Gonadorelin Hydrochloride

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$C_{55}H_{75}N_{17}O_{13},HCl$ 1218.5 *33515-09-2*

Action and use

Gonadotrophin-releasing hormone.

Preparation

Gonadorelin Injection

Definition

Gonadorelin Hydrochloride is the chloride form of a hypothalamic peptide that stimulates the release of follicle stimulating hormone and luteinising hormone from the pituitary gland. It contains not less than 88.0% and not more than 98.0% of the peptide $C_{55}H_{75}N_{17}O_{13}$, calculated with reference to the anhydrous substance. It is obtained by synthesis.

Characteristics

A white or slightly yellowish white powder.

Soluble in *water* and in a 1% v/v solution of *glacial acetic acid*; sparingly soluble in *methanol*.

Identification

A. In tests A and B for Related peptides, the principal spot in the chromatogram obtained with solution (2) is similar in position and size to that in the chromatogram obtained with solution (4).

B. In the Assay, the position of the principal peak in the chromatogram obtained with solution (1) corresponds to that of the principal peak in the chromatogram obtained with solution (2).

Tests

Clarity and colour of solution

A 1.0% w/v solution is *clear*, Appendix IV A, and not more intensely coloured than *reference solution* Y_5, Appendix IV B, Method II.

Light absorption

Absorbance of a 0.01% w/v solution at the maximum at 278 nm, 0.55 to 0.59, Appendix II B, calculated with reference to the peptide content as determined in the Assay.

Specific optical rotation

In a 1% w/v solution containing 1% v/v of *glacial acetic acid,* −54 to −63, calculated with reference to the peptide content as determined in the Assay, Appendix V F.

Chloride

0.83 mg dissolved in 15 ml of *water* complies with the *limit test for chlorides*, Appendix VII (6%).

Amino acids

Operate an amino acid analyser according to the manufacturer's instructions. Standardise the apparatus either with the calibration mixture supplied by the manufacturer or with a mixture containing equimolar amounts of ammonia, glycine and the L-form of the following amino acids:

lysine	threonine	alanine	leucine
histidine	serine	valine	tyrosine
arginine	glutamic acid	methionine	phenylalanine
aspartic acid	proline	isoleucine	

plus half the equimolar amount of L-cystine.

Dissolve 30 mg of DL-*norleucine* (internal standard) in a mixture of equal volumes of *hydrochloric acid* and *water* and dilute to 100 ml with the same mixture of solvents. Place 1.0 mg of the substance being examined in a rigorously cleaned hard-glass tube (10 cm × 6 mm), add an accurately measured volume of the internal standard solution containing an amount of DL-*norleucine* corresponding to about half the expected number of moles of gonadorelin, immerse the tube in a freezing mixture at −5°, evacuate to a pressure not exceeding 0.133 kPa and seal. Heat for 16 hours at 110° to 115°, cool, open the tube, transfer the contents to a 10 ml flask with the aid of five 0.2 ml quantities of *water* and evaporate to dryness over *potassium hydroxide* at a pressure of 2 kPa. Take up the residue in *water* and repeat the evaporation; repeat these operations. Dissolve the residue in a suitable buffer solution (pH 2.2) and dilute to a suitable volume with the same buffer solution. Apply an aliquot of the solution to the amino acid analyser. Choose conditions such that the peak given by the amino acid present in the largest amount produces almost maximum deflection on the chart paper.

Express the content of each amino acid in moles. Calculate the relative proportions of the amino acids taking one seventh of the sum of the number of moles of histidine, glutamic acid, leucine, proline, glycine and arginine as equal to one. The values fall within the following limits: serine 0.7 to 1.05; glutamic acid 0.95 to 1.05; proline 0.95 to 1.05; glycine 1.9 to 2.1; leucine 0.9 to 1.1; tyrosine 0.7 to 1.05; histidine 0.95 to 1.05; arginine 0.95 to 1.05. Lysine and isoleucine are absent; not more than traces of other amino acids are present.

The determination is not valid unless the number of moles of norleucine recovered, corrected for the volume of test solution applied, is within ±5 per cent of the amount taken for hydrolysis.

Related peptides

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using *silica gel G* as the coating substance and a mixture of 6 volumes of *glacial acetic acid*, 14 volumes of *water*,

45 volumes of *methanol* and 60 volumes of *chloroform* as the mobile phase. Apply separately to the plate 10 µl of each of four solutions in *water* containing (1) 1.0% w/v of the substance being examined, (2) 0.10% w/v of the substance being examined, (3) 0.020% w/v of the substance being examined and (4) 0.10% w/v of *gonadorelin EPCRS*. After removal of the plate, allow it to dry in air for 5 minutes. Place the plate in a closed tank of chlorine gas, prepared by the addition of 3 ml of *hydrochloric acid* to 10 ml of a 5% w/v solution of *potassium permanganate* in an evaporating dish placed at the bottom of the tank, and allow to stand for 2 minutes. Dry in a current of cold air until a sprayed area of the plate below the line of application does not give a blue colour with 0.05 ml of *potassium iodide and starch solution*; avoid prolonged exposure to air. Spray the plate with a *potassium iodide and starch solution*. Any *secondary spot* in the chromatogram obtained with solution (1) is not more intense than the spot in the chromatogram obtained with solution (3) (2%).

B. Complies with test A but using a mixture of 20 volumes of 13.5M *ammonia*, 45 volumes of *methanol* and 60 volumes of *chloroform* as the mobile phase.

Water

Not more than 7.0% w/w when determined by the following method. Use dry glassware throughout; siliconised glassware may be used. Carry out the method for *gas chromatography*, Appendix III B, using the following solutions. For solution (1) dilute 50 µl of *anhydrous methanol* (internal standard) with sufficient *propan-2-ol R1* to produce 100 ml. For solution (2) dissolve 4 mg of the substance being examined in 1 ml of *propan-2-ol R1*. For solution (3) dissolve 4 mg of the substance being examined in 1 ml of solution (1). For solution (4) add 10 µl of *water* to 50 µl of solution (1).

The chromatographic procedure may be carried out using (a) a stainless steel column (1 m × 2 mm) packed with porous polymer beads (60 to 80 mesh) (Chromosorb 102 is suitable) and maintained at 120°, (b) *helium* as the carrier gas and (c) a thermal conductivity detector maintained at 150°.

From the chromatograms obtained and taking into account any water detectable in solution (1), calculate the percentage w/w of water taking 0.9972 g as its weight per ml at 20°.

Assay

Carry out the method for *liquid chromatography*, Appendix III D, using two solutions in a mixture of 85 volumes of a 1% w/v solution of *orthophosphoric acid* adjusted to pH 3.0 with *triethylamine* and 15 volumes of *acetonitrile* containing (1) 0.01% w/v of the substance being examined and (2) 0.01% w/v of *gonadorelin EPCRS*.

The chromatographic procedure may be carried out using (a) a stainless steel column (15 cm × 4.6 mm) packed with *stationary phase C* (5 µm) (Spherisorb ODS 1 is suitable), (b) as the mobile phase with a flow rate of 1 ml per minute, a mixture of a 1% w/v solution of *orthophosphoric acid* adjusted to pH 3.0 with *triethylamine* and *acetonitrile*, the mixture being adjusted so that the retention time of gonadorelin is about 10 minutes (a mixture of 75 volumes of a 1% w/v solution of *orthophosphoric acid* adjusted to pH 3.0 with *triethylamine* and 25 volumes of *acetonitrile* is usually suitable) and (c) a detection wavelength of 220 nm.

The assay is not valid unless the *column efficiency* is at least 20,000 theoretical plates per metre.

Calculate the content of the peptide, $C_{55}H_{75}N_{17}O_{13}$, using the declared content of $C_{55}H_{75}N_{17}O_{13}$ in *gonadorelin EPCRS*.

Storage

Gonadorelin Hydrochloride should be protected from light and moisture and stored at a temperature of 2° to 8°.

Labelling

The label states (1) the weight of the peptide in the container; (2) the date after which the material is not intended to be used; (3) the conditions under which it should be stored.