(4) Iron—Dissolve 1.0 g of Zinc Oxide in 50 mL of diluted hydrochloric acid (1 in 2), dissolve 0.1 g of ammonium peroxodisulfate in this solution, and extract with 20 mL of 4-methyl-2-pentanone. Add 30 mL of acetic acid-sodium acetate buffer solution for Iron Limit Test, pH 4.5, to the 4-methyl-2-pentanone layer, extract again, and use the layer of the buffer solution as the test solution. Separately, perform the test in the same manner with 1.0 mL of Standard Iron Solution, and use the layer so obtained as the control solution. Add 2 mL each of L-ascorbic acid solution for Iron Limit Test (1 in 100) to the test solution and the control solution, respectively, mix, allow to stand for 30 minutes, add 5 mL of an ethanol (95) solution of α,α′-dipyridyl (1 in 200) and water to make 50 mL. After allowing to stand for 30 minutes, compare the color of the both liquids against a white back: the color of the liquid from the test solution is not stronger than that from the control solution (not more than 10 ppm).

(5) Lead—To 2.0 g of Zinc Oxide add 20 mL of water, then add 5 mL of acetic acid (100) with stirring, and heat on a water bath until solution is complete. Cool, and add 5 drops of potassium chromate TS: no turbidity is produced.

(6) Arsenic—Dissolve 0.5 g of Zinc Oxide in 5 mL of dilute hydrochloric acid, use this solution as the test solution, and perform the test using the Apparatus B (not more than 4 ppm).

Loss on ignition Not more than 1.0% (1 g, 850°C, 1 hour).

Assay Weigh accurately about 0.8 g of Zinc Oxide, previously ignited at 850°C for 1 hour, dissolve in 2 mL of water and 3 mL of hydrochloric acid, and add water to exactly 100 mL. Pipet 10 mL of this solution, add 80 mL of water, then add a solution of sodium hydroxide (1 in 50) until a slight precipitate is produced. Add 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 0.04 g of eriochrome black T-sodium chloride indicator).

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 4.069 mg of ZnO

Containers and storage Containers—Tight containers.

Zinc Sulfate

硫酸亜鉛

$ZnSO_4.7H_2O$: 287.56

Zinc Sulfate contains not less than 99.0% and not more than 102.0% of $ZnSO_4.7H_2O$.

Description Zinc Sulfate occurs as colorless crystals or a white, crystalline powder. It is odorless, and has an astringent, characteristic taste.

It is very soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

The pH of a solution of Zinc Sulfate (1 in 20) is between 3.5 and 6.0.

It effloresces in dry air.

Identification A solution of Zinc Sulfate (1 in 20) responds to the Qualitative Tests for zinc salt and for sulfate.

Purity (1) Acid—Dissolve 0.25 g of Zinc Sulfate in 5 mL of water, and add 1 drop of methyl orange TS: no red color develops.

(2) Heavy metals—Dissolve 1.0 g of Zinc Sulfate in 10 mL of water contained in a Nessler tube. Add 20 mL of potassium cyanide TS, and mix well. Add 2 drops of sodium sulfide TS, and allow the mixture to stand for 5 minutes. Observe vertically against a white background, the color of the solution is not more intense than the following control solution.

Control solution: To 1.0 mL of Standard Lead Solution add 10 mL of water and 20 mL of potassium cyanide TS, and mix well. Add 2 drops of sodium sulfide TS (not more than 10 ppm).

(3) Alkali earth metals and alkali metals—Dissolve 2.0 g of Zinc Sulfate in 150 mL of water, add a suitable amount of ammonium sulfide TS to complete the precipitation, and add water to make exactly 200 mL. Shake well, and filter through a dry filter paper. Discard the first 20 mL of the filtrate, take exactly 100 mL of the subsequent filtrate, evaporate to dryness, and ignite as directed under the Residue on N,gnition: the mass of the residue is not more than 5.0 mg.

(4) Arsenic—Prepare the test solution with 1.0 g of Zinc Sulfate according to Method 1, and perform the test using Apparatus B (not more than 2 ppm).

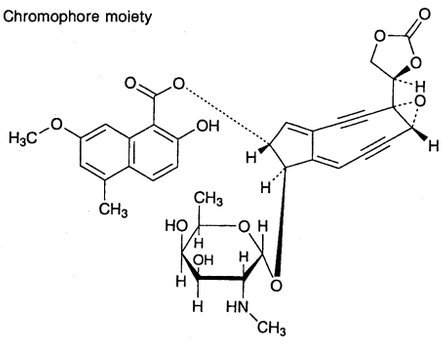
Assay Weigh accurately about 0.3 g of Zinc Sulfate, and dissolve in water to make exactly 100 mL. Measure exactly 25 mL of this solution, add 100 mL of water and 2 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 0.04 g of eriochrome black T-sodium chloride indicator).

Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 2.8756 mg of $ZnSO_4.7H_2O$

Containers and storage Containers—Tight containers.

Zinostatin Stimalamer

ジノスタチン スチマラマー

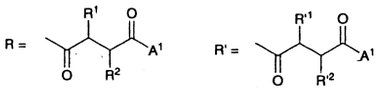


(4*S*,6*R*,11*R*,12*R*)-11-[α-D-2,6-Dideoxy-2-(methylamino)-galactopyranosyloxy]-4-[(4*R*)-2-oxo-1,3-dioxolan-4-yl]-

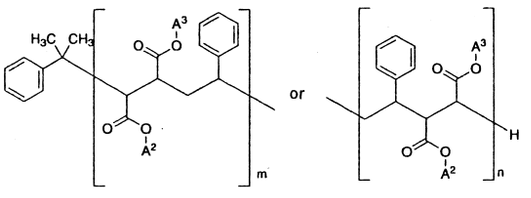
5-oxatricyclo[8.3.0.0^{4,6}]trideca-1(13),9-diene-2,7-diyne-12-yl 2-hydroxy-7-methoxy-5-methylnaphthalene-1-carboxylate
[*123760-07-6*]

Apoprotein moiety bonded to styrene-maleic acid alternate copolymer

R-Ala-Ala-Pro-Thr-Ala-Thr-Val-Thr-Pro-Ser-Ser-Gly-Leu-Ser-Asp-Gly-Thr-Val-
R'
Val-Lys-Val-Ala-Gly-Ala-Gly-Leu-Gln-Ala-Gly-Thr-Ala-Tyr-Asp-Val-Gly-Gln-Cys-Ala-Trp-Val-Asp-Thr-Gly-Val-Leu-Ala-Cys-Asn-Pro-Ala-Asp-Phe-Ser-Ser-Val-Thr-Ala-Asp-Ala-Asp-Gly-Ser-Ala-Ser-Thr-Ser-Leu-Thr-Val-Arg-Arg-Ser-Phe-Glu-Gly-Phe-Leu-Phe-Asp-Gly-Thr-Arg-Trp-Gly-Thr-Val-Asp-Cys-Thr-Thr-Ala-Ala-Cys-Gln-Val-Gly-Leu-Ser-Asp-Ala-Ala-Gly-Asn-Gly-Pro-Glu-Gly-Val-Ala-Ile-Ser-Phe-Asn



R^1 and R^2, and R'^{1} and R'^{2} are different each other as follows, respectively.



A^1=H or NH_4

A^2, A^3=H, NH_4 or C_4H_9 (no C_4H_9 appears at the same time at A^2 and A^3)

Average m+n=about 5.5

Zinostatin Stimalamer consists 1 molecule of zinostatin, consisting of chromophore and apoprotein (polypeptide consisting of 113 amino acid residues) and 2 molecules of partially butyl-esterified styrene-maleic acid alternate copolymer, and has average molecular mass of about 15,000. The alternate copolymer is bound an amido bond to α-amino group of alanine of N-terminal and to ε-amino group of lysine 20 of the apoprotein.

Zinostatin Stimalamer contains not less than 900 μg (potency) per mg, calculated on the anhydrous basis. The potency of Zinostatin Stimalamer is expressed as mass (potency) of zinostatin stimalamer.

Description Zinostatin Stimalamer occurs as a pale yellow powder.

It is freely soluble in water, and practically insoluble in ethanol (95).

Identification (1) Dissolve 0.01 g of Zinostatin Stimalamer in 1 mL of sodium hydroxide TS, and add a drop of copper (II) sulfate TS: a purple color develops.

(2) Dissolve 1 mg of Zinostatin Stimalamer in 1 mL of 0.05 mol/L phosphate buffer solution, pH 7.0, add 0.5 mL of a solution of trichloroacetic acid (1 in 5), and shake: a white precipitate is formed.

(3) Determine the absorption spectra of solutions of Zinostatin Stimalamer and Zinostatin Stimalamer Reference Standard in 0.05 mol/L phosphate buffer solution, pH 7.0 (1 in 2500) as directed under the Ultraviolet-visible Spectrophotometry, and compare these spectra: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectra of Zinostatin Stimalamer and Zinostatin Stimalamer Reference Standard as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare these spectra: both spectra exhibit similar intensities of absorption at the same wave numbers.

Absorbance $E^{1\%}_{1\,cm}$ (268 nm): 15.5 - 18.5 (4 mg calculated on the anhydrous basis, 0.05 mol/L phosphate buffer solution, pH 7.0, 10 mL).

Optical rotation $[\alpha]^{20}_D$: -30.0 - $-38.0°$ (0.02 g calculated on the anhydrous basis, 0.05 mol/L phosphate buffer solution, pH 7.0, 5 mL, 100 mm).

pH Dissolve 0.01 g of Zinostatin Stimalamer in 1 mL of water: the pH of the solution is between 4.5 and 5.5.

Purity (1) Clarity and color of solution—Being specified separately.

(2) Heavy metals—Weigh accurately 0.040 g of Zinostatin Stimalamer, place in a crucible, carbonize and incinerate according to Method 2, add 2 mL of hydrochloric acid, and evaporate on a water bath to dryness. After cooling, weigh the residue W_T g. Then, moisten the residue with 0.1 mL of diluted hydrochloric acid (1 in 5), add 1 mL of water, 85 μL of diluted ammonia TS (1 in 2) and 0.1 mL of dilute acetic acid, and add water so that the mass is W_T + 2.0 g. Adjust the pH of this solution to 3.2 to 3.4 with diluted ammonia TS (1 in 20) or diluted hydrochloric acid (1 in 50), add water so that the mass is W_T + 2.5 g, and use this solution as the test solution. Separately, prepare the blank solution in the same manner without the sample. Separately, take 2 mL of nitric acid, 5 drops of sulfuric acid and 2 mL of hydrochloric acid, and evaporate to dryness according to Method 2. After cooling, weigh the residue W_S g. Then, moisten the residue with 0.1 mL of diluted hydrochloric acid (1 in 5), and proceed in the same manner as directed in the preparation of the test solution. After adjusting the pH of the solution so obtained to 3.2 to 3.4, add 80 μL of Standard Lead Solution, and add water so that the mass is W_S + 2.5 g, and use this solution as the control solution. Add 10 μL each of diluted sodium sulfide TS (1 in 6) to the test solution, the blank solution and the control solution, mix, and allow to stand for 5 minutes. Determine the absorbances, A_T, A_O and A_S of the test solution, the blank solution and the control solution at 400 nm as directed under the Ultraviolet-visible Spectrophotometry: $A_T - A_O$ is not larger than $A_S - A_O$ (not more than 20 ppm).

(3) Related substances—Being specified separately.

(4) Inorganic salts of manufacturing process origin—Being specified separately.

Water Not more than 12.0% (0.01 g, coulometric titration).

Assay Perform the test according to the Cylinder-plate method as directed under the Microbial Assay for Antibiotics according to the following conditions. Perform the procedures of (3), (4) and (5) without exposure to direct or indirect sunlight.

(1) Test organism—*Micrococcus luteus* ATCC 9341

(2) Culture medium—Use the medium i in 3) Medium for other organisms under (1) Agar media for seed and base

layer. Adjust the pH of the medium so that it will be 7.9 to 8.1 after sterilization.

(3) Standard solution—Weigh accurately an amount of Zinostatin Stimalamer Reference Standard equivalent to about 0.02 g (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 50 mL, and use this solution as the high concentration standard solution. Pipet 5 mL of the high concentration standard solution, add 0.1 mol/L phosphate buffer solution, pH 8.0, to make exactly 20 mL, and use this solution as the low concentration standard solution.

(4) Sample solution—Weigh accurately an amount of Zinostatin Stimalamer equivalent to about 0.02 g (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 50 mL, and use this solution as the high concentration sample solution. Pipet 5 mL of the high concentration sample solution, add 0.1 mol/L phosphate buffer solution, pH 8.0, to make exactly 20 mL, and use this solution as the low concentration sample solution.

(5) Procedure—Allow to stand at 3 to 5°C for 2 hours before incubation.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and not exceeding −20°C.