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Novel process for the preparation of cetorelix lyophilisate

Cetorelix is a decapeptide with a terminal acid amide group that is used as acetate salt. The synthesis and some pharmacological effects are described in European patent application 299 402.

It should be possible to administer the active substance subcutaneously in a dose of 0.1 to 20 mg. Aqueous solutions of the decapeptide are unstable, autoclaving in the final container is not possible. Under sterilisation conventional in the pharmacopoeias, the decapeptide tends to decompose. To obtain an injectable solution it was therefore necessary to develop a lyophilisate.

The amount of active substance in the solution to be lyophilised is, however, so small that, in low active substance concentrations, only a loose fluff results on the glass wall of the ampoule after drying the solution free of auxiliary substances which fluff is carried out of the vial with the stream of steam used for sterilisation. It is therefore necessary to use a bulking agent that forms a stable cake. In high concentrations this auxiliary substance can be dispensed with. The following auxiliary substances may be considered as bulking agents: hexitols, in particular mannitol, glucitol, sorbitol, such as D-sorbitol, dulcitol, allitol, altritol (for example D- and L-altritol), iditol (for example D- and L-iditol), their optically active forms (D- and L-forms) as well as the corresponding racemates. Mannitol is used in particular, such as D-mannitol, L-mannitol, DL-mannitol, sorbitol and/or dulcitol and of these preferably D-mannitol. The hexitol used may also be mixtures of the hexitols named, for example mixtures of mannitol and sorbitol and/or dulcitol. Since dulcitol is less water soluble than, for example, mannitol, the dulcitol content in the aqueous solution should not exceed for example 3 percent by weight. Mannitol and sorbitol, on the other hand, can for example be mixed in all ratios.

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REPLACED SHEETS	SG
☒ Description : paged to paged 1	
☒ Claim(s) : paged to paged 2	
☐ Drawing(s) : paged to paged 3	
☒ Abstract : paged to paged 4	
DATE OF RECEIPT	

Apart from hexitol it is also possible to add other, conventional pharmaceutical auxiliary substances, such as amino acids, such as alanine, glycine, lysine, phenylalanine, asparaginic acid, glutaminic acid, leucine, lactose, polyvinylpyrrolidone, glucose, fructose, albumin and equivalent bulking agents. Urea and sodium chloride may also be used as bulking agents. The total amount of such substances in the solution which is used for freeze-drying, is for example 0 - 16.9 parts by weight, for example 0.1 - 7 parts by weight, based on 1 part by weight of cetrorelix. In the finished lyophilisate the total amount of such auxiliary substances may be up to 16.9 parts by weight, based on one part by weight of hexitol. In detail, the amount of such auxiliary substances depends on the amount of hexitol present and to such an extent that the total amount of hexitol and such other auxiliary substances in the finished lyophilisate is not more than a maximum of 17 parts by weight, based on 1 part by weight of cetrorelix. If only 0.1 part by weight of hexitol is present in the lyophilisate, it is thus possible to have up to 16.9 parts by weight of other auxiliary substances; if, for example, 8.5 parts by weight of hexitol are present, the amount of other auxiliary substances may for example be up to 8.5 parts by weight, based on 1 part by weight of cetrorelix.

It was, however, found during the development work on the lyophilisate that the active substance behaves in a very different and unpredictable manner on processing. The first batches led to good results, but it soon transpired that difficulties occur during sterile filtration and faulty batches resulted.

It is known from the literature, for example from Powell, M.F.; Pharmaceutical Research, 1258-1263 (8)1991; Dathe, M: Int. J. Peptide Protein Res. 344-349 (36) 1990; Szejtli, J.: Pharmaceutical Technology International 16-22, 1991 that oligopeptides, particularly those with terminal acid amide function, tend to form gels. During sterile filtration this is apparent from the speed of filtration, indeed, the increased viscosity of such solutions can even often already be detected



organoleptically. A gelatinous layer remains behind on the sterile filter. It is then no longer possible to prepare a medicament with an exactly defined active substance content.

Table 1 lists various results of the first 11 batches.

The active substance contents fluctuate between 100% and 36%.

Table 1: Cetrorelix acetate

Batch	Dosage	Active substance content
		%
1	100 µg	100
2	500 µg	100
3	500 µg	90
4	500 µg	36
5	500 µg	100
6	500 µg	85
7	1 mg	80
8	1 mg	100
9	2 mg	100
10	2 mg	80
11	2 mg	100

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To avoid this gel formation, the literature lists the following additives which were tried out experimentally:

Organic solvents may be considered, for example acetonitrile, n-butanol, tertiary butanol, ethanol, isopropanol, octanol and benzyl alcohol. It is also possible to use salts and buffer solutions, such as acetate buffer, citrate buffer, sodium chloride, sodium phosphate, sodium EDTA, sodium bicarbonate, phosphate buffer, guanidine acetate, urea.

Polymers may also be used, such as gelatin, polyethylene glycol 600, hydroxyethyl starch, polyvinylpyrrolidone, polyvinyl alcohol. The addition of amino acids, for example alanine, glycine, lysine, phenylalanine, asparaginic acid, glutaminic acid and leucine has also been described.

Acids that were used were citric acid, caprylic acid, octanic acid, hydrochloric acid, sulphuric acid and acetic acid.

Physiologically acceptable surfactants that may be used are benzalkonium chloride, cetyl alcohol, bile acids, lecithins, polysorbates, Spans^(R) and Pluronic^(R).

Carbohydrates and cyclodextrins such as glucose, lactose, mannitol, saccharose, alpha-, beta- and gamma cyclodextrins, hydroxypropyl-alpha- and beta-cyclodextrins, hydroxyethyl cyclodextrins and methyl cyclodextrins were already used. These auxiliary substances were tested as filtration supporting agents to prevent gel formation.

No satisfactory solution of the problem could, however, be detected. Only acidification with acetic acid showed partial successes. Here, too, it was, however, recurring necessary to accept high filtration losses. It was then surprisingly found that cetorelix can be easily dissolved in 30% volume/volume acetic acid. The solution is then filled up to a final concentration of 3% cetorelix with water for injection purposes and mannitol is added. Although it is stated in the literature that the terminal amide group hydrolyses easily in acid medium, this was not found in the case of cetorelix. Solutions prepared according to this method caused no difficulties during



filtration. The correct amounts of active substance were always found.

The filtration speed attains values that ensure satisfactory production sequences. A general process for sterile lyophilisation is described in Sucker, Fuchs and Speiser (Publishers) "Pharmazeutische Technologie" 2nd edition 1991, Thieme-Verlag, Stuttgart-New York on pages 557 - 559. A further description of the lyophilisation process used is given in DE-OS 37 35 614.

The lyophilisate is used in the treatment of female sterility. One therapeutic process has hitherto consisted in stimulating follicle maturation using human menopause gonadotrophin and then triggering ovulation by administering human chorion gonadotrophin. The ovulation triggered thereby occurred 32 hours later. The ova collected thereby are available for extracorporeal sterilisation.

A disadvantage of this treatment with agonists is the fact that up to 10 follicles mature in the stimulation phase. This elevated follicle maturation leads to hormone level peaks of the LH. These peaks result in an early stage of follicle maturation and an ovulation at an unpredicted point in time. This impaired ovulation occurs in about 25% of treated cases and is a disadvantage since the cycle that displays disturbed ovulation of this kind cannot be used for the collection of ova and has to be repeated about 1 month later for the entire treatment.

Another disadvantage of the conventional simulation treatment is the long treatment duration of 4 weeks which is needed to achieve satisfactory suppression. The agonists continue to display a hyperstimulation syndrome in 1-2% of cases in which the follicle cells hypertrophy. The risk of hyperstimulation is particularly great in the case of polycystic ovaries. The hyperstimulation syndrome is a severe side effect which can lead to fatalities.

It has now been found that the antagonist cetrorelix displays the following advantages particularly in this treatment:

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Treatment with cetorelix over 5 days is sufficient to achieve total suppression. The hyperstimulation syndrome cannot arise. In addition, less HMG is used in the 2nd phase of therapy, the ovulation triggering phase. This gives this in-vitro fertilisation treatment a not inconsiderable cost advantage. In-vitro fertilisation is, for example, used when a tube anomaly is present. To perform this treatment it is necessary to precisely monitor the cycle and to establish the time of ovulation as precisely as possible. This has hitherto only been achieved to a limited extent since preovulatory LH increase often occurred too early due to stimulation with HMG/HCG, or was not maintained for a sufficiently long period. Avoidance of this premature increase is, however, of critical importance for the success of the treatment in order to precisely determine the time of fertilisation. This reduces the physical and mental burden on the patients and makes optimum use of hospital logistics. To achieve this objective with great reliability it is necessary to suppress endogenous hormone production (LH-FSH, oestradiol) as completely as possible to simultaneously stimulate follicle maturation through administration of exogenous gonadotrophins (HMG/HCG) and to monitor the hormone status at any time. It is only when a sufficiently large number of follicles have been achieved (4-6), having approximately the same degree of maturation, that ovulation is triggered by administering an HCG bolus injection.

Use of an antagonist makes treatment substantially more successful and safer for the patients.

Another area of use of the cetorelix lyophilisate according to the present invention is to protect the gonads in male patients. Male patients are pre-treated with cetorelix lyophilisate and the activity of the gonads is reinforced. As a result, other harmful noxious agents, such as a radiation therapy or treatment with cytostatics, have no or only small possibilities of affecting the sensitive tissue of the gonads.

Example of the method of preparation:



Approx. 1.5 litres of water for injection purposes are prepared in a suitable glass vessel. 210 g water for injection purposes are prepared in another glass vessel and 91.17 g acetic acid are added. The amount of cetorelix acetate calculated (1.62 - 1.695 g, depending on the content of the batch used) is dissolved in the prepared 30% acetic acid with stirring. This solution is transferred to the glass vessel with 1.5 litres of water for injection purposes, 82.2 g mannitol are added, dissolved and made up to 3039 g with water for injection purposes.

In-process checks:

pH value: 2.5 - 3.0
Density: 1.009 - 1.017 g/cm³ at 20°C
Refractive index: 1.227 - 1.340 at 440 nm and 20°C

Sterilisation of the solution is effected by filtration through an appropriate membrane filter (pore size 0.2 µm) under aseptic conditions. 100 ml first runnings should be discarded. The filters should be sterilised with superheated steam. Cetorelix freeze-dried solution is stored protected from recontamination. The solution is immediately filled into colourless injection bottles DIN 2R, hydrolytic class I under aseptic conditions and provided with sterile freeze-drying stoppers. The nominal filling amount is 2.0 ml = 2.026 g.

The 2 ml injection bottles were rinsed in an injection bottle washing machine, dried with hot air and sterilised. The cleaned freeze-drying stoppers were autoclaved. The closed injection bottles were transferred to a freeze-drying installation and frozen at a plate temperature of -40°C. Drying is carried out using a drying programme at a plate temperature of -40°C rising to +20°C. The installation is then flooded with sterile nitrogen, the bottles are closed in the installation and the stoppers secured with crimped caps.



The injection bottles are checked visually for faulty closures and outer faults. Faulty injection bottles are removed and destroyed.

Cetrorelix lyophilisate 1 mg is a white, solid, freeze-dried cake in a colourless 2 ml injection bottle which is closed with grey freeze-drying stoppers and yellow flip-off crimped caps.

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Claims

1. Lyophilisate of a peptide with 3-15 amino acids and optionally one or several bulking agents, characterised in that 1 part by weight of peptide is dissolved in 100 - 10,000 parts by weight of acetic acid and then transferred to water and the solution so obtained lyophilised.
2. Lyophilisate according to Claim 1, characterised in that mannitol is used as bulking agent.
3. Lyophilisate according to Claim 1, characterised in that the peptide is cetorelix.
4. Use of the lyophilisate obtained according to Claim 1 or 2 for the treatment of female infertility.
5. Use of the lyophilisate obtained according to Claim 1 or 2 for gonad protection.
6. Use of the lyophilisate obtained according to Claim 1 or 2 to prepare a medicament for the treatment of female infertility.
7. Use of the lyophilisate obtained according to Claim 1 or 2 to prepare a medicament for gonad protection.

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