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Tetracosactide Zinc Injection

General Notices

Action and use

Corticotropic peptide.

DEFINITION

Tetracosactide Zinc Injection is a sterile aqueous suspension of Tetracosactide with zinc hydroxide.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Content of tetracosactide, $C_{136}H_{210}N_{40}O_{31}S$

80.0 to 110.0% of the stated amount of the peptide.

CHARACTERISTICS

A white, flocculent suspension which settles slowly and is readily resuspended. On examination under a microscope the majority of the particles are seen as amorphous or microcrystalline particles or aggregates thereof. The maximum dimension of single particles rarely exceeds 50 µm. Under high power magnification a considerable proportion of the particles can be seen to have no uniform shape.

IDENTIFICATION

A. Carry out the test for Identification described under Tetracosactide Injection using the following solutions. For solution (1) freeze dry a quantity of the well-shaken suspension containing 2 mg of the peptide and dissolve the residue in a mixture of 0.1 mL of *formic acid* and 0.1 mL of *water* with the aid of gentle heat. For solution (2) dissolve 10.45 g of *zinc chloride* in 20 mL of *water*, add 95 mL of *formic acid* followed by 4.2 g of *disodium hydrogen orthophosphate* and 4.0 g of *sodium chloride*. Dissolve 6.04 g of *sodium hydroxide* in 40 mL of *water*. Mix the two solutions carefully with cooling and add sufficient *water* to produce 200 mL. Dissolve 2.8 mg of *tetracosactide EPCRS* in 0.2 mL of the resulting solution. The principal spot in the electrophoretogram obtained with solution (1) corresponds to that in the electrophoretogram obtained with solution (2).
B. Evaporate 1 mL of the well-shaken suspension to dryness in a crucible and heat strongly until combustion of the organic material is complete. The residue is yellow while hot and becomes white on cooling.

TESTS

Alkalinity

pH, 7.8 to 9.2, Appendix V L.

Light absorption

Centrifuge 7 mL of the well-shaken suspension for 10 minutes at about 2000 *g*. Shake the clear, supernatant liquid with five 5-mL quantities of *chloroform*, previously washed with *water*. Discard the chloroform and centrifuge the aqueous phase for 5 minutes at about 2000 g. The *absorbance* of the clear, supernatant liquid at 276 nm, Appendix II B, is not more than 0.38 for preparations containing 1 mg of the peptide per mL and not more than 0.19 for preparations containing 0.5 mg of the peptide per mL.

Sediment volume

Transfer 3.0 mL of the well-shaken suspension to a cuvette 10 mm × 10 mm in cross section. Allow to stand for 5 hours. The depth of the sediment is between 8 and 25 mm and the supernatant liquid is clear.

Zinc

To a volume of the well-shaken suspension containing 1 mg of the peptide add 5 mL of 0.1M *hydrochloric acid* and sufficient *water* to produce 1000 mL. Carry out the method for *atomic absorption spectrophotometry*, Appendix II D, measuring at 214 nm and using *zinc standard solution* (*5 mg/mL Zn*), diluted if necessary with *water*, to prepare the standard solution. Preparations containing 1 mg of the peptide per mL contain 2.25 to 2.75 mg of zinc per mL; preparations containing 0.5 mg of the peptide per mL contain 1.35 to 1.65 mg of zinc per mL.

Tetracosactide sulfoxide

Carry out the test described under Tetracosactide Injection using the following solutions. For solution (1) mix a volume of the well-shaken suspension containing 1 mg of the peptide with 15 µL of *glacial acetic acid*. For solution (2) add 50 µL of a solution prepared by diluting 1 volume of *hydrogen peroxide solution* (*20 vol*) to 200 volumes to 1 mL of a 0.1% w/v solution of *tetracosactide EPCRS* in a 1% v/v solution of *glacial acetic acid* and allow to stand for 2 hours. The chromatogram obtained with solution (2) exhibits a peak due to tetracosactide and a significant peak with a shorter retention time, due to tetracosactide sulfoxide. In the chromatogram obtained with solution (1) the area of the peak due to tetracosactide sulfoxide is not more than 18% of the area of the peak due to tetracosactide. The peaks due to associated substances may be identified from the chromatogram obtained with solution (2) in the Assay.

ASSAY

Carry out the Assay described under Tetracosactide Injection using the following solutions. For solution (1) mix 1 mL of the well-shaken suspension with 15 µL of *glacial acetic acid*. For preparations containing 1 mg of the peptide per mL prepare solution (2) by dissolving quantities of *tetracosactide EPCRS* and *benzyl alcohol* in sufficient *water* to produce a solution containing the equivalent of 0.10% w/v of the peptide and 1.0% w/v of *benzyl alcohol* and adding 15 µL of *glacial acetic acid* per mL. For preparations containing 0.5 mg of the peptide per mL prepare solution (2) by dissolving quantities of *tetracosactide EPCRS* and *benzyl alcohol* in sufficient *water* to produce a solution containing the equivalent of 0.05% w/v of the peptide and 1.0% w/v of *benzyl alcohol* and adding 15 µL of *glacial acetic acid* per mL. If necessary, adjust the ratio of the volumes of *acetonitrile* and *water* in the mobile phase so that in the chromatogram obtained with solution (2) the *resolution factor* between the peaks due to benzyl alcohol (eluted before tetracosactide) and tetracosactide is between 1.5 and 2.0.

STORAGE

Tetracosactide Zinc Injection should be protected from light and stored at a temperature of 2° to 8°. It should not be allowed to freeze.

LABELLING

The strength is stated in terms of the equivalent amount of the peptide in mg per mL.