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THE UNITED STATES PHARMACOPEIAL CONVENTION
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(791): between 11.5 and 13.5.

Residual solvents (467): meets the requirements.

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Diluent—Transfer 500.0 mL of a 4.0 M sodium hydroxide solution to a 1000-mL volumetric flask, and add about 67.0 g of sodium disulfide and 36.0 g of ascorbic acid, each accurately weighed. Dilute with water to volume, and mix.

Assay preparation—Transfer an accurately weighed portion of the Sodium Thiosulfate to a 100-mL volumetric flask, add 50 mL of *Diluent*, and shake by mechanical means until the Topical Gel is dispersed. Dilute with water to volume, and mix.

Procedure—Transfer 30.0 mL of the *Assay preparation* to a suitable beaker, and titrate with 0.01 M lead perchlorate VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.01 M lead perchlorate is equivalent to 2.4018 mg of sodium sulfide ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$).

Sodium Thiosulfate

$\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ 248.19

Sulfuric acid, disodium salt, pentahydrate.

Sodium thiosulfate pentahydrate. [10102-17-7].

Anhydrous. 158.11 [7772-98-7].

Sodium Thiosulfate contains not less than 99.0 percent and not more than 100.5 percent of $\text{Na}_2\text{S}_2\text{O}_3$, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers.

Identification—

A. To a solution (1 in 10) add a few drops of iodine TS: the color is discharged.

B. A solution (1 in 10) responds to the tests for *Sodium* (191) and for *Thiosulfate* (191).

Water (921)—Dry about 1.0 g, accurately weighed, in vacuum at 45° for 16 hours: it loses between 32.0% and 37.0% of its weight.

Calcium—Dissolve 1 g in 20 mL of water, and add a few mL of ammonium oxalate TS: no turbidity is produced.

Heavy metals (231)—Dissolve 1 g in 10 mL of water, slowly add 5 mL of 3 N hydrochloric acid, evaporate on a steam bath nearly to dryness, and heat the residue at 150° for 1 hour. Add 15 mL of water to the residue, boil gently for 2 minutes, and filter. Heat the filtrate to boiling, and add sufficient bromine TS to produce a clear solution and provide a slight excess of bromine. Boil the solution to expel the excess bromine, cool to room temperature, add 1 drop of phenolphthalein TS, and neutralize with 1 N sodium hydroxide. Dilute with water to 25 mL: the limit is 0.002%.

Residual solvents (467): meets the requirements.

(Official January 1, 2007)

Assay—Dissolve about 800 mg of Sodium Thiosulfate, accurately weighed, in 30 mL of water. If necessary, adjust by the addition of 1 N hydrochloric acid to a pH of between 6.2 and 6.7 and titrate with 0.1 N iodine VS, adding 3 mL of starch TS as the endpoint is approached. Each mL of 0.1 N iodine is equivalent to 15.81 mg of $\text{Na}_2\text{S}_2\text{O}_3$.

Sodium Thiosulfate Injection

» Sodium Thiosulfate Injection is a sterile solution of Sodium Thiosulfate in freshly boiled Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$.

Packaging and storage—Preserve in single-dose containers, of Type I glass.

USP Reference standards (11)—*USP Endotoxin RS*.

Identification—It responds to the *Identification* tests under *Sodium Thiosulfate*.

Bacterial endotoxins (85)—It contains not more than 0.03 USP Endotoxin Unit per mg of sodium thiosulfate.

pH (791): between 6.0 and 9.5.

Residual solvents (467): meets the requirements.

(Official January 1, 2007)

Other requirements—It meets the requirements under *Injections* (1).

Assay—Transfer to a suitable container an accurately measured volume of Injection, containing about 1 g of sodium thiosulfate, and adjust by the addition of 3 N hydrochloric acid to a pH of between 6.2 and 6.7. Dilute with water to about 20 mL, and titrate with 0.1 N iodine VS, adding 3 mL of starch TS as the endpoint is approached. Each mL of 0.1 N iodine is equivalent to 24.82 mg of $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$.

Somatropin

FPITPLSRFL	DNAMLRHRL	HOLAFDTYQE	FEAYIPKEQ	KYSFLQNPOT
SLCFSES IPT	PSNREETOOK	SNLELLRSL	LLTOSWLEPV	QFLRSVFANS
LVYGASDSNV	YDLKOLEEG	LOTLMGRLED	GSPTGQTFK	QTSKFDITNS
HNDALLKNY	GLLYCFKDKM	DKVETFLRV	QCRSVEGSCG	F

$\text{C}_{990}\text{H}_{1528}\text{N}_{262}\text{O}_{300}\text{S}_7$ 22,125 [12629-01-5].

» 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. When prepared as a lyophilized powder, it contains not less than 910 µg of somatropin per mg, calculated on the anhydrous basis. When prepared as a bulk solution, it contains not less than 910 µg of somatropin per mg of total protein. 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.]

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 Endotoxin RS*; *USP Somatropin RS*.

Identification—

A: Proceed as directed in the test for *Chromatographic purity*, except to prepare a *Standard solution* by reconstituting a vial of USP Somatropin RS with the *Diluent* to obtain a solution having a known concentration of about 2.0 mg per mL. Chromatograph the *Standard solution* and the *Test solution* as directed for *Procedure*; the retention time of the somatropin peak in the chromatogram of the *Test solution* corresponds to that in the chromatogram of the *Standard solution*.

B: *Peptide Mapping* (see *Biotechnology-Derived Articles—Tests* (1047)).

Solution A—Prepare a filtered and degassed solution of trifluoroacetic acid in water (1 in 1000, v/v).

Solution B—Transfer 100 mL of water to a 1000-mL volumetric flask, add 1 mL of trifluoroacetic acid, dilute with acetonitrile to volume, and mix.

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments to either solution as necessary (see *System Suitability* under *Chromatography* (621)).

Tris buffer—Prepare a 0.05 M solution of tris(hydroxymethyl)aminomethane (Tris), and adjust with hydrochloric acid to a pH of 7.5.

Trypsin solution—Prepare a solution containing 1 mg of trypsin per mL of *Tris buffer*, and mix. Store in a freezer, if necessary.

Standard solution—Prepare a solution containing 2.0 mg of USP Somatropin RS per mL of the *Tris buffer*, and mix. Add 1 mL of this solution to a suitable tube, and add 30 μ L of *Trypsin solution*. Cap the tube, and place it in a water bath at 37° for 4 hours. [NOTE—If this solution is not injected immediately, store it in a freezer.]

Test solution—Prepare a solution containing 2.0 mg of Somatropin per mL of *Tris buffer*, and mix. Add 1 mL of this solution to a suitable tube, and add 30 μ L of *Trypsin solution*. Cap the tube, and place it in a water bath at 37° for 4 hours. [NOTE—If this solution is not injected immediately, store it in a freezer.]

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 214-nm detector and a 4.6-mm $\times$ 25-cm column that contains packing L7. The flow rate is 1 mL per minute, and the column temperature is maintained at 30°. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–20	100→80	0→20	linear gradient
20–40	80→75	20→25	linear gradient
40–65	75→50	25→50	linear gradient
65–70	50→20	50→80	linear gradient
70–71	20→100	80→0	linear gradient
71–86	100	0	isocratic, re-equilibration

Procedure—[NOTE—Condition the chromatographic system by running a blank gradient program prior to injecting the digests.] Separately inject equal volumes (about 100 μ L) of the *Standard solution* and the *Test solution*, and record the chromatograms; the chromatographic profile of the *Test solution* is similar to that of the *Standard solution*.

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

Buffer solution—Prepare a solution of 0.1 M ammonium bicarbonate, and adjust with sodium hydroxide to a pH of 8.0.

Standard solutions—Reconstitute the USP Somatropin RS, and dissolve in and dilute quantitatively with *Buffer solution* to obtain solutions having known concentrations between 10 and 100 μ g per mL.

Test solutions—Prepare a solution of Somatropin, and dissolve in and dilute quantitatively with *Buffer solution* to obtain solutions having concentrations similar to those of the *Standard solutions*. [NOTE—Do not agitate while mixing; swirl gently.]

Control solution—Use the *Buffer solution*.

Test animals—Select an appropriate number of only female or only male Sprague Dawley rats hypophysectomized at 25 to 30 days of age. After hypophysectomization, feed the rats on rat chow and

5% dextrose water for at least 72 hours. After 72 hours, feed the rats on rat chow and filtered and deionized water adjusted with 1 N hydrochloric acid to a pH of 3.0 ± 0.25 . Weigh the rats when they are 37 to 44 days old, and retain only healthy rats. Reweigh the remaining rats 7 days later, and use only those rats that are in good health and have not gained or lost more than 10% of their body weight in the previous 7-day period.

Procedure—Randomly divide the rats into control, standard, and test groups, each group containing approximately 10 rats. Each day for 10 days inject subcutaneously 0.1 mL of the *Control solution*, *Standard solutions*, and *Test solutions* to the control, standard, and test groups, respectively. Record the body weight of each animal at the start of the test and at approximately 18 hours following the 10th injection. Determine the change in body weight for each rat during the 10-day period, and compute the potency of the *Test solution* relative to that of the *Standard solution* using appropriate statistical analysis. Calculate the mean potency in USP Somatropin Units per mg; not less than 2 USP Somatropin Units per mg is found. Using appropriate statistical methods, calculate the width, *L*, of a 95% confidence interval for the estimated logarithm of the relative potency; *L* is not more than 0.40, which corresponds to confidence limits between 63% and 158% of the calculated potency. If *L* is more than 0.40, repeat the test until the results from two or more tests, combined by appropriate statistical methods, meet this criterion.

Microbial limits (61)—The total aerobic microbial count does not exceed 300 cfu per g, the test being performed on about 0.2 to 0.3 g of powder, accurately weighed.

Bacterial endotoxins (85)—It contains not more than 10 USP Endotoxin Units per mg.

Water, Method 1c (921): not more than 10%, when prepared as a lyophilized powder.

Chromatographic purity—

Diluent—Prepare a solution of 0.05 M Tris in water, and adjust with hydrochloric acid to a pH of 7.5.

Mobile phase—Degas the *Diluent*, mix with *n*-propyl alcohol (71:29, v/v), and filter. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Resolution solution—Prepare a solution of 2.0 mg of Somatropin per mL of the *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 hours. [NOTE—Use within 48 hours after preparation, or store the solution in a refrigerator until ready to use.]

Test solution—Prepare a solution of 2.0 mg of Somatropin per mL of the *Diluent* immediately before use. [NOTE—Maintain the solutions between 2° and 8°, and use within 24 hours. If an automatic injector is used, maintain the temperature between 2 and 8°.]

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm $\times$ 25-cm column that contains packing L26 and is maintained at 45°. The flow rate is about 0.5 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*; the resolution, *R*, between somatropin and its adjacent peak is not less than 1.0; and the tailing factor of the somatropin peak (major peak) is between 0.9 and 1.1.

Procedure—Inject about 20 μ L of the *Test solution*, record the chromatograms, and measure the peak responses. Calculate the percentage of impurities in the portion of Somatropin taken by the formula:

$$100A_1/(A_1 + A_2),$$

in which *A*₁ is the sum of the responses of all the peaks other than the somatropin peak (major peak) and disregarding any peak due to the solvent; and *A*₂ is the response of the somatropin peak; not more than 6.0% of total impurities is found.

Limit of high molecular weight proteins—

Phosphate buffer, **Mobile phase**, **Diluent**, **Resolution solution**, and **Chromatographic system**—Proceed as directed in the *Assay*.

Test solution—Prepare as directed for the *Assay preparation*.

Packaging and storage—Preserve in room temperature or in a cool place.

Identification—Proceed as directed for *Sodium Sulfate*.

Procedure—Inject about 20 μ L of the *Test solution*, record the chromatogram, and measure the areas of the main peak and of the peaks eluting prior to the main peak, excluding the solvent peaks. Calculate the percentage of high molecular weight proteins in the portion of Somatropin taken by the formula:

$$100A_H/(A_H + A_M),$$

in which A_H is the sum of the areas of the high molecular weight peaks, and A_M is the area of the monomer peak in the chromatogram of the *Test solution*: not more than 4% of high molecular weight proteins is found.

Total protein (see *Spectrophotometry and Light-Scattering* (851))—

Phosphate buffer—Prepare a 0.025 M solution of monobasic potassium phosphate in water, and adjust with sodium hydroxide to a pH of 7.0.

Test solution—Dissolve an accurately weighed quantity of Somatropin in *Phosphate buffer* to obtain a solution having an absorbance value between 0.5 and 1.0 at the wavelength of maximum absorbance at about 280 nm.

Procedure—Determine the absorbance of the *Test solution* using a spectrophotometric cell of path length 1-cm, at the wavelength of maximum absorbance at around 280 nm and at 320 nm, using *Phosphate buffer* as the blank. Calculate the protein content, in mg, in the portion of Somatropin taken by the formula:

$$V(A_{\text{max}} - A_{320})/0.82,$$

in which V is the volume of the *Test solution*; and A_{max} and A_{320} are the absorbance values of the *Test solution* at the wavelength of maximum absorbance and at 320 nm, respectively.

Assay—

Phosphate buffer—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 to a pH of 7.0, and dilute with water to 1000 mL.

Mobile phase—Prepare a filtered and degassed mixture of the *Phosphate buffer* and isopropyl alcohol (97:3, v/v). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Diluent—Prepare a mixture of water and *Phosphate buffer* (5:1).

Resolution solution—Place 1 vial of USP Somatropin RS in an oven at 50° for 12 to 24 hours. Remove from the oven, and dissolve the contents of the vial in *Diluent* to obtain a solution having a known concentration of about 1 mg per mL with the content of the vial between 1% and 2%.

Standard preparation—Reconstitute a vial of USP Somatropin RS with the *Diluent* to obtain a solution having a known concentration of about 1.0 mg per mL.

Assay preparation—Dissolve an accurately weighed quantity of Somatropin in *Diluent*, or dilute a bulk solution of Somatropin with *Diluent*, to obtain a solution having a concentration of about 1 mg per mL. [NOTE—If necessary, the amount of protein in solution can be determined by the test for *Total protein*.]

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 214-nm detector and a 3.9-mm $\times$ 30-cm column that contains packing L33 and is maintained at ambient temperature. The flow rate is 0.6 mL per minute. Chromatograph the *Resolution solution* as directed for *Procedure*: the resolution, R , (determined as the ratio of the valley height, between the dimer and the monomer, and the dimer peak height) is not more than 0.4; and the tailing factor of the monomer peak (major peak) is not more than 1.7.

Procedure—Separately inject equal volumes (about 20 μ L) of the *Standard preparation* and the *Assay preparation*, record the chromatograms for not less than twice the retention time of the somatropin monomer peak (major peak), and measure the peak responses for the monomer. Calculate the concentration, in mg per mL, of somatropin in the *Assay preparation* by the formula:

$$C_S(r_U/r_S),$$

in which C_S is the concentration, in mg per mL, of USP Somatropin RS in the *Standard preparation*; and r_U and r_S are the peak responses of the monomer in the *Assay preparation* and the *Standard preparation*, respectively.

Somatropin for Injection

» Somatropin for Injection is a sterile, lyophilized mixture of Somatropin with one or more suitable buffering and stabilizing agents. It contains not less than 89.0 percent and not more than 110.0 percent of the amount of somatropin stated on the label. Manufacturers must demonstrate a correlation between the assay and a validated and approved growth-promotion based bioassay. [NOTE—One mg of anhydrous Somatropin is equivalent to 3.0 USP Somatropin Units.]

Packaging and storage—Preserve in tight containers, and store between 2° and 8°.

Labeling—The labeling states that the material is of recombinant DNA origin.

USP Reference standards (11)—USP Endotoxin RS. USP Somatropin RS.

Identification—It meets the requirements for *Identification test A* under *Somatropin*.

Bioidentity—[NOTE—If the bulk material used to prepare Somatropin for Injection was tested and meets the requirements, it is not necessary to perform this test.] It meets the requirements for *Bioidentity* under *Somatropin*.

Bacterial endotoxins (85)—It contains not more than 20 USP Endotoxin Units per mg of somatropin.

Sterility (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

Chromatographic purity—Proceed as directed for the *Chromatographic purity* test under *Somatropin*: not more than 12% of total impurities is found.

Limit of high molecular weight proteins—Proceed as directed in the test for *Limit of high molecular weight proteins* under *Somatropin*, except to use the *Assay preparation* as the *Test solution*: not more than 6% of high molecular weight proteins is found.

Assay—

Phosphate buffer, *Mobile phase*, *Diluent*, *Resolution solution*, *Standard preparation*, and *Chromatographic system*—Proceed as directed in the *Assay* under *Somatropin*.

Assay preparation—Dissolve the contents of a suitable number of containers in *Diluent* to obtain a concentration of 1 mg of somatropin per mL.

Procedure—Proceed as directed under *Somatropin*. Calculate the quantity of somatropin, in mg of somatropin per container, by the formula:

$$C(V/N)(r_U/r_S),$$

in which C is the concentration, in mg per mL, of USP Somatropin RS in the *Standard preparation*; V is the total volume of the *Assay preparation*; N is the number of containers used to obtain the *Assay preparation*; and r_U and r_S are the peak responses of the monomer in the *Assay preparation* and the *Standard preparation*, respectively.

Sorbitol Solution

USP Reference standards (11)—USP Sorbitol Hydrochloride RS.

» Sorbitol Solution is an aqueous solution containing not less than 64.0 percent of D-sorbitol ($C_6H_{14}O_6$). The amounts of total sugars, other polyhydric alcohols, and