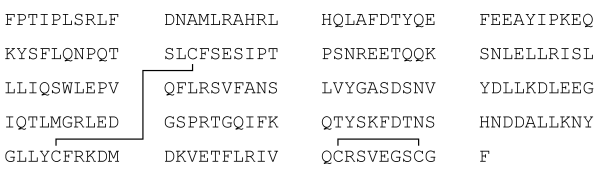
STORAGE

In an airtight container protected from light, at a temperature of 2 °C to 8 °C. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

01/2008:0951

SOMATROPIN

Somatropinum



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

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. During 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. The limit is approved by the competent authority.

Host-cell- and vector-derived DNA. 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 the reference solution.

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

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
65 - 70	50 → 20	50 → 80
70 - 71	20 → 100	80 → 0
71 - 85	100	0

Flow rate: 1 ml/min.

Detection: spectrophotometer at 214 nm.

Injection: 100 µl.

System suitability: the chromatograms obtained with the test solution and the reference solution are 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 the reference solution.

TESTS

Related proteins. Liquid chromatography (*2.2.29*): use the normalisation procedure. *Maintain the solutions at 2-8 °C and use within 24 h. If an automatic injector is used, maintain it at 2-8 °C.*

Test solution. Prepare a solution of the substance to be examined in *0.05 M tris-hydrochloride buffer solution pH 7.5 R*, containing 2.0 mg/ml of somatropin.

Reference solution. Prepare a solution of *somatropin CRS* in *0.05 M tris-hydrochloride buffer solution pH 7.5 R*, containing 2.0 mg/ml of somatropin.

Resolution solution. Dissolve the contents of a vial of *somatropin/desamidosomatropin resolution mixture CRS* in *0.05 M tris-hydrochloride buffer solution pH 7.5 R* to obtain a concentration of 2 mg/ml of somatropin.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm;
– *stationary phase*: a suitable singly end-capped butylsilyl silica gel, with a granulometry of 5 µm and a porosity of 30 nm; a silica saturation column is placed between the pump and the injector valve;
– *temperature*: 45 °C.

Mobile phase: *propanol R, 0.05 M tris-hydrochloride buffer solution pH 7.5 R* (29:71 *V/V*).

Flow rate: 0.5 ml/min.

Detection: spectrophotometer at 220 nm.

Preconditioning of the column: rinse with 200-500 ml of a 0.1 per cent *V/V* solution of *trifluoroacetic acid R* in a 50 per cent *V/V* solution of *acetonitrile R*; repeat as necessary, to improve column performance.

Injection: 20 µl.

Relative retention with reference to somatropin (retention time = about 33 min; if necessary adjust the concentration of *propanol R* in the mobile phase): desamidosomatropin = about 0.85.

System suitability: resolution solution:

– *resolution*: minimum 1.0 between the peaks due to desamidosomatropin and somatropin;
– *symmetry factor*: 0.9 to 1.8 for 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*, containing 1.0 mg/ml of somatropin.

Reference solution. 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.

Resolution solution. 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: resolution solution:

– *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 *water 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*; 2 peaks (I_1, I_2) eluting prior to the principal peak and at least 2 peaks (I_3, I_4) eluting after the principal peak are clearly visible.

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 dosage forms 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.

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

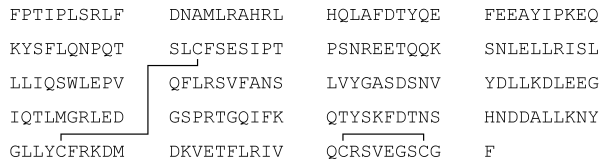
STORAGE

In an airtight container, at a temperature of 2 °C to 8 °C. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

01/2008:0950

SOMATROPIN CONCENTRATED SOLUTION

Somatropini solutio concentrata



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

DEFINITION

Solution containing a protein having the structure (191 amino-acid residues) of the major component of growth hormone produced by the human pituitary. It may contain buffer salts and other auxiliary substances.

Content: 91.0 per cent to 105.0 per cent of the amount of somatropin stated on the label.

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 concentrated solution is produced by a method based on recombinant DNA (rDNA) technology. During the course of product development, it must be demonstrated that the manufacturing process produces a product having a biological activity of at least 2.5 IU/mg, using a validated bioassay based on growth promotion and approved by the competent authority.

Somatropin concentrated solution complies with the following additional requirements.

Host-cell-derived proteins. The limit is approved by the competent authority.

Host-cell- and vector-derived DNA. The limit is approved by the competent authority.

CHARACTERS

Appearance: clear or slightly turbid, colourless solution.

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 the reference solution.

C. Peptide mapping (*2.2.55*).

SELECTIVE CLEAVAGE OF THE PEPTIDE BONDS

Test solution. Dilute the solution to be examined with *0.05 M tris-hydrochloride buffer solution pH 7.5 R* so that it contains 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.

Note: If a 2 mg/ml somatropin concentration is not obtainable, a similar digest relationship (micrograms of trypsin per milligram of somatropin) may be used.

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

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
65 - 70	50 → 20	50 → 80
70 - 71	20 → 100	80 → 0
71 - 85	100	0

Flow rate: 1 ml/min.

Detection: spectrophotometer at 214 nm.

Injection: 100 µl.