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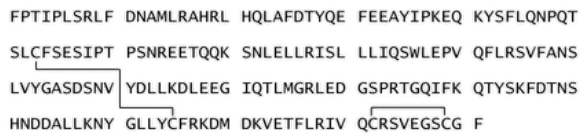
Somatropin



$C_{990}H_{1528}N_{262}O_{300}S_7$ 22,124.77 CAS RN®: 12629-01-5; UNII: NQX9KB6PCL.

DEFINITION

Somatropin is a protein hormone consisting of 191 amino acid residues, and its structure corresponds to the major component of the growth hormone extracted from human pituitary glands. It is produced as a lyophilized powder or bulk solution by methods based on recombinant DNA technology. The presence of host-cell DNA and host-cell protein impurities in Somatropin is process specific—the limits of these impurities are determined by validated methods. Manufacturers must demonstrate a correlation between the *Assay* and a validated and approved growth-promotion-based bioassay. It may contain excipients.

[NOTE—One mg of anhydrous Somatropin is equivalent to 3.0 USP Somatropin Units.]

IDENTIFICATION

• **A.** The retention time of the somatropin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Chromatographic Purity*, except that a *Standard solution* is also chromatographed and prepared by reconstituting a vial of USP Somatropin RS with *Diluent* to obtain a known concentration of about 2 mg/mL.

• **B. PEPTIDE MAPPING**

Solution A: Trifluoroacetic acid and water (1:999). Filter, and degas.

Solution B: Water, trifluoroacetic acid, and acetonitrile (100:1:899)

Solution C: 0.05 M solution of tris(hydroxymethyl)aminomethane (Tris). Adjust with hydrochloric acid to a pH of 7.5.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
20	80	20
40	75	25
65	50	50
70	20	80
71	100	0
86	100	0

Trypsin solution: 1 mg/mL of trypsin in *Solution C*. Store in a freezer, if necessary.

Standard stock solution: 2.0 mg/mL of USP Somatropin RS in *Solution C*

Standard solution: To 1 mL of the *Standard stock solution* add 30 µL of *Trypsin solution*. Cap the tube, and place it in a water bath at 37° for 4 h. [Note—If this solution is not injected immediately, store it in a freezer.]

Sample stock solution: 2.0 mg/mL of Somatropin in *Solution C*

Sample solution: To 1 mL of the *Sample stock solution* add 30 µL of *Trypsin solution*. Cap the tube, and place it in a water bath at 37° for 4 h. [Note—If this solution is not injected immediately, store it in a freezer.]

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 25-cm; packing L7

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 100 µL

Analysis

Samples: *Standard solution* and *Sample solution*, separately injected

[Note—Condition the chromatographic system by running a blank gradient program before injecting the digests.]

Acceptance criteria: The chromatographic profile of the *Sample solution* is similar to that of the *Standard solution*.

Change to read:

• **C.** ▲**Somatropin Bioidentity Test ⟨126⟩**▲ (CN 1-May-2023) **:** Meets the requirements

[Note—The bioidentity test may be performed either on the Somatropin bulk drug substance or on the finished pharmaceutical product.]

ASSAY

• **Somatropin Content**

Buffer solution: Dissolve 5.18 g of dibasic sodium phosphate and 3.65 g of monobasic sodium phosphate in 950 mL of water. Adjust with phosphoric acid or sodium hydroxide solution to a pH of 7.0. Dilute with water to 1000 mL.

Mobile phase: Isopropyl alcohol and *Buffer solution* (3:97). Filter, and degas.

Diluent: *Buffer solution* and water (1:1.5)

System suitability solution: Place 1 vial of USP Somatropin RS in an oven at 50° for 12–24 h. Remove from the oven, and dissolve the contents of the vial in *Diluent* to obtain a solution with a known concentration of about 1 mg/mL and a dimer content of 1%–2%.

Standard solution: Known concentration of about 1 mg/mL of USP Somatropin RS in *Diluent*

Sample solution: About 1 mg/mL of accurately weighed Somatropin in *Diluent*, or by diluting a bulk solution of Somatropin with *Diluent*. [Note—If necessary, the amount of protein in the solution can be determined with the test for *Total Protein Content*.]

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 7.8-mm × 30-cm; packing L33

Column temperature: Ambient

Flow rate: 0.6 mL/min

Injection volume: 20 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NMT 0.4 for the ratio of the valley height, between the dimer and the monomer, and the dimer peak height

Tailing factor: NMT 1.7 for the monomer (major) peak

Analysis

Samples: *Standard solution* and *Sample solution*, separately injected

Record the chromatograms for NLT twice the retention time of the somatropin monomer (major) peak, and measure the peak responses for the monomer.

Calculate the concentration, in mg/mL, of somatropin in the *Sample solution* taken:

$$\text{Result} = (r_U/r_S) \times C_S$$

r_U = peak response of the monomer from the *Sample solution*

r_S = peak response of the monomer from the *Standard solution*

C_S = concentration of USP Somatropin RS in the *Standard solution* (mg/mL)

Acceptance criteria: NLT 910 µg of somatropin/mg on the anhydrous basis. When prepared as a bulk solution, it contains NLT 910 µg of somatropin/mg of total protein.

IMPURITIES

• **Chromatographic Purity**

Diluent: 0.05 M Tris in water. Adjust with hydrochloric acid to a pH of 7.5.

Mobile phase: *n*-Propyl alcohol and degassed *Diluent* (29:71). Filter.

System suitability solution: 2.0 mg/mL of Somatropin in *Diluent*. Pass through a filter to sterilize or add sodium azide to a final concentration of 0.01%, and allow to stand at room temperature for 24 h. [Note—Use within 48 h of preparation, or store the solution in a refrigerator until ready to use.]

Sample solution: 2.0 mg/mL of Somatropin in *Diluent*. [Note—Maintain the solutions between 2° and 8°, and use within 24 h. If an automatic injector is used, maintain the temperature between 2° and 8°.]

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; packing L26

Column temperature: 45°

Flow rate: 0.5 mL/min

Injection volume: 20 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 1.0 between somatropin and its adjacent peak

Tailing factor: 0.9–1.8 for the somatropin (major) peak

Analysis

Sample: *Sample solution*

Calculate the percentage of impurities in the portion of Somatropin taken:

$$\text{Result} = [A_U/(A_U + A_S)] \times 100$$

A_U = sum of all the peak responses other than the somatropin (major) peak and disregarding any peak due to the solvent from the *Sample solution*

A_S = peak response of somatropin from the *Sample solution*

Acceptance criteria: NMT 6.0% of total impurities

• **Limit of High Molecular Weight Proteins**

Buffer solution, Mobile phase, Diluent, System suitability solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Measure the areas of the main peak and of the peaks eluting before the main peak, excluding the solvent peaks.

Calculate the percentage of high molecular weight proteins in the portion of Somatropin taken:

$$\text{Result} = [A_H/(A_H + A_M)] \times 100$$

A_H = sum of the areas of the high molecular weight peaks

A_M = peak area of the monomer from the *Sample solution*

Acceptance criteria: NMT 4% of high molecular weight proteins

SPECIFIC TESTS

• **Total Protein Content**

(See *Ultraviolet-Visible Spectroscopy ⟨857⟩*.)

The method is used in the calculation of total protein in the assay of bulk solution.

Buffer solution: 0.025 M solution of monobasic potassium phosphate in water. Adjust with sodium hydroxide to a pH of 7.0.

Sample solution: Mix a weighed quantity of Somatropin bulk solution with a weighed quantity of *Buffer solution* to obtain a solution with an absorbance value between 0.5 and 1.0 at the wavelength of maximum absorbance at 280 nm.

Analysis: Determine the absorbance of the *Sample solution* using a spectrophotometric cell of path length 1 cm, at the wavelength of maximum absorbance around 280 nm and at 320 nm, using *Buffer solution* as the blank.

Calculate the protein content, in mg, in the portion of Somatropin taken:

$$\text{Result} = (A_{max} - A_{320}) \times (V/0.82)$$

A_{max} = absorbance value of the *Sample solution* at the wavelength of maximum absorbance

A_{320} = absorbance value of the *Sample solution* at 320 nm

V = volume of the *Sample solution*

• **Microbial Enumeration Tests ⟨61⟩** and **Tests for Specified Microorganisms ⟨62⟩:** The total aerobic microbial count is NMT 3×10^2 cfu/g, the test being performed on 0.2–0.3 g of powder, accurately weighed.

• **Water Determination ⟨921⟩, *Method I, Method Ic*:** NMT 10%, when prepared as a lyophilized powder

• **Bacterial Endotoxins Test ⟨85⟩:** NMT 10 USP Endotoxin Units/mg of Somatropin

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers, and store between −10° and −25°.

• **Labeling:** The labeling states that the material is of recombinant DNA origin.

• **USP Reference Standards ⟨11⟩**

USP Somatropin RS

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
SOMATROPIN	Rebecca C. Potts Associate Scientific Liaison	BIO2 Biologics Monographs 2 - Proteins
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	BIO2 Biologics Monographs 2 - Proteins

Chromatographic Database Information: Chromatographic Database

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