Somatropin for Injection

Somatropin for injection is a freeze-dried, sterile preparation of a protein having the structure (191 amino-acid residues) of the major component of growth hormone produced by the human pituitary.

Somatropin contains not less than 89 per cent and not more than 105 per cent of the stated potency. By convention, for the purpose of labelling somatropin preparations 1 mg of anhydrous somatropin is equivalent to 3.0 IU of biological activity.

Somatropin for injection is prepared either from somatropin or from somatropin concentrated solution, or by a method based on recombinant DNA (rDNA) technology in which the injectable preparation is produced without the isolation of an intermediate solid or liquid bulk. In the later case, 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. The purified preparation, to which buffers and stabilizers may be added, is filtered through a bacteria-retentive filter, aseptically distributed in sterile containers of glass type I (6.2.1) and freeze-dried. The containers are immediately sealed so as to exclude microbial contamination and moisture.

Somatropin for injection complies with the following additional requirements.

**Usual strength:** 5 mg (15 IU), 6 mg (18 IU), 10 mg (30 IU) and 12 mg (36 IU)

**Host cell derived proteins (HCP).** Not more than 100 ppm.

**Host cell or vector derived DNA.** Not more than 10 ng per dose.

**Description.** White or almost white powder

**Identification**

Where Somatropin for injection is prepared from Somatropin or from Somatropin concentrated solution compliance with the requirements for host cell derived proteins, host cell and vector-derived DNA, identification test A, identification test C, and charged variants need not be reconfirmed by the manufacturer during subsequent product of Somatropin for injection.

A. Determine by capillary electrophoresis (capillary zone electrophoresis) (2.4.32) as described in the test for charged variants with the following modifications.

Inject test solution (b) under pressure or vacuum, in the following sequence: sample injection for at least 3 seconds then CZE buffer injection for 1 second.
In the electropherogram obtained with the *test solution (b)* only 1 principal peak corresponding to somatropin, is detected and no doubling of this peak is observed.

B. Determine by Liquid chromatography (2.4.14) as described in the test for related proteins
In the chromatogram obtained with the test solution the retention time of principal peak is similar to that of the principal peak in the chromatogram obtained with reference solution.

C. Determine by Peptide mapping (2.3.47).
*Test solution.* Prepare a solution of the substance to be examined in 0.05 M *tris-hydrochloride buffer* solution pH 7.5 to obtain a solution containing 2.0 mg per ml of somatropin and transfer about 1.0 ml to a tube made from a suitable material such as polypropylene. Prepare a 1 mg per ml solution of *trypsin* in 0.05 M *tris-hydrochloride buffer* solution pH 7.5 and add 30 μl to the solution of the preparation under examination. Cap the tube and place in a water-bath at 37° for at 4 hours. Remove sample from the water-bath and stop the reaction immediately, for example by freezing. If analyzed immediately using an automatic injector, maintain at 2° to 8°.

*Reference solution.* Prepare reference solution as per the procedure given for the test solution, ensuring that all procedures are carried out simultaneously, and under identical conditions but using somatropin RS instead of the substance under examination.

**Chromatographic system**
- A stainless steel column 25 cm x 4.6 mm, packed with octylsilyl silica gel (5-10 μm) with a pore size of 30 nm,
- column temperature, 30°
- mobile phase: A. dilute 1 ml of *trifluoroacetic acid* to 1000 ml with water,
  - B. to 100 ml of water, add 1.0 ml of *trifluoroacetic acid* and dilute to 1000 ml with *acetonitrile*,
- flow rate: 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 214 nm,
- Injection volume: 100 μl.

<table>
<thead>
<tr>
<th>Time (in min)</th>
<th>Mobile phase A (per cent v/v)</th>
<th>Mobile phase B (per cent v/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>40</td>
<td>75</td>
<td>25</td>
</tr>
<tr>
<td>65</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>70</td>
<td>20</td>
<td>80</td>
</tr>
</tbody>
</table>

Equilibrate at initial conditions for at least 15 minutes. Carry out a blank run using the above-mentioned gradient.
Inject the test solution and the reference solution. The chromatogram obtained with each solution is qualitatively similar to the reference chromatogram of somatropin RS digest. The profile of the chromatogram obtained with the test solution corresponds to that of the chromatogram obtained with the reference solution.

D. Determine by Size-exclusion chromatography (2.4.16) as described in assay. 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. Determine by liquid chromatography (2.4.14) using the normalization procedure.

Test solution. Prepare the solution of the preparation under examination in 0.05 M tris-hydrochloride buffer solution pH 7.5, to obtain a concentration of 2 mg per ml of somatropin.

Reference solution (a). Prepare a solution of somatropin RS in 0.05 M tris-hydrochloride buffer solution pH 7.5, to obtain a concentration of 2 mg per ml of somatropin.

Reference solution (b). Dissolve the contents of a vial of somatropin/desamidosomatropin RS in 0.05 M tris-hydrochloride buffer solution pH 7.5 to obtain a concentration of 2 mg per ml of somatropin.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, singly end-capped butylsilylsilica gel (5 μm) and with pore size of 30 nm; a silica saturation column is placed between the pump and the injector valve,
- column temperature: 45°C,
- mobile phase: a mixture of 29 volumes of propanol and 71 volumes of 0.05 M tris-hydrochloride buffer solution pH 7.5
- flow rate: 0.5 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 μl

Inject reference solution (b). The test is not valid unless the resolution between the peaks corresponding to desamidosomatropin and somatropin is at least 1.0.

Inject reference solution (a) and test solution, the sum of the areas of all the peaks other than the principal peak is not more than 13.0 per cent of the total area of all the peaks. Relative retention with reference to somatropin: for desamidosomatropin is about 0.85.

Dimer and related substances of higher molecular mass. Determine by size-exclusion chromatography (2.4.16). use the normalization procedure.

Test solution. Dilute the preparation under examination in 0.025 M phosphate buffer solution pH 7.0, to obtain a concentration of 1.0 mg per ml of somatropin.

Reference solution. Dissolve the contents of a vial of somatropin RS in 0.025 M phosphate buffer solution pH 7.0
and dilute with the same solution to obtain a concentration of 1.0 mg per ml.

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

Chromatographic system

– a stainless steel column 30cm x 7.8 mm, packed with hydrophilic silica gel, of a grade suitable for fractionation of globular proteins in the relative molecular mass range of 5000 to 150 000,
– column temperature. ambient,
– mobile phase: a mixture of 3 volumes of 2-propanol and 97 volumes of 0.063 M tris-hydrochloride buffer solution pH 7.0; filter and degas,
– flow rate: 0.6 ml per minute,
– spectrophotometer set at 214 nm,
– injection volume: 20 μl

Inject reference solution (a) and test solution. Relative retention time with reference to somatropin monomer: related substances of higher molecular mass is about 0.65; somatropin dimer is about 0.9.

Inject the reference solution (b), the resolution between the peaks due to somatropin dimer and somatropin monomer is not less than 2.5. Calculate the content of dimers, oligomers and aggregates. The sum of the peaks with retention times less than that of the principal peak is not more than 6.0 per cent.

Charged variants. Determine by Capillary electrophoresis (2.4.32).

All the solutions should be filtered through 0.45μm membrane filter before use.

CZE buffer. Dissolve 13.2 gram of ammonium phosphate in 1000 ml of water and adjust the pH to 6.0 using phosphoric acid.

Test solution (a). Dilute the preparation under examination to 1 mg per 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 RS in water and dilute with the same solvent to obtain a concentration of 1 mg per ml

Capillary System

- material. Uncoated fused silica,
- size: effective length = at least 70 cm, internal diameter= 50μm,
- temperature. 30°,
- spectrophotometer set at 200 nm,

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

Preconditioning of the capillary. Rinse with 1 M sodium hydroxide for 20 min, with water for 20 min and with the CZE buffer for 20 min at 20 psi.
Between-run rinsing. Rinse using 0.1 M sodium hydroxide for 2 minutes and with the CZE buffer for 6 minutes.

Rinsing times may be adapted according to the length of the capillary and the equipment used.

Inject test solution (a) and the reference solution under pressure or vacuum, using the following sequence: sample injection for at least 3 seconds then CZE buffer injection for 1 second. 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 minutes using the CZE buffer as the electrolyte in both buffer reservoirs. It is recommended to use fresh buffer for each separation to decrease the risk of ion depletion.

The electropherogram obtained with test solution (a) and reference solution are similar to the electropherogram of somatropin supplied with somatropin RS. Two peaks (I₁, I₂) eluting prior to the principal peak and at least 2 peaks (I₃, I₄) eluting after the principal peak are clearly visible. Peak I₂ corresponds to the cleaved form and peak I₄ corresponds to the deaminated forms, eluting as a doublet. Relative migration with reference to somatropin: deamidated forms is 1.02 to 1.11. In the electropherogram obtained with the test solution the area of the peak due to deaminated forms is not more than 6.5 per cent; the area of the peak other than principal peak is not more than 2.0 per cent and sum of areas of any peaks other than the principal peak is not more than 11.5 per cent of the total area of all the peaks.

\[
\text{Relative Migration peak } i = \frac{\text{Migration time of peak } i}{\text{Migration time of Somatropin}}
\]

Note: “i” represents a peak number.

In the identification of charged variants by capillary electrophoresis the deamidated forms is not more than 6.5 per cent, for each impurity not more than 2.0 per cent and total impurities not more than 11.5 per cent.

Water (2.3.43). Not more than 3.0 per cent, determined by the semi-micro determination of water.

Bacterial endotoxins (2.2.3). Not more than 5 IU per mg of protein.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay
Determine by Size-exclusion chromatography (2.4.16) as described in the test for dimer and related substances of higher molecular mass.

Calculate the content of somatropin from the declared content of somatropin RS.
**Storage.** Store in a sterile, airtight, tamper-proof container, at a temperature of 2° to 8°.

**Labelling.** The label states (1) the content of Somatropin in the container, in milligrams; (2) the composition and volume of the liquid to be added for reconstitution; (3) the time within which the reconstituted solution shall be used and the storage conditions during this period; (4) the name and quantity of any excipient; (5) the storage temperature; (6) that the preparation shall not be shaken during reconstitution.