Inject 20 µl of the resolution solution. Desamido-somatropin appears as a small peak at a retention time of about 0.85 relative to the principal peak. The test is not valid unless the resolution between the peaks corresponding to somatropin and desamido-somatropin is at least 1.0 and the symmetry factor of the somatropin peak is 0.9 to 1.8.

Inject 20 µl of the test solution. In the chromatogram obtained, the sum of the areas of all the peaks, apart from the principal peak, is not greater than 6.0 per cent of the total area of the peaks. Disregard any peak due to the solvent.

Dimer and related substances of higher molecular mass. Examine by size-exclusion chromatography (*2.2.30*) as described under Assay.

Inject 20 µl of the test solution. In the chromatogram obtained with the test solution, the sum of the areas of any peak with a retention time less than that of the principal peak is not greater than 4.0 per cent of the total area of the peaks. Disregard any peak due to the solvent.

Isoform distribution. Examine by isoelectric focusing.

Test solution (a). Dilute the solution to be examined with *0.025 M phosphate buffer solution pH 7.0 R* so as to contain 2.0 mg/ml of somatropin.

Test solution (b). Add 0.1 ml of test solution (a) to 1.9 ml of *0.025 M phosphate buffer solution pH 7.0 R*.

Reference solution (a). Prepare a solution of *somatropin CRS* in *0.025 M phosphate buffer solution pH 7.0 R*, containing 2.0 mg/ml of somatropin.

Reference solution (b). Use an isoelectric point calibration solution in the pH range of 2.5 to 6.5, prepared and used according to the manufacturer's instructions.

Operate the apparatus in accordance with the manufacturer's instructions. The isoelectric focusing procedure may be carried out using a pre-cast gel 245 mm × 110 mm × 1 mm, with a pH in the range 4.0 to 6.5. Apply to the gel 15 µl of each solution. Use as the anode solution a 14.7 g/l solution of *glutamic acid R* in phosphoric acid (50 g/l H_3PO_4) and as the cathode solution an 89.1 g/l solution of *β-alanine R*. Adjust the operating conditions to 2000 V and 25 mA. Allow focusing to take place for 2.5 h at a constant voltage and at a power of not more than 25 W. Immerse the gel for 30 min in a solution containing 115 g/l of *trichloroacetic acid R* and 34.5 g/l of *sulphosalicylic acid R*, and then for 5 min in a mixture of 8 volumes of *acetic acid R*, 25 volumes of *ethanol R* and 67 volumes of deionised *water R* (de-stain solution). Stain the gel by immersion in a 1.15 g/l solution of *acid blue 83 R* in de-stain solution at 60 °C for 10 min, and then place the gel in de-stain solution until excess stain is removed.

The test is not valid unless the distribution of bands in the electropherogram obtained with reference solution (b) corresponds to the manufacturer's indications. The electropherogram obtained with reference solution (a) contains a major band with an isoelectric point of approximately five, and a slightly more acidic minor band at approximately 4.8. In the electropherogram obtained with test solution (a), no band apart from the major band is more intense than the major band in the electropherogram obtained with test solution (b) (5 per cent).

Bacterial endotoxins (*2.6.14*): less than 5 IU in the volume that contains 1 mg of somatropin, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for removal of bacterial endotoxins.

ASSAY

Examine by size-exclusion chromatography (*2.2.30*).

Test solution. Dilute the solution to be examined with *0.025 M phosphate buffer solution pH 7.0 R* so as to contain 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 solvent to obtain a concentration of 1.0 mg/ml.

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

The chromatographic procedure may be carried out using:

- a stainless steel column 0.30 m long and 7.8 mm in internal diameter packed with *hydrophilic silica gel for chromatography R* of a grade suitable for fractionation of globular proteins in the molecular mass range 5000 to 150 000,
- as mobile phase at a flow rate of 0.6 ml/min a mixture (filtered and degassed) consisting of 3 volumes of *2-propanol R* and 97 volumes of *0.063 M phosphate buffer solution pH 7.0 R*,
- as detector a spectrophotometer set at 214 nm.

Inject 20 µl of the resolution solution. In the chromatogram obtained, the main peak elutes at a retention time of approximately 12 min to 17 min and the peaks corresponding to the somatropin dimer and to the higher molecular weight proteins at relative retention times of 0.90 and 0.65 respectively, relative to the main peak. The resolution, defined by the ratio of the height above the baseline of the valley separating the monomer and dimer peaks to the height of the dimer peak, is not greater than 0.4.

Inject 20 µl of the test solution and 20 µl of the reference solution.

Calculate the content of somatropin ($C_{990}H_{1528}N_{262}O_{300}S_7$) from the peak areas in the chromatograms obtained with the test solution and the reference solution and the declared content of ($C_{990}H_{1528}N_{262}O_{300}S_7$) in *somatropin CRS*.

STORAGE

Store in an airtight container at a temperature of −20 °C. Avoid repeated freezing and thawing. If the solution is sterile, store in a sterile, airtight, tamper-proof container.

LABELLING

The label states:

- the content of somatropin in milligrams per millilitre,
- the name and concentration of any auxiliary substance,
- where applicable, that the solution is free from bacterial endotoxins.

01/2005:0952
corrected

SOMATROPIN FOR INJECTION

Somatropinum ad iniectabilium

DEFINITION

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. It contains not less than 89.0 per cent and not more than 105.0 per cent of the amount of

somatropin[3] ($C_{990}H_{1528}N_{262}O_{300}S_7$) stated on the label. It complies with the requirements of the monographs on *Parenteral preparations (0520)*.

PRODUCTION

Somatropin for injection is prepared either from *Somatropin (0951)* or from *Somatropin bulk solution (0950)*, 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 latter 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 stabilisers may be added, is filtered through a bacteria-retentive filter, aseptically distributed in sterile containers of glass type I (*3.2.1*) 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.

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.

Where somatropin for injection is prepared from Somatropin (0951) or from Somatropin bulk solution (0950), compliance with the requirements for host-cell-derived proteins, host-cell- and vector-derived DNA and with identification test C need not be reconfirmed by the manufacturer during subsequent production of somatropin for injection.

CHARACTERS

A white or almost white powder.

IDENTIFICATION

A. Examine the electropherograms obtained in the test for isoform distribution. In the electropherogram obtained with test solution (a), the principal band corresponds in position to that in the electropherogram obtained with reference solution (a).

B. Examine the chromatograms obtained in the test for related proteins. The retention time of the principal peak in the chromatogram obtained with the test solution is similar to that of the principal peak in the chromatogram obtained with the reference solution.

C. Examine by peptide mapping.

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 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 the temperature at 2 °C to 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.

Examine by liquid chromatography (*2.2.29*).

The chromatographic procedure may be carried out using:

– a stainless steel column 0.25 m long and 4.6 mm in internal diameter packed with *octylsilyl silica gel for chromatography R* (5 µm to 10 µm),

– as mobile phase at a flow rate of 1 ml/min:

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*,

following the elution conditions as described in the table below (if necessary, the gradient or the temperature of the column may be modified to improve separation of the digest):

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

– as detector a spectrophotometer set at 214 nm,

maintaining the temperature of the column at 30 °C.

Equilibrate the column with mobile phase A for at least 15 min. Carry out a blank run using the above-mentioned gradient.

Inject 100 µl of the test solution and 100 µl of the reference solution. The test is not valid unless the chromatogram obtained with each solution is qualitatively similar to the chromatogram of somatropin digest supplied with *somatropin CRS*. 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. The retention time of the principal peak in the chromatogram obtained with the test solution is similar to that of the principal peak in the chromatogram obtained with the reference solution.

TESTS

Related proteins. Examine by liquid chromatography (*2.2.29*).

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 (somatropin/desamido-somatropin resolution mixture). 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. Either filter through a sterile filter or add *sodium azide R* to a concentration of 0.1 mg/ml and allow to stand at room temperature for 24 h.

Maintain the solutions at 2 °C to 8 °C and use within 24 h. If an automatic injector is used, maintain the temperature at 2 °C to 8 °C.

(3) 1 mg of anhydrous somatropin ($C_{990}H_{1528}N_{262}O_{300}S_7$) is equivalent to 3.0 IU of biological activity.

The chromatographic procedure may be carried out using:
- a stainless steel column 0.25 m long and 4.6 mm in internal diameter packed with 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 to be placed between the pump and the injector valve,
- as mobile phase at a flow rate of 0.5 ml/min a mixture of 29 volumes of *propanol R* and 71 volumes of *0.05 M tris-hydrochloride buffer solution pH 7.5 R*,
- as detector a spectrophotometer set at 220 nm,

maintaining the temperature of the column at 45 °C.

Prior to use, rinse the column with 200 ml to 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.

Inject 20 µl of the reference solution. If necessary, adjust the concentration of *propanol R* in the mobile phase so that the retention time of the principal peak is about 33 min.

Inject 20 µl of the resolution solution. Desamido-somatropin appears as a small peak at a retention time of about 0.85 relative to the principal peak. The test is not valid unless the resolution between the peaks corresponding to somatropin and desamido-somatropin is at least 1.0 and the symmetry factor of the somatropin peak is 0.9 to 1.8.

Inject 20 µl of the test solution. In the chromatogram obtained, the sum of areas of all the peaks, apart from the principal peak, is not greater than 13 per cent of the total area of the peaks. Disregard any peak due to the solvent.

Dimer and related substances of higher molecular mass. Examine by size-exclusion chromatography (*2.2.30*) as described under Assay.

Inject 20 µl of the test solution. In the chromatogram obtained with the test solution, the sum of the areas of any peak with a retention time less than that of the principal peak is not greater than 6.0 per cent of the total area of the peaks. Disregard any peak due to the solvent.

Isoform distribution. Examine by isoelectric focusing.

Test solution (a). Prepare a solution of the substance to be examined in *0.025 M phosphate buffer solution pH 7.0 R*, containing 2.0 mg/ml of somatropin.

Test solution (b). Add 0.1 ml of test solution (a) to 1.5 ml of *0.025 M phosphate buffer solution pH 7.0 R*.

Reference solution (a). Prepare a solution of *somatropin CRS* in *0.025 M phosphate buffer solution pH 7.0 R*, containing 2.0 mg/ml of somatropin.

Reference solution (b). Use an isoelectric point calibration solution in the pH range of 2.5 to 6.5, prepared and used according to the manufacturer's instructions.

Operate the apparatus in accordance with the manufacturer's instructions. The isoelectric focusing procedure may be carried out using a pre-cast gel 245 mm × 110 mm × 1 mm, with a pH in the range 4.0 to 6.5. Apply to the gel 15 µl of each solution. Use as the anode solution a 14.7 g/l solution of *glutamic acid R* in phosphoric acid (50 g/l H_3PO_4) and as the cathode solution an 89.1 g/l solution of *β-alanine R*. Adjust the operating conditions to 2000 V and 25 mA. Allow focusing to take place for 2.5 h at a constant voltage and at a power of not more than 25 W. Immerse the gel for 30 min in a solution containing 115 g/l of *trichloroacetic acid R* and 34.5 g/l of *sulphosalicylic acid R*, and then for 5 min in a mixture of 8 volumes of *acetic acid R*, 25 volumes of *ethanol R* and 67 volumes of deionised *water R* (de-stain solution). Stain the gel by immersion in a 1.15 g/l solution of *acid blue 83 R* in de-stain solution at 60 °C for 10 min, and then place the gel in de-stain solution until excess stain is removed.

The test is not valid unless the distribution of bands in the electropherogram obtained with reference solution (b) corresponds to the manufacturer's indications. The electropherogram obtained with reference solution (a) contains a major band with an isoelectric point of approximately five, and a slightly more acidic minor band at approximately 4.8. In the electropherogram obtained with test solution (a), no band apart from the major band is more intense than the major band in the electropherogram obtained with test solution (b) (6.25 per cent).

Water (*2.5.32*). Not more than 3.0 per cent, unless otherwise justified and authorised, determined by the micro determination of water.

Bacterial endotoxins (*2.6.14*): less than 5 IU/mg.

ASSAY

Examine by size-exclusion chromatography (*2.2.30*).

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 solvent to obtain a concentration of 1.0 mg/ml.

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

The chromatographic procedure may be carried out using:
- a stainless steel column 0.30 m long and 7.8 mm in internal diameter packed with *hydrophilic silica gel for chromatography R* of a grade suitable for fractionation of globular proteins in the molecular mass range 5000 to 150 000,
- as mobile phase at a flow rate of 0.6 ml/min a mixture (filtered and degassed) consisting of 3 volumes of *2-propanol R* and 97 volumes of *0.063 M phosphate buffer solution pH 7.0 R*,
- as detector a spectrophotometer set at 214 nm.

Inject 20 µl of the resolution solution. In the chromatogram obtained, the main peak elutes at a retention time of approximately 12 min to 17 min and the peaks corresponding to the somatropin dimer and to the higher molecular weight proteins at relative retention times of 0.90 and 0.65 respectively, relative to the main peak. The resolution defined by the ratio of the height above the baseline of the valley separating the monomer and dimer peaks, to the height of the dimer peak, is not greater than 0.4.

Inject 20 µl of the test solution and 20 µl of the reference solution.

Calculate the content of somatropin ($C_{990}H_{1528}N_{262}O_{300}S_7$) from the peak areas in the chromatograms obtained with the test solution and the reference solution and the declared content of ($C_{990}H_{1528}N_{262}O_{300}S_7$)in *somatropin CRS*.

STORAGE

Store in a sterile, airtight, tamper-proof container, at a temperature of 2 °C to 8 °C.

LABELLING

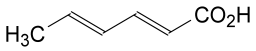
The label states:
- the content of somatropin in the container, in milligrams,
- the composition and volume of the liquid to be added for reconstitution,

– the time within which the reconstituted solution shall be used and the storage conditions during this period,
– the name and quantity of any added substance,
– the storage temperature,
– that the preparation shall not be shaken during reconstitution.

01/2005:0592

SORBIC ACID

Acidum sorbicum



$C_6H_8O_2$ M_r 112.1

DEFINITION

Sorbic acid contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of (*E,E*)-hexa-2,4-dienoic acid, calculated with reference to the anhydrous substance.

CHARACTERS

A white or almost white, crystalline powder, slightly soluble in water, freely soluble in alcohol.

IDENTIFICATION

First identification: A, C.

Second identification: A, B, D.

A. Melting point (*2.2.14*): 132 °C to 136 °C.

B. Dissolve 50.0 mg in *water R* and dilute to 250.0 ml with the same solvent. Dilute 2.0 ml of this solution to 200.0 ml with *0.1 M hydrochloric acid*. Examined between 230 nm and 350 nm (*2.2.25*), the solution shows a maximum at 264 nm. The specific absorbance at the maximum is 2150 to 2550.

C. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *sorbic acid CRS*.

D. Dissolve 0.2 g in 2 ml of *alcohol R* and add 0.2 ml of *bromine water R*. The solution is decolorised.

TESTS

Solution S. Dissolve 1.25 g in *alcohol R* and dilute to 25 ml with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Aldehydes. Dissolve 1.0 g in a mixture of 30 ml of *water R* and 50 ml of *2-propanol R*, adjust the solution to pH 4 with *0.1 M hydrochloric acid* or *0.1 M sodium hydroxide* and dilute to 100 ml with *water R*. To 10 ml of the solution add 1 ml of *decolorised fuchsin solution R* and allow to stand for 30 min. Any colour in the solution is not more intense than that in a standard prepared at the same time by adding 1 ml of *decolorised fuchsin solution R* to a mixture of 1.5 ml of *acetaldehyde standard solution (100 ppm C_2H_4O) R*, 4 ml of *2-propanol R* and 4.5 ml of *water R* (0.15 per cent, calculated as C_2H_4O).

Heavy metals (*2.4.8*). 12 ml of solution S complies with limit test B for heavy metals (10 ppm). Prepare the standard using 5 ml of *lead standard solution (1 ppm Pb) R* and 5 ml of *alcohol R*.

Water (*2.5.12*). Not more than 1.0 per cent, determined on 2.000 g by the semi-micro determination of water.

Sulphated ash (*2.4.14*). Not more than 0.2 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.1000 g in 20 ml of *alcohol R*. Using 0.2 ml of *phenolphthalein solution R* as indicator, titrate with *0.1 M sodium hydroxide* until a pink colour is obtained.

1 ml of *0.1 M sodium hydroxide* is equivalent to 11.21 mg of $C_6H_8O_2$.

STORAGE

Store protected from light.

01/2005:1040

SORBITAN LAURATE

Sorbitani lauras

DEFINITION

Mixture usually obtained by partial esterification of sorbitol and its mono- and di-anhydrides with lauric acid.

CHARACTERS

Appearance: brownish-yellow, viscous liquid.

Solubility: practically insoluble, but dispersible in water, miscible with alcohol.

Relative density: about 0.98.

IDENTIFICATION

A. It complies with the test for hydroxyl value (see Tests).

B. It complies with the test for iodine value (see Tests).

C. It complies with the test for composition of fatty acids (see Tests).

TESTS

Acid value (*2.5.1*): maximum 7.0, determined on 5.0 g.

Hydroxyl value (*2.5.3, Method A*): 330 to 358.

Iodine value (*2.5.4*): maximum 10.

Peroxide value (*2.5.5*): maximum 5.0.

Saponification value (*2.5.6*): 158 to 170.

Carry out the saponification for 1 h.

Composition of fatty acids. Gas chromatography (*2.4.22, Method C*).

Prepare reference solution (a) as indicated in tables 2.4.22.-1 and 2.4.22.-2.

Composition of the fatty acid fraction of the substance:
– *caproic acid*: maximum 1.0 per cent,
– *caprylic acid*: maximum 10.0 per cent,
– *capric acid*: maximum 10.0 per cent,
– *lauric acid*: 40.0 per cent to 60.0 per cent,
– *myristic acid*: 14.0 per cent to 25.0 per cent,
– *palmitic acid*: 7.0 per cent to 15.0 per cent,
– *stearic acid*: maximum 7.0 per cent,
– *oleic acid*: maximum 11.0 per cent,
– *linoleic acid*: maximum 3.0 per cent.

Heavy metals (*2.4.8*): maximum 10 ppm.

2.0 g complies with limit test D. Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Water (*2.5.12*): maximum 1.5 per cent, determined on 1.00 g.

Total ash (*2.4.16*): maximum 0.5 per cent.

STORAGE

Protected from light.