Edition: BP 2025 (Ph. Eur. 11.6 update)

Somatropin

General Notices

(Ph. Eur. monograph 0951)

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FPTIPLSRLF  DNAMLRAHRL  HQLAFDTYQE  FEEAYIPKEQ   40
KYSFLQNPQT  SLCFSESIPT  PSNREETQQK  SNLELLRISL   80
LLIQSWLEPV  QFLRSVFANS  LVYGASDSNV  YDLLKDLEEG  120
IQTLMGRLED  GSPRTGQIFK  QTYSKFDTNS  HNDDALLKNY  160
GLLYCFRKDM  DKVETFLRIV  QCRSVEGSCG  F           191
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disulfide bridges: 53-165, 182-189

$C_{990}H_{1528}N_{262}O_{300}S_7$ 22 125

Action and use

Growth hormone.

Preparations

Somatropin Injection

Somatropin Powder for Injection

Ph Eur

DEFINITION

Protein having the structure (191 amino-acid residues) of the major component of growth hormone produced by the human pituitary.

Content

91.0 per cent to 105.0 per cent (anhydrous substance).

By convention, for the purpose of labelling somatropin preparations, 1 mg of anhydrous somatropin ($C_{990}H_{1528}N_{262}O_{300}S_7$) is equivalent to 3.0 IU of biological activity.

PRODUCTION

Somatropin is produced by a method based on recombinant DNA (rDNA) technology. In the course of product development, it must be demonstrated that the manufacturing process produces a product having a biological activity of not less than 2.5 IU/mg, using a validated bioassay based on growth promotion and approved by the competent authority.

Somatropin complies with the following additional requirements.

Host-cell-derived proteins (*2.6.34*)

The limit is approved by the competent authority.

Host-cell- and vector-derived DNA (2.6.35)

The limit is approved by the competent authority.

CHARACTERS

Appearance

White or almost white powder.

IDENTIFICATION

A. Capillary electrophoresis (2.2.47) as described in the test for charged variants with the following modifications.

Injection Test solution (b); under pressure or vacuum, using the following sequence: sample injection for at least 3 s then CZE buffer injection for 1 s.

Results In the electropherogram obtained, only 1 principal peak, corresponding to somatropin, is detected: no doubling of this peak is observed.

B. Examine the chromatograms obtained in the test for related proteins.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

C. Peptide mapping (2.2.55).

SELECTIVE CLEAVAGE OF THE PEPTIDE BONDS

Test solution Prepare a solution of the substance to be examined in *0.05 M tris-hydrochloride buffer solution pH 7.5 R* to obtain a solution containing 2.0 mg/mL of somatropin and transfer about 1.0 mL to a tube made from a suitable material such as polypropylene. Prepare a 1 mg/mL solution of *trypsin for peptide mapping R* in *0.05 M tris-hydrochloride buffer solution pH 7.5 R* and add 30 µL to the solution of the substance to be examined. Cap the tube and place in a water-bath at 37 °C for 4 h. Remove from the water-bath and stop the reaction immediately, for example by freezing. If analysed immediately using an automatic injector, maintain at 2-8 °C.

Reference solution Prepare at the same time and in the same manner as for the test solution, but using *somatropin CRS* instead of the substance to be examined.

CHROMATOGRAPHIC SEPARATION Liquid chromatography (2.2.29).

Column:

— *size*: *l* = 0.25 m, *Ø* = 4.6 mm;

— *stationary phase*: *octylsilyl silica gel for chromatography R* (5-10 µm) with a pore size of 30 nm;

— *temperature*: 30 °C.

Mobile phase:

— *mobile phase A*: dilute 1 mL of *trifluoroacetic acid R* to 1000 mL with *water for chromatography R*;

— *mobile phase B*: to 100 mL of *water for chromatography R*, add 1 mL of *trifluoroacetic acid R* and dilute to 1000 mL with *acetonitrile R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 20	100 → 80	0 → 20
20 - 40	80 → 75	20 → 25
40 - 65	75 → 50	25 → 50

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
65 - 70	50 → 20	50 → 80

Flow rate 1 mL/min.

Detection Spectrophotometer at 214 nm.

Injection 100 µL.

System suitability The chromatogram obtained with the reference solution is qualitatively similar to the chromatogram of somatropin digest supplied with *somatropin CRS*.

Results The profile of the chromatogram obtained with the test solution corresponds to that of the chromatogram obtained with the reference solution.

D. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

Related proteins

Liquid chromatography (*2.2.29*): use the normalisation procedure. *Maintain the solutions at 2-8 °C and use within 24 h.*

Solution A Dilute 5 µL of *strong hydrogen peroxide solution R* to 100 mL with *0.025 M phosphate buffer solution pH 7.0 R*.

Test solution Prepare a solution of the substance to be examined in an appropriate buffer, for example *0.025 M phosphate buffer solution pH 7.0 R*, to obtain a concentration of 1.6 mg/mL.

Reference solution (a) Dissolve the contents of a vial of *somatropin CRS* in *0.025 M phosphate buffer solution pH 7.0 R* to obtain a concentration of 1.6 mg/mL.

Reference solution (b) Dissolve the contents of a vial of *somatropin CRS* in solution A to obtain a concentration of 1.6 mg/mL. Allow to stand for 1-7 days to obtain a sufficient amount of sulfoxide forms.

Column:

— *size*: *l* = 0.25 m, Ø = 4.0 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (7 µm) with a pore size of 100 nm;

— *temperature*: 45 °C.

Mobile phase:

— *mobile phase A*: dissolve 82.6 g of *ammonium sulfate R* and 34.5 g of *sodium dihydrogen phosphate monohydrate R* in 2950 mL of *water for chromatography R*; add 25.0 mL of *perchloric acid R* and 2000 mL of *acetonitrile R1*, and dilute to 5000 mL with *water for chromatography R*;

— *mobile phase B*: to 4000 mL of *acetonitrile R1*, add 1000 mL of *water for chromatography R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 60	77 → 72	23 → 28
60 - 61	72 → 77	28 → 23
61 - 72	77	23

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 215 nm.

Autosampler Set at 2-8 °C.

Injection 20 µL.

Relative retention With reference to somatropin (retention time = about 40 min): [MetO^{125}]somatropin (double peak) = about 0.40; [β-Asp^{107}]somatropin = about 0.78; [MetO^{14}]somatropin = about 0.91; 130,131-anhydrosomatropin = about 1.27; [seco-130/131]somatropin = about 1.31.

If necessary, adjust the percentage of mobile phase B in the gradient so that the main peak elutes at about 40 min.

System suitability Reference solution (b):

— *peak-to-valley ratio*: minimum 2.0, where H_p = height above the baseline of the peak due to [MetO^{14}]somatropin and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to somatropin.

Limit:

— *total*: maximum 6.0 per cent.

Dimer and related substances of higher molecular mass

Size-exclusion chromatography (2.2.30): use the normalisation procedure.

Test solution Prepare a solution of the substance to be examined in *0.025 M phosphate buffer solution pH 7.0 R* to obtain a concentration of 1.0 mg/mL.

Reference solution (a) Dissolve the contents of a vial of *somatropin CRS* in *0.025 M phosphate buffer solution pH 7.0 R* and dilute with the same solution to obtain a concentration of 1.0 mg/mL.

Reference solution (b) Place 1 vial of *somatropin CRS* in an oven at 50 °C for a period sufficient to generate 1-2 per cent of dimer (typically 12-24 h). Dissolve its contents in *0.025 M phosphate buffer solution pH 7.0 R* and dilute with the same solution to obtain a concentration of 1.0 mg/mL.

Column:

— *size*: *l* = 0.30 m, Ø = 7.8 mm;

— *stationary phase*: *hydrophilic silica gel for chromatography R* of a grade suitable for fractionation of globular proteins in the relative molecular mass range of 5000 to 150 000.

Mobile phase *2-propanol R*, *0.063 M phosphate buffer solution pH 7.0 R* (3:97 *V/V*); filter and degas.

Flow rate 0.6 mL/min.

Detection Spectrophotometer at 214 nm.

Injection 20 µL.

Relative retention With reference to somatropin monomer (retention time = 12 min to 17 min): related substances of higher molecular mass = about 0.65; somatropin dimer = about 0.9.

System suitability Reference solution (b):

— *peak-to-valley ratio*: minimum 2.5, where H_p = height above the baseline of the peak due to the dimer and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to the monomer.

Limit:

— *sum of the peaks with retention times less than that of the principal peak*: maximum 4.0 per cent.

Charged variants

Capillary electrophoresis (2.2.47).

Test solution (a) Prepare a solution of the substance to be examined containing 1 mg/mL of somatropin.

Test solution (b) Mix equal volumes of test solution (a) and the reference solution.

Reference solution Dissolve the contents of a vial of *somatropin CRS* in *0.05 M tris-hydrochloride buffer solution pH 7.5 R* and dilute with the same solvent to obtain a concentration of 1 mg/mL.

Capillary:

— *material*: uncoated fused silica;

— *size*: effective length = at least 70 cm, Ø = 50 µm.

Temperature 30 °C.

CZE buffer 13.2 g/L solution of *ammonium phosphate R* adjusted to pH 6.0 with *phosphoric acid R* and filtered.

Detection Spectrophotometer at 200 nm.

Set the autosampler to store the samples at 4 °C during analysis.

Preconditioning of the capillary Rinse with *1 M sodium hydroxide* for 20 min, with *water R* for 10 min and with the CZE buffer for 20 min.

Between-run rinsing Rinse with *0.1 M sodium hydroxide* for 2 min and with the CZE buffer for 6 min.

NOTE: rinsing times may be adapted according to the length of the capillary and the equipment used.

Injection Test solution (a) and the reference solution; under pressure or vacuum, using the following sequence: sample injection for at least 3 s then CZE buffer injection for 1 s.

The injection time and pressure may be adapted in order to meet the system suitability criteria.

Migration Apply a field strength of 217 V/cm (20 kV for capillaries of 92 cm total length) for 80 min, using the CZE buffer as the electrolyte in both buffer reservoirs.

Relative migration With reference to somatropin: deamidated forms = 1.02 to 1.11.

System suitability Reference solution:

— the electropherogram obtained is similar to the electropherogram of somatropin supplied with *somatropin CRS*. The peak due to deamidated somatropin, I_4, elutes after and is completely separated from the peak due to somatropin, with a relative migration time (RMT) of at least 1.02. Other commonly observed peaks may include: I_1 (RMT = 0.93 – 0.96), I2 (RMT = 0.96 – 0.98) and I_3 (RMT = 1.01 – 1.03).

NOTE: peak I_2 corresponds to the cleaved form and peak I_4 corresponds to the deamidated forms, eluting as a doublet.

Limits:

— *deamidated forms*: maximum 5.0 per cent;

— *any other impurity*: for each impurity, maximum 2.0 per cent;

— *total*: maximum 10.0 per cent.

Water (*2.5.32*)

Maximum 10.0 per cent.

Bacterial endotoxins (*2.6.14*)

Less than 5 IU/mg, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for removal of bacterial endotoxins.

ASSAY

Size-exclusion chromatography (*2.2.30*) as described in the test for dimer and related substances of higher molecular mass with the following modification.

Injection Test solution and reference solution (a).

Calculate the content of somatropin ($C_{990}H_{1528}N_{262}O_{300}S_7$) from the assigned content of $C_{990}H_{1528}N_{262}O_{300}S_7$ in *somatropin CRS*.

STORAGE

In an airtight container, at a temperature of 2 °C to 8 °C. If the substance is sterile, the container is also sterile and tamper-evident.

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