding items 1b-21b, wherein the pharmaceutically acceptable preservative is comprised in an amount of from 0.1 to 20.0 mg/ml, such as of from about 0.5 to 15 mg/ml, for example about 0.5, 1.0, 2.0, 3.0, 5.0 or 15.0 mg/ml, such as of from about 1.0 to 5.0 mg/ml, for example about 1.0, 2.0, 3.0 or 5.0 mg/ml.

Item 23b: The liquid pharmaceutical composition according to any of the preceding items 1b-22b, wherein the pharmaceutical composition comprises, for example consists of,

- Cetorelix acetate in a concentration of from about 0.10 to about 0.60 mg/ml of Cetorelix (e.g. in a concentration of about 0.49 mg/ml Cetorelix),
- poloxamer 188 in a concentration of from about 0.5 to about 10.0 mg/ml (e.g. of from about 1.0 to about 5.0 mg/ml, such as about 1.5 mg/ml),
- mannitol (e.g. D-mannitol) in a concentration of from about 30.0 to about 60.0 mg/ml (e.g. in a concentration of from about 35.0 to about 55.0 mg/ml, such as about 54.8 mg/ml),
- acetic acid in a concentration to adjust the pH of the pharmaceutical composition to a value of from about 4.0 to about 6.0 (e.g. a value of from about 3.5 to about 5.0, for example about 3.5, 4.5 or 5.0, such as about 4.5)
- a pharmaceutically acceptable preservative in a concentration of from 0.1 to 20.0 mg/ml (e.g. in a concentration of from about 0.5 to 15 mg/ml, for example about 0.5, 1.0, 2.0, 3.0, 5.0 or 15.0 mg/ml), and
- water for injection.

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Item 24b: The liquid pharmaceutical composition according to any of items 1b to 23b, which is a multi-dose formulation.

Item 25b: A pharmaceutical dosage form comprising the liquid pharmaceutical composition according to any of items 1b to 24b in a container.

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Item 26b: The pharmaceutical dosage form according to item 25b, wherein the container is an ampoule or vial such as a sterile vial, an injection device such as a pre-filled syringe, or an autoinjector.

- 5     Item 27b: The pharmaceutical dosage form according to items 25b or 26b, which is a parenteral dosage form, for example for injection.

Item 28b: The pharmaceutical dosage form according to any of items 25b to 27b, which is a multiple-dose dosage form.

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Item 29b: A liquid pharmaceutical composition according to any of items 1b to 24b or a pharmaceutical dosage form according to any of items 25b to 28b for use in inhibiting premature luteinizing hormone surges in woman undergoing controlled ovarian stimulation or for use in preventing premature ovulation during controlled ovarian stimulation, for example, for use in preventing premature ovulation during controlled ovarian stimulation, followed by oocyte pick-up and assisted reproductive technique.

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Item 30b: A method for preparing a liquid pharmaceutical composition according to any of items 1b to 24b or a pharmaceutical dosage form according to any of items 25b to 28b, comprising the steps of:

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a) providing a first composition comprising an osmotic agent and a stabilizing agent selected from the group consisting of poloxamer having an average molecular weight of from about 7,000 to about 12,000 g/mol;

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b) providing a second composition comprising Cetorelix, or a pharmaceutically acceptable salt thereof, and an organic (e.g. acetic acid);

c) mixing the first composition of step a) and the second composition of step b) to obtain a liquid composition,

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d) adding a pharmaceutically acceptable preservative to the mixture obtained in step c);

and

e) adjusting the pH by adding a pharmaceutically acceptable acid (e.g. acetic acid) or a pharmaceutically acceptable base (e.g. sodium hydroxide),

wherein a pharmaceutically acceptable solvent is present in the first composition and in the second composition.

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Item 31b: The method of item 30b, wherein the first and second composition comprise a pharmaceutically acceptable solvent, which is the same or different for each, for example wherein the solvent is the same and it is water for injection.

- 5     Item 32b: The method of items 30b or 31b, wherein step a) comprises mixing the stabilizing agent, the osmotic agent and the pharmaceutically acceptable solvent (e.g. water) to obtain the first composition.

- 10    Item 33b: The method of any of items 30b to 32b, wherein step b) comprises mixing Cetorelix, or a pharmaceutically acceptable salt thereof, with a pharmaceutically acceptable solvent, for example water, and an organic acid to obtain the second composition.

- 15    Item 34b: The method of any of items 30b to 33b, wherein the second composition comprises an aqueous solution of Cetorelix or a pharmaceutically acceptable salt thereof (e.g. Cetorelix acetate).

- 20    Item 35b: The method of any of items 30b to 34b, further comprising the step of adjusting the pH of the liquid composition obtained in step c) or d) to a predetermined value by adding a pharmaceutically acceptable acid, for example acetic acid, or a pharmaceutically acceptable base, for example sodium hydroxide.

- 25    Item 36b: The method of item 35b, wherein the predetermined pH value is of from about 3.5 to about 6.0, such as of from about 3.5 to about 5.0, for example about 3.5, 4.5 or 5.0, for example of from about 4.0 to about 5.0, for example about 4.5.

- 30    Item 37b: The method of any of items 30b to 36b, wherein in step e) the pH is adjusted to a value of from about 3.5 to about 6.0, such as of from about 3.5 to about 5.0, for example about 3.5, 4.5 or 5.0, for example of from about 4.0 to about 5.0, for example about 4.5.

- Item 38b: The method of any of items 30b to 37b, wherein step e) further comprises adding a pharmaceutically acceptable solvent, for example water, to achieve the final weight of the liquid pharmaceutical composition.

- 35    Item 39b: The method of any of items 30b to 38b, further comprising a step f) of sterile filtration of the liquid composition obtained in step e) to obtain a sterile liquid composition.

Item 40b: The method of any of items 30b to 39b, further comprising filling the liquid composition obtained in step c), d), or e) in a container, such as an ampoule, vial or an injection device, such as a syringe or an autoinjector.

- 5 Item 41b: A liquid pharmaceutical composition according to any of items 1b to 24b or a pharmaceutical dosage form according to any of items 25b to 28b for use in suppressing serum estradiol levels in a woman at risk of OHSS, post ovarian stimulation with gonadotropins and oocyte retrieval; or for use to down-regulate the pituitary pre-ovarian stimulation in post-pubertal/premenopausal females attempting to preserve their fertility
- 10 options with medically assisted reproduction and oocytes/embryos cryopreservation prior to potential sterilizing effects of chemotherapeutic agents and pelvic radiation, or the need to surgically remove the gonads; or for use to random start controlled ovarian stimulation using an antagonist protocol, regardless of the phase of the menstrual cycle in post-pubertal/premenopausal females to preserve fertility options with medically assisted
- 15 reproduction and cryopreservation oocytes or embryos prior to potential sterilizing effects of chemotherapeutic agents and pelvic radiation, or the need to surgically remove the gonads; or for use to provide protective effect on ovarian function, when used during chemotherapy in post-pubertal/premenopausal females; or for use to effect pituitary down-regulation in hormone controlled cryopreserved embryo/blastocyst replacement treatment
- 20 cycle.

Item 42b: A liquid pharmaceutical composition according to any of items 1b to 24b or a pharmaceutical dosage form according to any of items 25b to 28b, which is to be administered as a combination therapy with further active agents.

25

## Figures

- Fig. 1 shows fluorescence spectra of liquid Cetorelix compositions including (a, b) and lacking (c, d) a stabilizing agent. Spectrum "a" corresponds to Composition 1, spectrum "b" corresponds to Composition 2, spectrum "c" corresponds to Comparative Composition 3 and spectrum "d" corresponds to Comparative Composition 4. The spectrum at time zero is compared with the spectrum after storage for two weeks at 40°C, after storage for four weeks at 25°C, and the spectrum of commercially available Cetrotide®.

- Fig. 2 shows fluorescence spectra of comparative Cetorelix compositions including arginine monohydrochloride (a), proline (b) and phenol (c) as a stabilizing agent. Spectrum "a" corresponds to Comparative Composition 5, spectrum "b" corresponds to Comparative

Composition 6, and spectrum "c" corresponds to Comparative Composition 7. The spectrum at time zero is compared with the spectrum after storage for two weeks at 40°C, after storage for four weeks at 25°C, and the spectrum of commercially available Cetrotide®.

5

The present invention is further described by, but not limited to, the following examples.

### Example 1

10 Four different Cetrotirelix acetate compositions were prepared as follows.

Composition 1: Cetrotirelix acetate was dissolved in a mixture of D-mannitol, benzyl alcohol and water for injection. Then, diluted acetic acid was added in an amount to adjust the pH of the composition to a value of 5.0. The amount of D-mannitol in the final composition was  
15 37.80 mg/ml, the amount of benzyl alcohol in the final composition was 10.00 mg/ml and the amount of Cetrotirelix acetate in the final composition was **0.25 mg/ml** Cetrotirelix pure peptide in its acetate salt form. The osmolality of the final composition was 316 mOsm/kg. The composition was sterile filtered and filled in a glass vial.

20 Composition 2: Cetrotirelix acetate was dissolved in a mixture of D-mannitol, poloxamer 188 and water for injection. Then, acetic acid was added in an amount to adjust the pH of the composition to a value of 5.0. The amount of D-mannitol in the final composition was 55.00 mg/ml, the amount of poloxamer 188 in the final composition was 0.50 mg/ml and the amount of Cetrotirelix acetate in the final composition was **0.25 mg/ml** Cetrotirelix pure  
25 peptide in its acetate salt form. The osmolality of the final composition was 325 mOsm/kg. The composition was sterile filtered and filled in a glass vial.

Comparative composition 3: Cetrotirelix acetate was dissolved in a mixture of D-mannitol and water for injection. Then, acetic acid was added in an amount to adjust the pH of the  
30 composition to a value of 5.0. The amount of D-mannitol in the final composition was 55.00 mg/ml and the amount of Cetrotirelix acetate in the final composition was **0.25 mg/ml** Cetrotirelix pure peptide in its acetate salt form. The osmolality of the final composition was 322 mOsm/kg. The composition was sterile filtered and filled in a glass vial.

35 Comparative composition 4: Cetrotirelix acetate was dissolved in a mixture of D-mannitol and water for injection. Then, glutamic acid was added in an amount to adjust the pH of the composition to a value of 5.0. The amount of D-mannitol in the final composition was 55.00

mg/ml and the amount of Cetorelix acetate in the final composition was 0.25 mg/ml Cetorelix pure peptide in its acetate salt form. The osmolality of the final composition was 322 mOsm/kg. The composition was sterile filtered and filled in a glass vial.

- 5 The compositions were examined for their stability and pharmaceutical properties by the following methods.

pH measurement

- 10 pH measurements were performed on a calibrated Seven Multi pH meter (Mettler Toledo), equipped with a microelectrode.

No pH changes were observed for the compositions (i.e. for compositions 1 to 4) after two weeks of storage at 40°C (RH 75%) and after four weeks of storage at 25°C (RH 60%).

15

Osmolality

The osmolality was determined by measuring the freezing point of formulations, using a OSMOMAT 030-D (Gonotec).

20

Visual inspection for particle formation

The visual inspections were carried out by two different operators, classifying the formulation as:

- 25 - FVP: if the formulation was deemed free of visible particles;  
- +: if few particles were observed;  
- ++: if there were particles;  
- +++: if many particles/large particulate/precipitation/flakes were observed;  
- H: Hurricane, tornado, e.g., because sedimented or floating particles were  
30 observed;  
- Gel: if the formulation appeared gelled.

Visual inspection was performed directly after preparation of the compositions (time 0), after two weeks of storage at 40°C (RH 75%) and after four weeks of storage at 25°C (RH 60%).

35

#### Sub-visible particle detection by Aura

An Aura instrument (Halo Labs) was used to count and characterize sub-visible particles. This instrument performs various photophysical microscopy measurements, enabling the  
5 characterization of the physical nature (count, size, and morphology) and the identity (protein / non-protein) of particulates within the sample.

Samples were loaded onto a proprietary filter plate and vacuum dried, leaving particles behind on the surface of the filter membrane.

10

Aura uses proprietary patented backgrounded membrane imaging (BMI) whereby a background image of the filter membrane is first taken and then subtracted from the sample image to increase particle resolution. In addition, fluorescence membrane microscopy (FMM) was used to identify particles stained with fluorescent dyes (Thioflavin T), enabling  
15 the differentiation between protein versus non-protein species within the sample.

Sub-visible particles were detected directly after preparation of the compositions (time 0), after two weeks of storage at 40°C (RH 75%) and after four weeks of storage at 25°C (RH 60%).

20

#### Protein content via SoloVPE

A SoloVPE instrument (C Technologies) is a UV-Vis spectrophotometer that allows measuring the peptide content using the Beer-Lambert law. Measurements were performed  
25 directly after preparation of the compositions (time 0), after two weeks of storage at 40°C (RH 75%) and after four weeks of storage at 25°C (RH 60%).

#### Turbidity by nephelometry

30 The clarity/turbidity of the samples was analyzed using Turbidimeter mod. 2100 ANIS (Hach Lange). The results are given in Nephelometric Turbidity Units (NTU).

A liquid is considered clear (limpid) if its brightness or clarity is  $\leq 3$  NTU. More in detail:

- a liquid is considered clear if its opalescence is  $\leq 3$  NTU;
- 35 • a liquid is considered slightly opalescent if its opalescence is  $> 3$  NTU but  $\leq 6$  NTU;
- a liquid is considered opalescent if its opalescence is  $> 6$  NTU but  $\leq 18$  NTU; and

• a liquid is considered highly opalescent if its opalescence is > 18 NTU but ≤ 30 NTU.

Stability via intrinsic fluorescence analysis

5 The intrinsic fluorescence analyzed via a spectrofluorometer (Uncle, Unchained Labs) was used to monitor possible changes in the stability of the compositions: the fluorescence spectrum of each formulation was compared with the Cetrotide® freeze-dry fluorescence spectrum, and then also after stressed and accelerated storage conditions the fluorescent profile was monitored.

Results

a) Particle formation

15 The Cetrotide compositions were examined for the presence of visible and sub-visible particles by visual inspection and Aura, respectively.

Table 1. Visual inspection of compositions 1-4

| Composition | time 0 | 2 weeks (40°C) | 4 weeks (25°C) |
|-------------|--------|----------------|----------------|
| 1           | +      | FVP            | FVP            |
| 2           | FVP    | FVP            | FVP            |
| 3           | ++     | ++             | ++             |
| 4           | ++     | ++             | ++             |

20 While compositions including a stabilizer according to the present invention showed no or only few particles after preparation and storage, comparative compositions 3 and 4 included a clearly visible amount of particles.

Sub-visible particles detected through BMI (all particles per ml present in the sample) are reported in Table 2, while proteinaceous particles detected by FMM are reported in Table 3. Compositions 1-3 contained comparative amounts of sub-visible (proteinaceous) particles.

25

Table 2. Sub-visible particles by Aura (BMI)

| Composition | time 0    |            | 2 weeks (40°C) |            | 4 weeks (25°C) |            |
|-------------|-----------|------------|----------------|------------|----------------|------------|
|             | SVP > 5µm | SVP > 25µm | SVP > 5µm      | SVP > 25µm | SVP > 5µm      | SVP > 25µm |
| 1           | 2880      | 120        | 2120           | 40         | 2520           | 120        |
| 2           | 1040      | 80         | 2360           | 240        | 5680           | 240        |
| 3           | 1200      | 120        | 2600           | 120        | 1720           | 40         |
| 4           | 16960     | 200        | 4640           | 40         | 6440           | 320        |

Table 3. Sub-visible proteinaceous particles

| Composition | time 0    |            | 2 weeks (40°C) |            | 4 weeks (25°C) |            |
|-------------|-----------|------------|----------------|------------|----------------|------------|
|             | SVP > 5µm | SVP > 25µm | SVP > 5µm      | SVP > 25µm | SVP > 5µm      | SVP > 25µm |
| 1           | 1720      | 120        | 1160           | 40         | 1960           | 120        |
| 2           | 920       | 80         | 2080           | 240        | 3880           | 160        |
| 3           | 1040      | 80         | 1160           | 80         | 1520           | 0          |
| 4           | 16760     | 200        | 1720           | 0          | 6120           | 320        |

5 b) Peptide content

The peptide content was monitored at time zero, after two weeks of storage at 40°C (RH 75%) and after four weeks of storage at 25°C (RH 60%) by SoloVPE. No peptide loss was seen in any of these conditions for compositions 1-4.

c) Turbidity

- 10 The turbidity of the compositions was examined at time zero, after two weeks of storage at 40°C (RH 75%) and after four weeks of storage at 25°C (RH 60%) by nephelometry. The results are shown in table 4.

Table 4. NTU values obtained for compositions 1-4

| Composition | time 0 | 2 weeks (40°C) | 4 weeks (25°C) |
|-------------|--------|----------------|----------------|
| 1           | 2      | 2              | 3              |
| 2           | 3      | 2              | 2              |
| 3           | 4      | 4              | 3              |
| 4           | 3      | 4              | 2              |

Clear formulations ( $\leq 3$  NTU) were obtained for the compositions according to the invention comprising a stabilizing agent. The comparative compositions were slightly opalescent.

#### 5 d) Stability

The stability of the compositions was evaluated via intrinsic fluorescence at time zero, after two weeks of storage 40°C (RH 75%) and after four weeks of storage at 25°C (RH 60%).

As it can be seen in Figure 1, only formulations containing a stabilizing agent were able to maintain the same fluorescent peptide profile after storage, i.e. showed good stability (Fig. 1a and 1b). The comparative compositions showed altered fluorescence intensities after storage, indicating peptide aggregation (Fig. 1c and 1d).

#### Example 2

Five different Cetorelix acetate compositions including a stabilizing agent were examined. Besides composition 1 (including benzyl alcohol) and composition 2 (including poloxamer 188) according to the invention as described in Example 1, the following comparative compositions were manufactured.

Comparative composition 5: Cetorelix acetate was dissolved in a mixture of D-mannitol, arginine monohydrochloride and water for injection. Then, diluted acetic acid was added in an amount to adjust the pH of the composition to a value of 5.0. The amount of D-mannitol in the final composition was 49.19 mg/ml, the amount of arginine monohydrochloride in the final composition was 3.16 mg/ml and the amount of Cetorelix acetate in the final composition was 0.25 mg/ml Cetorelix pure peptide in its acetate salt form. The osmolality of the final composition was 325 mOsm/kg. The composition was sterile filtered and filled in a glass vial.

Comparative composition 6: Cetorelix acetate was dissolved in a mixture of D-mannitol, proline and water for injection. Then, acetic acid was added in an amount to adjust the pH of the composition to a value of 5.0. The amount of D-mannitol in the final composition was 49.19 mg/ml, the amount of proline in the final composition was 3.45 mg/ml and the amount of Cetorelix acetate in the final composition was 0.25 mg/ml Cetorelix pure peptide in its acetate salt form. The osmolality of the final composition was 322 mOsm/kg. The composition was sterile filtered and filled in a glass vial.

Comparative composition 7: Cetorelix acetate was dissolved in a mixture of D-mannitol, phenol and water for injection. Then, acetic acid was added in an amount to adjust the pH of the composition to a value of 5.0. The amount of D-mannitol in the final composition was 35.30 mg/ml, the amount of phenol in the final composition was 10.00 mg/ml and the amount of Cetorelix acetate in the final composition was 0.25 mg/ml Cetorelix pure peptide in its acetate salt form. The osmolality of the final composition was 318 mOsm/kg. The composition was sterile filtered and filled in a glass vial.

Compositions 1, 2, and 5-7 were analyzed as set forth in Example 1.

Results

a) Particle formation

The Cetorelix compositions were examined for the presence of visible and sub-visible particles by visual inspection and Aura, respectively.

Table 5. Visual inspection of compositions 1-2 and 5-7

| Composition | time 0 | 2 weeks (40°C) | 4 weeks (25°C) |
|-------------|--------|----------------|----------------|
| 1           | +      | FVP            | FVP            |
| 2           | FVP    | FVP            | FVP            |
| 5           | ++     | ++             | ++             |
| 6           | ++     | ++             | ++             |
| 7           | +      | +              | FVP            |

While compositions including a stabilizer according to the present invention showed no or only few particles after preparation and storage, comparative compositions 5 and 6, comprising amino acid stabilizers, included a clearly visible amount of particles.

Sub-visible particles detected through BMI (all particles per ml present in the sample) are reported in Table 6, while proteinaceous particles detected by FMM are reported in Table 7. It was found that the presence of proline and phenol stabilizing agents (comparative compositions 6 and 7) lead to a significantly increased amount of sub-visible particles in the liquid Cetorelix composition.

Table 6. Sub-visible particles by Aura (BMI)

| Composition | time 0    |            | 2 weeks (40°C) |            | 4 weeks (25°C) |            |
|-------------|-----------|------------|----------------|------------|----------------|------------|
|             | SVP > 5µm | SVP > 25µm | SVP > 5µm      | SVP > 25µm | SVP > 5µm      | SVP > 25µm |
| 1           | 2880      | 120        | 2120           | 40         | 2520           | 120        |
| 2           | 1040      | 80         | 2360           | 240        | 5680           | 240        |
| 5           | 1760      | 80         | 720            | 0          | 1840           | 160        |
| 6           | 7120      | 120        | 1280           | 120        | 32920          | 440        |
| 7           | 4280      | 480        | 9000           | 160        | 5640           | 440        |

Table 7. Sub-visible proteinaceous particles

| Composition | time 0    |            | 2 weeks (40°C) |            | 4 weeks (25°C) |            |
|-------------|-----------|------------|----------------|------------|----------------|------------|
|             | SVP > 5µm | SVP > 25µm | SVP > 5µm      | SVP > 25µm | SVP > 5µm      | SVP > 25µm |
| 1           | 1720      | 120        | 1160           | 40         | 1960           | 120        |
| 2           | 920       | 80         | 2080           | 240        | 3880           | 160        |
| 5           | 1600      | 80         | 480            | 0          | 1140           | 120        |
| 6           | 6880      | 80         | 960            | 80         | 32280          | 400        |
| 7           | 1480      | 240        | 5240           | 160        | 3360           | 440        |

b) Peptide content

The peptide content was monitored at time zero, after two weeks of storage at 40°C (RH 75%) and after four weeks of storage at 25°C (RH 60%) by SoloVPE. Due to significant

interference from phenol in the SoloVPE measurement, no result are available for composition 7.

Table 8. Peptide content detected by SoloVPE

| Composition | time 0 | 2 weeks (40°C) | 4 weeks (25°C) |
|-------------|--------|----------------|----------------|
| 1           | 0.25   | 0.25           | 0.25           |
| 2           | 0.25   | 0.25           | 0.25           |
| 5           | 0.03   | 0.03           | 0.09           |
| 6           | 0.23   | 0.27           | 0.26           |

- 5 The presence of arginine stabilizing agent (comparative composition 5) lead to a severe loss in peptide, while no peptide loss was seen in any conditions for compositions 1, 2 and 6.

c) Turbidity

- 10 The turbidity of the compositions was examined at time zero, after two weeks of storage at 40°C (RH 75%) and after four weeks of storage at 25°C (RH 60%) by nephelometry. To evaluate the impact of shaking stress on peptide stability, all formulations were additionally investigated after being subjected to a shaking stress of 300 rpm for 24 hours at room temperature. The results are shown in table 9.

Table 9. NTU values obtained for compositions 1-2 and 5-7

| Composition | time 0 | 2 weeks (40°C) | 4 weeks (25°C) | shaking stress |
|-------------|--------|----------------|----------------|----------------|
| 1           | 2      | 2              | 3              | 2              |
| 2           | 3      | 2              | 2              | 2              |
| 5           | 4      | 2              | 3              | 5              |
| 6           | 3      | 4              | 3              | 3              |
| 7           | 6      | 2              | 3              | 48             |

15

Clear formulations ( $\leq 3$  NTU) were obtained for compositions 1 and 2 according to the invention. The comparative compositions were slightly opalescent.

Shaking stress did not have a significant impact on compositions 1-2 and 5-6, while the phenol-stabilized composition 7 was very opalescent and particle formation could be detected after shaking.

#### d) Stability

- 5 The stability of the compositions was evaluated via intrinsic fluorescence at time zero, after two weeks of storage 40°C (RH 75%) and after four weeks of storage at 25°C (RH 60%). For compositions 1-6 the fluorescence was analyzed using an emission wavelength of 275nm. Due to interference caused by the adsorption of phenol in the same area as Cetorelix, composition 7 was analyzed with an emission wavelength of 285nm.

10

Formulations containing a stabilizing agent according to the invention were able to maintain the same fluorescent peptide profile after storage, i.e. showed good stability (Fig. 1a and 1b). The comparative compositions showed altered fluorescence intensities after storage, indicating peptide aggregation (Fig. 2a and 2b).

15

#### **Example 3**

Computational investigations were also performed on Cetorelix.

#### Methods

- 20 The initial structure of Cetorelix was built in Molecular Operating Environment (MOE) 2020 software with default Amber10:EHT force field. The MD simulations were performed using NAMD software using CHARMM36m force field and TIP3P model for water. Nonbonded interactions were calculated with a cutoff of 12 Å, with a switching function starting at 10 Å. Particle mesh Ewald (PME) was used to account for long-range electrostatic interactions with a grid density of 1 Å<sup>-3</sup> and a PME interpolation order of 6. Rigid bonds for all hydrogen
- 25 atoms were enforced using the SHAKE algorithm. All simulations used for analysis were performed using periodic boundary conditions in the NPT ensemble. The structure of poloxamer was constructed using an in-house script that considers a total molecular weight of 8400 g/mol and a building block composition of 80% / 20% of polyoxyethylene / polyoxypropylene. The equilibration protocol of each system consisted in a temperature
- 30 ramp from 0 K to 300 K, the latter chosen as the target temperature for all systems in production runs. The simulation box contained either 1 single Cetorelix molecule or an ensemble of 4 Cetorelix molecules separated by at least 10 Å from each other. Each

system was simulated for 300 ns and was replicated three-folds. Analysis of the trajectories was performed in VMD using custom scripts.

### Results

5 In a first computational investigation, Cetorelix was simulated in the presence of either poloxamer 188 or benzyl alcohol. The results suggest that the mechanism of stabilization of both excipients is tightly bound to their interaction with hydrophobic regions of Cetorelix. By preventing the Cetorelix' exposure to the aqueous environment, both poloxamer 188 and benzyl alcohol effectively reduce the propensity for self-interaction between peptide molecules, thereby lowering the risk of aggregation.

10 In a second computational investigation, Cetorelix was simulated in the presence of either lactic acid or acetic acid. The results suggest that the negatively charged conjugated base from the buffer strongly interacts with positively charged residues in Cetorelix, leading to increased aggregation between Cetorelix molecules. Besides through the neutralization of the positive charge, aggregation is further facilitated by buffer coordination effect of multiple  
15 residues from different Cetorelix molecules. To minimize the quantity of conjugated base in the formulation, the use of free acid to fix the pH is preferred over buffer solutions. Furthermore, buffer agents with lower pKa values may reduce the risk of aggregation, as they can be used to reach a lower pH value with a relatively lower amount of acid.

### 20 **Example 4**

#### **Manufacturing method for Examples 4.1 and 4.2:**

A concentrated peptide solution (range 2 – 3 mg/mL Cetorelix pure peptide) is prepared by dissolving Cetorelix pure peptide (powder) in a 1 mg/mL acetic acid solution. The concentrated solution is then diluted with a diluent solution containing the remaining  
25 excipients (Mannitol and Poloxamer 188 for Example 4.1 / Mannitol and Benzyl alcohol for Example 4.2). The pH is checked and adjusted to target with diluted acetic acid or sodium hydroxide. Water is finally added to final weight. The solution is stirred for not less than 15 minutes. The solution is sterilized through filtration on a 0.22 µm membrane and filled in the relevant container.

30

**Example 4.1**

| <b>Ingredient</b>                    | <b>Amount (mg/mL)</b> |
|--------------------------------------|-----------------------|
| Cetrorelix (pure peptide)            | 0.25                  |
| Glacial Acetic Acid 100%             | 0.1                   |
| D – Mannitol                         | 54.8                  |
| Poloxamer 188                        | 1.5                   |
| Diluted acetic acid/sodium hydroxide | q.s. to pH 4.7        |
| Water for Injection                  | q.s. to 1 mL          |

q.s. = quantum statis (i.e. sufficient quantity to reach 1 mL in total)

**Example 4.2**

5

| <b>Ingredient</b>                    | <b>Amount (mg/mL)</b> |
|--------------------------------------|-----------------------|
| Cetrorelix (pure peptide)            | 0.25                  |
| Glacial Acetic Acid 100%             | 0.1                   |
| D – Mannitol                         | 37.8                  |
| Benzyl Alcohol                       | 10                    |
| Diluted acetic acid/sodium hydroxide | q.s. to pH 4.7        |
| Water for Injection                  | q.s. to 1 mL          |

q.s. = quantum statis (i.e. sufficient quantity to reach 1 mL in total)

- Visible particles by visual inspection (according to Pharm. Eur. chapter 2.9.20, version 10 / USP chapter <790>, 2023 Edition):

| <b>Liquid formulation</b> | <b>Time 0</b>               | <b>4 weeks +25°C</b>        | <b>4 weeks +40°C</b>        |
|---------------------------|-----------------------------|-----------------------------|-----------------------------|
| <b>Example 4.1</b>        | free from visible particles | free from visible particles | free from visible particles |

|             |                                   |                                   |                                   |
|-------------|-----------------------------------|-----------------------------------|-----------------------------------|
| Example 4.2 | free from<br>visible<br>particles | free from<br>visible<br>particles | free from<br>visible<br>particles |
|-------------|-----------------------------------|-----------------------------------|-----------------------------------|

- Turbidity (NTU) by Nephelometry {according to European Pharmacopoeia chapter 2.2.1, version 10 (hereinafter sometimes simply referred to as "Pharm. Eur.") / United States Pharmacopoeia chapter <855>, 2023 Edition (hereinafter sometimes simply referred to as "USP")}:  
5

| Liquid<br>formulation | Time 0 | 4 weeks<br>+25°C | 4 weeks<br>+40°C |
|-----------------------|--------|------------------|------------------|
| Example 4.1           | 2      | 3                | 3                |
| Example 4.2           | 2      | 3                | 3                |

- pH measurement (according to Pharm. Eur. chapter 2.2.3, version 10 / USP chapter <791>, 2023 Edition):

| Liquid<br>formulation | Time 0 | 4 weeks<br>+25°C | 4 weeks<br>+40°C |
|-----------------------|--------|------------------|------------------|
| Example 4.1           | 4.7    | 4.7              | 4.6              |
| Example 4.2           | 4.7    | 4.6              | 4.6              |

- 10
- Osmolality (Osm/Kg) (according to Pharm. Eur. chapter 2.2.35, version 10 / USP chapter <785>, 2023 Edition):

| Liquid<br>formulation | Time 0 | 26 weeks<br>+5°C | 26 weeks<br>+25°C |
|-----------------------|--------|------------------|-------------------|
| Example 4.1           | 0.320  | 0.321            | 0.322             |
| Example 4.2           | 0.316  | 0.320            | 0.317             |

- Sub-visible Particles by Light Obscuration (PAMAS) (according to Pharm. Eur. chapter 2.9.12, version 10 / USP <787> and <788>, 2023 Edition):

| Liquid<br>Formulation | Results<br>particles/containe<br>r | Time 0 | 4 weeks<br>+25°C | 4 weeks<br>+40°C |
|-----------------------|------------------------------------|--------|------------------|------------------|
| Example 4.1           | ≥ 2 micron                         | 14275  | 1993             | 1776             |
|                       | ≥ 5 micron                         | 2880   | 506              | 643              |
|                       | ≥ 10 micron                        | 379    | 112              | 273              |
|                       | ≥ 25 micron                        | 1      | 3                | 13               |
| Example 4.2           | ≥ 2 micron                         | 4444   | 2485             | 5883             |
|                       | ≥ 5 micron                         | 903    | 602              | 1460             |
|                       | ≥ 10 micron                        | 109    | 128              | 295              |
|                       | ≥ 25 micron                        | 1      | 6                | 35               |

- Peptide content (mg/mL) by RP-HPLC:

| Liquid Formulation | Before Filtration | Time 0 | 4 weeks +25°C | 4 weeks +40°C | 8 weeks +25°C |
|--------------------|-------------------|--------|---------------|---------------|---------------|
| Example 4.1        | 0.24              | 0.20   | 0.20          | 0.19          | 0.21          |
| Example 4.2        | 0.24              | 0.22   | 0.22          | 0.21          | 0.22          |

- Purity:

As determined by HPLC analysis, Examples 4.1 and 4.2 do not show any increase of impurities under refrigerated conditions (+5°C±3°C) up to 78 weeks of storage. Under accelerated conditions (25°C ± 2°C RH 60% ± 5%) the increase in total impurities is moderate (less than 1% after 52 weeks).

Example 5

10 Prepared according to the method described in Example 4.

| Ingredient                           | Composition (mg/mL) |
|--------------------------------------|---------------------|
| Cetorelix (pure peptide)             | 0.22                |
| Glacial Acetic Acid 100%             | 0.1                 |
| D – Mannitol                         | 54.8                |
| Poloxamer 188                        | 1.5                 |
| Diluted acetic acid/sodium hydroxide | q.s. to pH 4.7      |
| Water for Injection                  | q.s. to 1 mL        |
| Density (g/cm3) at +20°C             | 1.018               |

| Test                                     | Unit | Time 0                    | 4 weeks +25°C             | 4 weeks +40°C             |
|------------------------------------------|------|---------------------------|---------------------------|---------------------------|
| Particles formation by visual inspection | -    | Free of visible particles | Free of visible particles | Free of visible particles |

| Test                                                                                                                                                                                  | Unit    | Time 0 | 4 weeks<br>+25°C | 4 weeks<br>+40°C |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|--------|------------------|------------------|
| (according to Pharm.<br>Eur. chapter 2.9.20,<br>version 10 / USP<br>chapter <790>, 2023<br>Edition)                                                                                   |         |        |                  |                  |
| <b>pH measurement</b><br>(according to Pharm.<br>Eur. chapter 2.2.3,<br>version 10 / USP<br>chapter <791>, 2023<br>Edition)                                                           | -       | 4.7    | 4.7              | 4.7              |
| <b>Osmolality by<br/>freezing point</b><br>(according to Pharm.<br>Eur. chapter 2.2.35,<br>version 10 / USP<br>chapter <785>, 2023<br>Edition)                                        | Osm/Kg  | 0.318  | -                | -                |
| <b>Turbidity</b><br>by Nephelometry<br>(according to Pharm.<br>Eur. chapter 2.2.1,<br>version 10 / USP<br>chapter <855>, 2023<br>Edition)                                             | NTU     | 3      | 2                | 2                |
| <b>Sub-visible<br/>particles by light<br/>obscuration</b><br>(PAMAS)<br>(according to Pharm.<br>Eur. chapter 2.9.19,<br>version 10 / USP<br>chapter <787> and<br><788>, 2023 Edition) | ≥ 2 µm  | 9004   | 33069            | 22762            |
|                                                                                                                                                                                       | ≥ 5 µm  | 2481   | 8044             | 4780             |
|                                                                                                                                                                                       | ≥ 10 µm | 401    | 1074             | 615              |
|                                                                                                                                                                                       | ≥ 25 µm | 2      | 7                | 7                |

| Test                                            | Unit         | Time 0 | 4 weeks<br>+25°C | 4 weeks<br>+40°C |
|-------------------------------------------------|--------------|--------|------------------|------------------|
| Peptide Content<br>(as monitored by<br>RP-HPLC) | mg/mL        | 0.22   | 0.22             | 0.23             |
|                                                 | mg/container | 0.22   | 0.22             | 0.22             |

- Purity:

As determined by HPLC analysis, Example 5 does not show any increase of impurities under refrigerated conditions (+5°C±3°C) up to 26 weeks of storage. Under accelerated conditions (25°C± 2°C RH 60% ± 5%) the increase in total impurities is moderate (less than 0.5% after 26weeks).

Example 6

Manufacturing method (multidose formulations)

- 10
- A concentrated peptide solution (range 2 – 3 mg/mL Cetorelix pure peptide) is prepared by dissolving Cetorelix pure peptide (powder) in a 1 mg/mL acetic acid solution. The concentrated solution is then diluted with a diluent solution containing Mannitol and Poloxamer 188. Upon homogenization, the relevant preservative is added and the solution stirred until complete dissolution of the preservative. The pH is checked and adjusted to target with diluted acetic acid or sodium hydroxide. Water is finally added to final weight. The solution is stirred for not less than 15 minutes. The solution is sterilized through filtration on a 0.22 µm membrane and filled in the relevant container.
- 15

| Example      | Composition         |     |                                                    |                      |                    |                                         |
|--------------|---------------------|-----|----------------------------------------------------|----------------------|--------------------|-----------------------------------------|
|              | pH adjuster         | pH  | Excipients                                         |                      |                    | Active Pharmaceutical Ingredient        |
|              | Acetic Acid (mg/mL) |     | Bacteriostatic agent                               | Poloxamer188 (mg/mL) | D-Mannitol (mg/mL) | Cetorelix Acetate (pure peptide, mg/mL) |
|              |                     |     |                                                    |                      |                    |                                         |
| Example 6-01 | 0.1                 | 4.5 | Phenol (5 mg/mL)                                   | 1.5                  | 54.8               | 0.76                                    |
| Example 6-02 | 0.1                 | 5.0 | Chlorbutanol (5 mg/mL)                             | 1.5                  | 54.8               | 0.76                                    |
| Example 6-03 | 0.1                 | 3.5 | Methyl paraben (2 mg/mL) + propylparaben (1 mg/mL) | 1.5                  | 54.8               | 0.59                                    |

|              |     |     |                                                    |     |      |      |
|--------------|-----|-----|----------------------------------------------------|-----|------|------|
| Example 6-04 | 0.1 | 3.5 | Benzalkonium chloride (0.5 mg/mL)                  | 1.5 | 54.8 | 0.76 |
| Example 6-05 | 0.1 | 5.0 | Chlorobutanol (5 mg/mL)                            | 1.5 | 54.8 | 0.42 |
| Example 6-06 | 0.1 | 4.5 | Phenoxyethanol (5 mg/mL)                           | 1.5 | 54.8 | 0.42 |
| Example 6-07 | 0.1 | 5.0 | Benzalkonium chloride (0.5 mg/mL)                  | 1.5 | 54.8 | 0.42 |
| Example 6-08 | 0.1 | 5.0 | Benzyl alcohol (15 mg/mL)                          | 1.5 | 54.8 | 0.76 |
| Example 6-09 | 0.1 | 5.0 | m-cresol (3 mg/mL)                                 | 1.5 | 54.8 | 0.59 |
| Example 6-10 | 0.1 | 5.0 | Benzyl alcohol (15 mg/mL)                          | 1.5 | 54.8 | 0.42 |
| Example 6-11 | 0.1 | 4.5 | Methyl paraben (2 mg/mL) + propylparaben (1 mg/mL) | 1.5 | 54.8 | 0.59 |
| Example 6-12 | 0.1 | 3.5 | Phenol (5 mg/mL)                                   | 1.5 | 54.8 | 0.42 |
| Example 6-13 | 0.1 | 5.0 | Phenoxyethanol (5 mg/mL)                           | 1.5 | 54.8 | 0.76 |
| Example 6-14 | 0.1 | 4.5 | m-cresol (3 mg/mL)                                 | 1.5 | 54.8 | 0.42 |
| Example 6-15 | 0.1 | 4.5 | Phenoxyethanol (5 mg/mL)                           | 1.5 | 54.8 | 0.59 |

Results from screening of different peptide concentrations, pH values and bacteriostatic agents:

- 5
- Visible particles by visual inspection (according to Pharm. Eur. chapter 2.9.20, version 10 / USP chapter <790>, 2023 Edition):

| Liquid Formulation | Time 0                      | 4 weeks +25°C               |
|--------------------|-----------------------------|-----------------------------|
| Example 6-01       | free from visible particles | free from visible particles |
| Example 6- 02      | free from visible particles | free from visible particles |
| Example 6-03       | free from visible particles | free from visible particles |
| Example 6-05       | free from visible particles | free from visible particles |
| Example 6-06       | free from visible particles | free from visible particles |
| Example 6-08       | free from visible particles | free from visible particles |
| Example 6-09       | free from visible particles | free from visible particles |
| Example 6-10       | free from visible particles | free from visible particles |
| Example 6-11       | free from visible particles | free from visible particles |
| Example 6-12       | free from visible particles | free from visible particles |
| Example 6-13       | free from visible particles | free from visible particles |
| Example 6-14       | free from visible particles | free from visible particles |
| Example 6-15       | free from visible particles | free from visible particles |

- 10
- Turbidity (NTU) by Nephelometry (according to European Pharmacopoeia chapter 2.2.1, version 10 (hereinafter sometimes simply referred to as "Pharm. Eur.") / United States Pharmacopoeia chapter <855>, 2023 Edition (hereinafter sometimes simply referred to as "USP")):

| Liquid Formulation | Time 0 | 4 weeks<br>+25°C |
|--------------------|--------|------------------|
| Example 6-01       | 12     | 11               |
| Example 6-02       | 14     | 13               |
| Example 6-03       | 2      | 2                |
| Example 6-05       | 7      | 5                |
| Example 6-06       | 4      | 3                |
| Example 6-08       | 14     | 13               |
| Example 6-09       | 15     | 16               |
| Example 6-10       | 6      | 6                |
| Example 6-11       | 12     | 14               |
| Example 6-12       | 3      | 2                |
| Example 6-13       | 9      | 11               |
| Example 6-14       | 11     | 9                |
| Example 6-15       | 6      | 6                |

- pH measurement (according to Pharm. Eur. chapter 2.2.3, version 10 / USP chapter <791>, 2023 Edition):

| Liquid Formulation | Time 0 | 4 weeks<br>+25°C |
|--------------------|--------|------------------|
| Example 6-01       | 4.4    | 4.6              |
| Example 6-02       | 5.0    | 5.0              |
| Example 6-03       | 3.4    | 3.6*             |
| Example 6-05       | 4.9    | 5.0              |
| Example 6-06       | 4.5    | 4.6              |
| Example 6-08       | 5.0    | 5.0              |
| Example 6-09       | 5.0    | 5.1              |
| Example 6-10       | 5.0    | 5.0              |
| Example 6-11       | 4.5    | 4.6              |
| Example 6-12       | 3.5    | 3.6*             |
| Example 6-13       | 5.0    | 5.0              |
| Example 6-14       | 4.5    | 4.6              |
| Example 6-15       | 4.4    | 4.5              |

5

\*The samples underwent retesting due to the anomalous values obtained in the initial test compared to the target.

- Sub-visible Particles by Light Obscuration (PAMAS) (according to Pharm. Eur. chapter 2.9.12, version 10 / USP <787> and <788>, 2023 Edition):

10

| Liquid Formulation | Results particles/container | Time 0 | 4 weeks<br>+25°C |
|--------------------|-----------------------------|--------|------------------|
| Example 6-01       | ≥ 2 micron                  | 3207   | 7320             |
|                    | ≥ 5 micron                  | 546    | 71299            |
|                    | ≥ 10 micron                 | 104    | 154              |
|                    | ≥ 25 micron                 | 13     | 3                |
| Example 6-02       | ≥ 2 micron                  | 1984   | 2508             |
|                    | ≥ 5 micron                  | 360    | 462              |
|                    | ≥ 10 micron                 | 69     | 93               |
|                    | ≥ 25 micron                 | 4      | 4                |
| Example 6-03       | ≥ 2 micron                  | 783    | 1220             |
|                    | ≥ 5 micron                  | 225    | 260              |
|                    | ≥ 10 micron                 | 65     | 47               |

|                     |             |      |      |
|---------------------|-------------|------|------|
|                     | ≥ 25 micron | 5    | 3    |
| <b>Example 6-05</b> | ≥ 2 micron  | 1429 | 1893 |
|                     | ≥ 5 micron  | 402  | 342  |
|                     | ≥ 10 micron | 126  | 33   |
|                     | ≥ 25 micron | 18   | 2    |
| <b>Example 6-06</b> | ≥ 2 micron  | 3120 | 723  |
|                     | ≥ 5 micron  | 913  | 102  |
|                     | ≥ 10 micron | 263  | 28   |
|                     | ≥ 25 micron | 23   | 4    |
| <b>Example 6-08</b> | ≥ 2 micron  | 1751 | 1748 |
|                     | ≥ 5 micron  | 253  | 294  |
|                     | ≥ 10 micron | 33   | 49   |
|                     | ≥ 25 micron | 4    | 3    |
| <b>Example 6-09</b> | ≥ 2 micron  | 2355 | 4878 |
|                     | ≥ 5 micron  | 346  | 723  |
|                     | ≥ 10 micron | 59   | 81   |
|                     | ≥ 25 micron | 3    | 3    |
| <b>Example 6-10</b> | ≥ 2 micron  | 1176 | 980  |
|                     | ≥ 5 micron  | 330  | 68   |
|                     | ≥ 10 micron | 111  | 12   |
|                     | ≥ 25 micron | 7    | 1    |
| <b>Example 6-11</b> | ≥ 2 micron  | 1037 | 3566 |
|                     | ≥ 5 micron  | 178  | 703  |
|                     | ≥ 10 micron | 34   | 57   |
|                     | ≥ 25 micron | 3    | 3    |
| <b>Example 6-12</b> | ≥ 2 micron  | 1328 | 551  |
|                     | ≥ 5 micron  | 328  | 143  |
|                     | ≥ 10 micron | 75   | 30   |
|                     | ≥ 25 micron | 3    | 2    |
| <b>Example 6-13</b> | ≥ 2 micron  | 230  | 1736 |
|                     | ≥ 5 micron  | 58   | 124  |
|                     | ≥ 10 micron | 20   | 28   |
|                     | ≥ 25 micron | 3    | 2    |
| <b>Example 6-14</b> | ≥ 2 micron  | 2307 | 3299 |
|                     | ≥ 5 micron  | 528  | 595  |
|                     | ≥ 10 micron | 156  | 69   |
|                     | ≥ 25 micron | 8    | 2    |
| <b>Example 6-15</b> | ≥ 2 micron  | 530  | 1645 |
|                     | ≥ 5 micron  | 96   | 137  |
|                     | ≥ 10 micron | 31   | 18   |
|                     | ≥ 25 micron | 4    | 3    |

- Peptide content (mg/mL) by RP-HPLC:

| Liquid Formulation  | Before Filtration | Time 0 | 4 weeks +25°C |
|---------------------|-------------------|--------|---------------|
| <b>Example 6-01</b> | 0.75              | 0.70   | 0.70          |
| <b>Example 6-02</b> | 0.78              | 0.74   | 0.66          |
| <b>Example 6-03</b> | 0.61              | 0.58   | 0.58          |
| <b>Example 6-05</b> | 0.47              | 0.37   | 0.34          |
| <b>Example 6-06</b> | 0.43              | 0.41   | 0.41          |
| <b>Example 6-08</b> | 0.77              | 0.73   | 0.68          |
| <b>Example 6-09</b> | 0.61              | 0.55   | 0.51          |
| <b>Example 6-10</b> | 0.44              | 0.41   | 0.40          |
| <b>Example 6-11</b> | 0.58              | 0.44   | 0.55          |
| <b>Example 6-12</b> | 0.43              | 0.46   | 0.42          |
| <b>Example 6-13</b> | 0.81              | 0.60   | 0.54          |
| <b>Example 6-14</b> | 0.43              | 0.44   | 0.41          |
| <b>Example 6-15</b> | 0.54              | 0.58   | 0.56          |

Antimicrobial effectiveness test

The efficacy of antimicrobial preservation test efficacy was performed on the following selected formulations (according to Pharm. Eur. chapters 2.6.12 and 5.1.3, version 10 / USP chapter <51> and <1117>, 2023 Edition):

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| Results at Time = 6 hours |                       |             |                                |             |                                |             |                                |             |                                |             |                                |                      |                                |
|---------------------------|-----------------------|-------------|--------------------------------|-------------|--------------------------------|-------------|--------------------------------|-------------|--------------------------------|-------------|--------------------------------|----------------------|--------------------------------|
| Microorganism             | Time<br>0<br>Sol<br>A | Ex.<br>6-01 |                                | Ex.<br>6-02 |                                | Ex.<br>6-03 |                                | Ex.<br>6-08 |                                | Ex.<br>6-09 |                                | Ex.<br>6-15          |                                |
|                           |                       | CFU/mL      | $\Delta\log_{10}$<br>vs<br>Ref | CFU/<br>mL  | $\Delta\log_{10}$<br>vs<br>Ref | CFU/mL      | $\Delta\log_{10}$<br>vs<br>Ref | CFU/<br>mL  | $\Delta\log_{10}$<br>vs<br>Ref | CFU/mL      | $\Delta\log_{10}$<br>vs<br>Ref | CFU/<br>mL           | $\Delta\log_{10}$<br>vs<br>Ref |
| <i>S. aureus</i>          | 8.1<br>$\times 10^4$  | <<br>$10^2$ | ><br>3                         | <<br>$10^2$ | ><br>3                         | <<br>$10^2$ | ><br>3                         | <<br>$10^2$ | ><br>3                         | <<br>$10^2$ | ><br>3                         | 9.0<br>$\times 10^2$ | 2                              |

| Results at Time =24 hours |                       |             |                                |             |                                |             |                                |             |                                |             |                                |                      |                                |
|---------------------------|-----------------------|-------------|--------------------------------|-------------|--------------------------------|-------------|--------------------------------|-------------|--------------------------------|-------------|--------------------------------|----------------------|--------------------------------|
| Microorganism             | Time<br>0<br>Sol<br>A | Ex.<br>6-01 |                                | Ex.<br>6-02 |                                | Ex.<br>6-03 |                                | Ex.<br>6-08 |                                | Ex.<br>6-09 |                                | Ex.<br>6-15          |                                |
|                           |                       | CFU/mL      | $\Delta\log_{10}$<br>vs<br>Ref | CFU/<br>mL  | $\Delta\log_{10}$<br>vs<br>Ref | CFU/mL      | $\Delta\log_{10}$<br>vs<br>Ref | CFU/<br>mL  | $\Delta\log_{10}$<br>vs<br>Ref | CFU/mL      | $\Delta\log_{10}$<br>vs<br>Ref | CFU/<br>mL           | $\Delta\log_{10}$<br>vs<br>Ref |
| <i>S. aureus</i>          | 8.1<br>$\times 10^4$  | <<br>$10^2$ | ><br>3                         | <<br>$10^2$ | ><br>3                         | <<br>$10^2$ | ><br>3                         | <<br>$10^2$ | ><br>3                         | <<br>$10^2$ | ><br>3                         | 6.5<br>$\times 10^2$ | 2                              |

| Results at Time =7 days |                       |             |                                |             |                                |             |                                |             |                                |             |                                |                      |                                |
|-------------------------|-----------------------|-------------|--------------------------------|-------------|--------------------------------|-------------|--------------------------------|-------------|--------------------------------|-------------|--------------------------------|----------------------|--------------------------------|
| Microorganism           | Time<br>0<br>Sol<br>A | Ex.<br>6-01 |                                | Ex.<br>6-02 |                                | Ex.<br>6-03 |                                | Ex.<br>6-08 |                                | Ex.<br>6-09 |                                | Ex.<br>6-15          |                                |
|                         |                       | CFU/mL      | $\Delta\log_{10}$<br>vs<br>Ref | CFU/<br>mL  | $\Delta\log_{10}$<br>vs<br>Ref | CFU/mL      | $\Delta\log_{10}$<br>vs<br>Ref | CFU/<br>mL  | $\Delta\log_{10}$<br>vs<br>Ref | CFU/mL      | $\Delta\log_{10}$<br>vs<br>Ref | CFU/<br>mL           | $\Delta\log_{10}$<br>vs<br>Ref |
| <i>S. aureus</i>        | 8.1<br>$\times 10^4$  | <<br>$10^2$ | ><br>3                         | <<br>$10^2$ | ><br>3                         | <<br>$10^2$ | ><br>3                         | <<br>$10^2$ | ><br>3                         | <<br>$10^2$ | ><br>3                         | 6.5<br>$\times 10^2$ | 2                              |
| <i>Candida Albicans</i> | 6.8<br>$\times 10^5$  | < $10^2$    | >4                             | < $10^2$    | >4                             | < $10^2$    | >4                             | < $10^2$    | >4                             | < $10^2$    | >4                             | 2.0<br>$\times 10^2$ | 4                              |

| Results at Time =14 days |                       |             |                                |             |                                |             |                                |             |                                |             |                                |             |                                |
|--------------------------|-----------------------|-------------|--------------------------------|-------------|--------------------------------|-------------|--------------------------------|-------------|--------------------------------|-------------|--------------------------------|-------------|--------------------------------|
| Microorganism            | Time<br>0<br>Sol<br>A | Ex.<br>6-01 |                                | Ex.<br>6-02 |                                | Ex.<br>6-03 |                                | Ex.<br>6-08 |                                | Ex.<br>6-09 |                                | Ex.<br>6-15 |                                |
|                          |                       | CFU/mL      | $\Delta\log_{10}$<br>vs<br>Ref | CFU/<br>mL  | $\Delta\log_{10}$<br>vs<br>Ref | CFU/<br>mL  | $\Delta\log_{10}$<br>vs<br>Ref | CFU/<br>mL  | $\Delta\log_{10}$<br>vs<br>Ref | CFU/<br>mL  | $\Delta\log_{10}$<br>vs<br>Ref | CFU/<br>mL  | $\Delta\log_{10}$<br>vs<br>Ref |
| <i>S. aureus</i>         | 8.1<br>$\times 10^4$  | < $10^2$    | >3                             | < $10^2$    | >3                             | < $10^2$    | >3                             | < $10^2$    | >3                             | < $10^2$    | >3                             | < $10^2$    | >3                             |
| <i>Candida Albicans</i>  | 6.8<br>$\times 10^5$  | < $10^2$    | >4                             | < $10^2$    | >4                             | < $10^2$    | >4                             | < $10^2$    | >4                             | < $10^2$    | >4                             | < $10^2$    | >4                             |

10 *S. aureus* : Staphylococcus Aureus ; Ex. = Example

Conclusion:

All samples met criteria of EP and USP considering both bacteria (*S. aureus*: Staphylococcus Aureus) and fungi (*C. Albicans*: Candida Albicans). The only exception is the Example 6-15, that is not conform for criterion A of EP (*S. aureus*).

5

- Purity:

As determined by HPLC analysis, Example 6 does not show any increase of impurities under both refrigerated (+5°C±3°C) and accelerated conditions (25°C± 2°C RH 60% ± 5%) up to 4 weeks of storage.

10

Example 7:

Prepared according to the method of Example 6.

| Example      | DP                  |     |                                   |                       |                     |                                          |
|--------------|---------------------|-----|-----------------------------------|-----------------------|---------------------|------------------------------------------|
|              | pH adjuster         | pH  | Excipients                        |                       |                     | API                                      |
|              | Acetic Acid (mg/mL) |     | Bacteriostatic agent              | Poloxamer 188 (mg/mL) | D- Mannitol (mg/mL) | Cetrorelix Acetate (pure peptide, mg/mL) |
| Example 7-01 | 0.1                 | 4.5 | Phenol (5 mg/mL)                  | 1.5                   | 54.8                | 0.59                                     |
| Example 7-02 | 0.1                 | 4.5 | Benzalkonium chloride (0.5 mg/mL) | 1.5                   | 54.8                | 0.59                                     |
| Example 7-03 | 0.1                 | 4.5 | Phenoxyethanol (10 mg/mL)         | 1.5                   | 54.8                | 0.59                                     |
| Example 7-04 | 0.1                 | 4.5 | Benzyl alcohol (15 mg/mL)         | 1.5                   | 54.8                | 0.59                                     |
| Example 7-05 | 0.1                 | 4.5 | m-cresol (3 mg/mL)                | 1.5                   | 54.8                | 0.59                                     |

- Visible particles by visual inspection (according to Pharm. Eur. chapter 2.9.20, version 10 / USP chapter <790>, 2023 Edition):

15

| Example      | Time 0                      | 4 weeks +25°C               |
|--------------|-----------------------------|-----------------------------|
| Example 7-01 | free from visible particles | free from visible particles |
| Example 7-02 | free from visible particles | free from visible particles |
| Example 7-03 | free from visible particles | free from visible particles |
| Example 7-04 | free from visible particles | free from visible particles |
| Example 7-05 | free from visible particles | free from visible particles |

- Turbidity (NTU) by Nephelometry {according to European Pharmacopoeia chapter 2.2.1, version 10 (hereinafter sometimes simply referred to as "Pharm. Eur.") / United States Pharmacopoeia chapter <855>, 2023 Edition (hereinafter sometimes simply referred to as "USP")}:  
5

| Example      | Time 0 | 4 weeks<br>+25°C |
|--------------|--------|------------------|
| Example 7-01 | 6      | 7                |
| Example 7-02 | 7      | 8                |
| Example 7-03 | 4      | 5                |
| Example 7-04 | 4      | 5                |
| Example 7-05 | 12     | 13               |

- pH measurement (according to Pharm. Eur. chapter 2.2.3, version 10 / USP chapter <791>, 2023 Edition):

| Example      | Time 0 | 4 weeks<br>+25°C |
|--------------|--------|------------------|
| Example 7-01 | 4.5    | 4.5              |
| Example 7-02 | 4.3    | 4.3              |
| Example 7-03 | 4.4    | 4.4              |
| Example 7-04 | 4.5    | 4.6              |
| Example 7-05 | 4.5    | 4.5              |

10

- Sub-visible Particles by Light Obscuration (PAMAS) (according to Pharm. Eur. chapter 2.9.12, version 10 / USP <787> and <788>, 2023 Edition):

| Example      | Results<br>particles/container | Time 0 | 4 weeks<br>+25°C |
|--------------|--------------------------------|--------|------------------|
| Example 7-01 | ≥ 2 micron                     | 961    | 1818             |
|              | ≥ 5 micron                     | 195    | 418              |
|              | ≥ 10 micron                    | 41     | 75               |
|              | ≥ 25 micron                    | 8      | 6                |
| Example 7-02 | ≥ 2 micron                     | 360    | 755              |
|              | ≥ 5 micron                     | 83     | 243              |
|              | ≥ 10 micron                    | 33     | 66               |
|              | ≥ 25 micron                    | 3      | 4                |
| Example 7-03 | ≥ 2 micron                     | 593    | 633              |
|              | ≥ 5 micron                     | 148    | 207              |
|              | ≥ 10 micron                    | 50     | 72               |
|              | ≥ 25 micron                    | 4      | 24               |
| Example      | ≥ 2 micron                     | 457    | 1801             |

|              |             |      |      |
|--------------|-------------|------|------|
| 7-04         | ≥ 5 micron  | 108  | 482  |
|              | ≥ 10 micron | 27   | 139  |
|              | ≥ 25 micron | 1    | 13   |
| Example 7-05 | ≥ 2 micron  | 1458 | 2276 |
|              | ≥ 5 micron  | 279  | 425  |
|              | ≥ 10 micron | 53   | 48   |
|              | ≥ 25 micron | 13   | 4    |

- Peptide content (mg/mL) by RP-HPLC:

| DP           | Before Filtration | Time 0 | 4 weeks +25°C |
|--------------|-------------------|--------|---------------|
| Example 7-01 | 0.59              | 0.58   | 0.55          |
| Example 7-02 | 0.59              | 0.58   | 0.55          |
| Example 7-03 | 0.61              | 0.58   | 0.56          |
| Example 7-04 | 0.61              | 0.59   | 0.55          |
| Example 7-05 | 0.57              | 0.58   | 0.54          |

5

Antimicrobial effectiveness test:

The efficacy of antimicrobial preservation test efficacy was performed on the following selected formulations (according to Pharm. Eur. chapters 2.6.12 and 5.1.3, version 10 / USP chapter <51> and <1117>, 2023 Edition).

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Results according to EP

| Results at T=6hrs |                       |              |                           |              |                           |                  |                           |
|-------------------|-----------------------|--------------|---------------------------|--------------|---------------------------|------------------|---------------------------|
| Microorganism     | Time 0 Sol A          | Example 7-02 |                           | Example 7-03 |                           | Example 7-04     |                           |
|                   |                       | CFU/mL       | Δlog <sub>10</sub> vs Ref | CFU/mL       | Δlog <sub>10</sub> vs Ref | CFU/mL           | Δlog <sub>10</sub> vs Ref |
| <i>S. aureus</i>  | 1.48 x10 <sup>5</sup> | 600          | 2                         | 800          | 2                         | <10 <sup>2</sup> | >3                        |

15

| Results at T=24hrs |                       |                  |                           |                  |                           |                  |                           |
|--------------------|-----------------------|------------------|---------------------------|------------------|---------------------------|------------------|---------------------------|
| Microorganism      | Time 0 Sol A          | Example 7-02     |                           | Example 7-03     |                           | Example 7-04     |                           |
|                    |                       | CFU/mL           | Δlog <sub>10</sub> vs Ref | CFU/mL           | Δlog <sub>10</sub> vs Ref | CFU/mL           | Δlog <sub>10</sub> vs Ref |
| <i>S. aureus</i>   | 1.48 x10 <sup>5</sup> | <10 <sup>2</sup> | >3                        | <10 <sup>2</sup> | >3                        | <10 <sup>2</sup> | >3                        |

Results according to USP and EP

| Results at T=7 days |                    |              |                          |              |                          |              |                          |
|---------------------|--------------------|--------------|--------------------------|--------------|--------------------------|--------------|--------------------------|
| Microorganism       | Time 0 Sol A       | Example 7-02 |                          | Example 7-03 |                          | Example 7-04 |                          |
|                     |                    | CFU/mL       | $\Delta\log_{10}$ vs Ref | CFU/mL       | $\Delta\log_{10}$ vs Ref | CFU/mL       | $\Delta\log_{10}$ vs Ref |
| <i>S. aureus</i>    | $1.48 \times 10^5$ | $<10^2$      | $>3$                     | $<10^2$      | $>3$                     | $<10^2$      | $>3$                     |
| <i>C. albicans</i>  | $1.21 \times 10^5$ | $<10^2$      | $>3$                     | $<10^2$      | $>3$                     | $<10^2$      | $>3$                     |

| Results at T=14 days |                    |              |                          |              |                          |              |                          |
|----------------------|--------------------|--------------|--------------------------|--------------|--------------------------|--------------|--------------------------|
| Microorganism        | Time 0 Sol A       | Example 7-02 |                          | Example 7-03 |                          | Example 7-04 |                          |
|                      |                    | CFU/mL       | $\Delta\log_{10}$ vs Ref | CFU/mL       | $\Delta\log_{10}$ vs Ref | CFU/mL       | $\Delta\log_{10}$ vs Ref |
| <i>S. aureus</i>     | $1.48 \times 10^5$ | $<10^2$      | $>3$                     | $<10^2$      | $>3$                     | $<10^2$      | $>3$                     |
| <i>C. albicans</i>   | $1.21 \times 10^5$ | $<10^2$      | $>3$                     | $<10^2$      | $>3$                     | $<10^2$      | $>3$                     |

**Conclusion APE test step 2:** All samples met criteria of EP and USP considering both bacteria (*S. aureus*) and fungi (*C. Albicans*).

- Purity:

As determined by HPLC analysis, Example 7 does not show any increase of impurities under both refrigerated ( $+5^{\circ}\text{C}\pm 3^{\circ}\text{C}$ ) and accelerated conditions ( $25^{\circ}\text{C}\pm 2^{\circ}\text{C}$  RH 60%  $\pm$  5%) up to 4 weeks of storage.

Example 8

- Osmolality (Osm/Kg; according to Pharm. Eur. chapter 2.2.35, version 10 / USP chapter <785>, 2023 Edition):

| DP            | Time 0 |
|---------------|--------|
| Example 6-01- | 387    |
| Example 6-02  | 351    |
| Example 6-03  | 353    |
| Example 6-05  | 349    |
| Example 6-06  | 361    |
| Example 6-08  | 485    |
| Example 6-09  | 357    |
| Example 6-10  | 472    |
| Example 6-11  | 388    |
| Example 6-12  | 387    |

|              |     |
|--------------|-----|
| Example 6-13 | 366 |
| Example 6-14 | 351 |
| Example 6-15 | 361 |
| Example 7-01 | 385 |
| Example 7-02 | 330 |
| Example 7-03 | 413 |
| Example 7-04 | 469 |
| Example 7-05 | 353 |

## Claims

1. A liquid pharmaceutical composition, comprising and preferably consisting of:
- Cetorelix or a pharmaceutically acceptable salt thereof, preferably Cetorelix acetate,
  - a stabilizing agent selected from poloxamer having an average molecular weight of 7,000-12,000 g/mol, benzyl alcohol, or a mixture thereof,
  - an osmotic agent, and
  - a pharmaceutically acceptable solvent comprising an organic acid.
2. The liquid pharmaceutical composition according to claim 1, wherein the stabilizing agent is poloxamer 188, preferably comprised in an amount of 0.5-10.0 mg/ml, more preferably 1.0-5.0 mg/ml, or benzyl alcohol, preferably comprised in an amount of 5.0-20.0 mg/ml, more preferably 7.5-15.0 mg/ml.
3. The liquid pharmaceutical composition according to any of the preceding claims, wherein the osmotic agent is selected from the group consisting of mannitol, glycerol, sorbitol, sodium chloride, potassium chloride, dextrose, sucrose, trehalose, or a combination thereof, preferably D-mannitol.
4. The liquid pharmaceutical composition according to any of the preceding claims, wherein the organic acid is acetic acid, lactic acid, glutamic acid, gluconic acid, succinic acid, or a mixture thereof, preferably acetic acid, preferably comprised in a concentration of 0.05-0.50 mg/ml, more preferably 0.10-0.30 mg/ml (e.g. 0.1 mg/mL).
5. The liquid pharmaceutical composition according to any of the preceding claims, wherein the pharmaceutically acceptable solvent is aqueous acetic acid or water.
6. The liquid pharmaceutical composition according to any of the preceding claims, wherein the pH of the pharmaceutical composition is 4.0-6.0, preferably 4.3-5.1, and more preferably 4.5-5.0.
7. The liquid pharmaceutical composition according to any of the preceding claims, wherein the pharmaceutical composition comprises and preferably consists of Cetorelix acetate in a concentration of 0.2 -0.3 mg/ml Cetorelix pure peptide in its acetate salt form, poloxamer 188 in a concentration of 1.5 mg/ml, D-mannitol in a concentration of 54.8 mg/ml, acetic acid in a concentration of 0.1 mg/ml, and water for injection.

8. The liquid pharmaceutical composition according to any of the preceding claims, wherein the pharmaceutical composition comprises and preferably consists of Cetorelix acetate in a concentration of 0.2 – 0.3 mg/ml Cetorelix pure peptide in its acetate salt form, benzyl alcohol in a concentration of 10 mg/ml, D-mannitol in a concentration of 37.8 mg/ml, acetic acid in a concentration of 0.1 mg/ml, and water for injection.
9. The liquid pharmaceutical composition according to any of the preceding claims, which is a single-dose formulation, or a multi-dose formulation comprising a pharmaceutically acceptable preservative in an effective amount.
10. The liquid pharmaceutical composition according to any of the preceding claims, which is in the form of a solution, preferably for parenteral administration.
11. A pharmaceutical dosage form comprising the liquid pharmaceutical composition according to any of claims 1-10 in a container, such as an ampoule or vial, preferably a sterile vial, or an injection device, preferably a pre-filled syringe or an autoinjector.
12. A liquid pharmaceutical composition according to any of claims 1-10 or a pharmaceutical dosage form according to claim 11 for use in inhibiting premature luteinizing hormone surges in woman undergoing controlled ovarian stimulation or for use in preventing premature ovulation during controlled ovarian stimulation, for example, for use in preventing premature ovulation during controlled ovarian stimulation, followed by oocyte pick-up and assisted reproductive technique.
13. A method for preparing a liquid pharmaceutical composition according to any of claims 1-10 or a pharmaceutical dosage form according to claim 11, comprising the steps of:
- a) providing a first composition comprising a stabilizing agent selected from poloxamer having an average molecular weight of 7,000-12,000 g/mol, benzyl alcohol, or a mixture thereof, and an osmotic agent, preferably D-mannitol;
  - b) providing a second composition comprising or consisting of Cetorelix or a pharmaceutically acceptable salt thereof, preferably Cetorelix acetate;
  - c) mixing the first composition of step a) and the second composition of step b) to obtain a liquid composition; and
  - d) optionally adding an organic acid to the mixture obtained in step c),
- wherein a pharmaceutically acceptable solvent is present in the first composition and/or wherein a pharmaceutically acceptable solvent is present in the second composition.

14. The method of claim 13, wherein a pharmaceutically acceptable solvent is present in the first and second composition, wherein the solvent of the first and second composition is the same or different, preferably wherein one of the solvents is water for injection and the  
5 other solvent is aqueous acetic acid.

15. The method of any of claims 13-14, further comprising the step of adjusting the pH of the liquid composition obtained in step c) to a predetermined value by adding a pharmaceutically acceptable acid, preferably acetic acid, or a pharmaceutically acceptable  
10 base, preferably sodium hydroxide, wherein the predetermined pH value is preferably 4.0-6.0, more preferably 4.3-5.1, and particularly 4.5-5.0.