

nus Antitoxin, Equine, in the Minimum Requirements for Biological Products.

Description Freeze-dried Tetanus Antitoxin, Equine, becomes a clear, colorless to light yellow-brown liquid or slightly white-turbid liquid on addition of solvent.

Thianthol

チアントール

Thianthol consists of dimethylthianthrene and ditoluene disulfide. It contains not less than 23.5% and not more than 26.5% of sulfur (S: 32.07).

Description Thianthol is a yellowish, viscous liquid. It has a faint, agreeable odor.

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

It, when cold, may separate crystals, which melt on warming.

Specific gravity d_{20}^{20} : 1.19 – 1.23

Identification To 0.1 g of Thianthol add cautiously 5 mL of sulfuric acid: a blue-purple color develops. Add 5 to 6 drops of nitric acid to the solution: the color of the solution changes to yellow-red with evolution of gas.

Purity (1) Acidity or alkalinity—Shake 10 g of Thianthol with 20 mL of water, allow to stand, and separate the water layer. The solution is neutral.

(2) Sulfate—To 10 mL of the water layer obtained in (1) add 2 to 3 drops of barium chloride TS: no opalescence is produced.

Residue on ignition Not more than 0.10% (1 g).

Assay Weigh accurately about 0.01 g of Thianthol, and proceed as directed in the sulfur determination of the Oxygen Flask Combustion Method, using a mixture of 5 mL of diluted sodium hydroxide TS (1 in 10) and 1.0 mL of hydrogen peroxide TS as an absorbing liquid.

Containers and storage Containers—Tight containers.

Compound Thianthol and Salicylic Acid Solution

複方チアントール・サリチル酸液

Compound Thianthol and Salicylic Acid Solution contains not less than 1.8 w/v% and not more than 2.2 w/v% of salicylic acid ($C_7H_6O_3$: 138.12), and not less than 1.8 w/v% and not more than 2.2 w/v% of phenol (C_6H_6O : 94.11).

Method of preparation

| | |
|----------------|--------|
| Thianthol | 200 mL |
| Salicylic Acid | 20 g |
| Phenol | 20 g |

| | |
|------------------|-----------------------|
| Olive Oil | 50 mL |
| Ether | 100 mL |
| Petroleum Benzin | a sufficient quantity |
| To make 1000 mL | |

Dissolve Salicylic Acid and Phenol in Ether, add Thianthol, Olive Oil and Petroleum Benzin to this solution, mix and dissolve to make 1000 mL.

Description Compound Thianthol and Salicylic Acid Solution is a light yellow liquid, having a characteristic odor.

Identification (1) Place 1 mL of Compound Thianthol and Salicylic Acid Solution to a porcelain dish, and evaporate on a water bath to dryness. To the residue add cautiously 5 mL of sulfuric acid: the color of the solution changes to yellow-red with evolution of gas (thianthol).

(2) Shake 10 mL of Compound Thianthol and Salicylic Acid Solution with 10 mL of sodium hydrogen carbonate TS, and separate the water layer. To 0.5 mL of the water layer add hydrochloric acid-potassium chloride buffer solution, pH 2.0, to make 50 mL, and to 5 mL of this solution add 5 mL of a solution of iron (III) nitrate enneahydrate (1 in 200): a red-purple color is produced (salicylic acid).

(3) Wash the upper phase obtained in (2) with 10 mL of sodium hydrogen carbonate TS, and extract with 10 mL of dilute sodium hydroxide TS. Shake 1 mL of the extract with 1 mL of sodium nitrate TS and 1 mL of dilute hydrochloric acid, and add 3 mL of sodium hydroxide TS: a yellow color is produced (phenol).

(4) To 1 mL of Compound Thianthol and Salicylic Acid Solution add 10 mL of ethanol (95), mix, and use this solution as the sample solution. Dissolve 0.01 g each of salicylic acid, phenol and thianthol in 5 mL each of ethanol (95), and use each solution as standard solutions (1), (2) and (3). Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 5 μ L each of the sample solution and the standard solutions on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetone and acetic acid (100) (45:5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): three spots obtained from the sample solution and the corresponding spots of standard solutions (1), (2) and (3) show the same R_f value. Spray evenly iron (III) chloride TS on the plate: the spot from standard solution (1) and the corresponding spot from the sample solution reveal a purple color.

Assay Measure exactly 2 mL of Compound Thianthol and Salicylic Acid Solution, add exactly 10 mL of the internal standard solution, then add 70 mL of diluted methanol (1 in 2), mix well, and add diluted methanol (1 in 2) to make 100 mL. Filter, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Weigh accurately about 0.2 g of salicylic acid for assay, previously dried in a desiccator (silica gel) for 3 hours, and about 0.2 g of phenol for assay, dissolve in diluted methanol (1 in 2) to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution and diluted methanol (1 in 2) to make 100 mL, and use this solution as the standard solution. With 5 μ L each of the sample solution and the standard solution, perform the test as directed under the Liquid Chromatography according to the following conditions, and calculate the ratios, Q_{Ta} and Q_{Tb} , of the peak area of salicylic

acid and phenol to that of the internal standard in the sample solution, and the ratios, Q_{Sa} and Q_{Sb} , of the peak area of salicylic acid and phenol to that of the internal standard in the standard solution.

$$\begin{aligned} & \text{Amount (mg) of salicylic acid (C}_7\text{H}_6\text{O}_3\text{)} \\ &= \text{amount (mg) of salicylic acid for assay} \\ & \times \frac{Q_{Ta}}{Q_{Sa}} \times \frac{1}{5} \end{aligned}$$

$$\begin{aligned} & \text{Amount (mg) of phenol (C}_6\text{H}_6\text{O)} \\ &= \text{amount (mg) of phenol for assay} \\ & \times \frac{Q_{Tb}}{Q_{Sb}} \times \frac{1}{5} \end{aligned}$$

Internal standard solution—A solution of theophylline in methanol (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 270 nm).

Column: A stainless steel column about 4 mm in inside diameter and 25 to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: Room temperature.

Mobile phase: A mixture of 0.1 mol/L phosphate buffer solution, pH 7.0, and methanol (3:1).

Flow rate: Adjust the flow rate so that the retention time of salicylic acid is about 6 minutes.

Selection of column: Dissolve 0.2 g of benzoic acid, 0.2 g of salicylic acid and 0.05 g of theophylline in 100 mL of diluted methanol (1 in 2). To 10 mL of this solution add 90 mL of diluted methanol (1 in 2). Proceed with 10 μ L of this solution under the above operating conditions. Use a column giving elution of benzoic acid, salicylic acid and theophylline in this order, and clearly dividing each peak.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and not exceeding 25°C.

Thrombin

トロンビン

Thrombin is prepared from prothrombin obtained from blood of man or bull, through interaction with added thromboplastin in the presence of calcium ions, sterilized and lyophilized.

It contains not less than 80% and not more than 150% of the labeled Units of thrombin.

Each mg contains not less than 10 Units of thrombin.

Description Thrombin is a white to light yellow, amorphous substance. Thrombin (500 Units) dissolves in 1.0 mL of isotonic sodium chloride solution clearly or with slight turbidity within 1 minute.

Loss on drying Not more than 3% (0.05 g, in vacuum, phosphorus (V) oxide, 4 hours).

Sterility Thrombin meets the requirements of the Sterility Test.

Assay (1) Fibrinogen solution—Weigh accurately about 0.03 g of fibrinogen, and dissolve in 3 mL of isotonic sodium chloride solution. Allow the solution to clot sufficiently with frequent shaking after the addition of about 3 Units of thrombin. Wash the precipitated clot thoroughly until the washings yield no turbidity on addition of silver nitrate TS, weigh the clot after drying at 105°C for 3 hours, and calculate the percentage of the clot in the fibrinogen. Dissolve the fibrinogen in isotonic sodium chloride solution so that the clot should be 0.20%, adjust the pH of the solution between 7.0 and 7.4 by addition of 0.05 mol/L dibasic sodium phosphate TS (or if necessary, use 0.5 mol/L disodium hydrogenphosphate TS), and dilute with isotonic sodium chloride solution to make a 0.10% solution.

(2) Procedure—Dissolve Thrombin Reference Standard in isotonic sodium chloride solution, and prepare four kinds of standard solutions which contain 4.0, 5.0, 6.2, and 7.5 Units in 1 mL. Transfer accurately 0.10 mL each of the standard solutions maintained at a given degree $\pm 1^\circ\text{C}$ between 20°C and 30°C to a small test tube, 10 mm in inside diameter, 100 mm in length, blow out 0.90 mL of the fibrinogen solution at the same temperature into the test tube from a pipet, start a stop watch simultaneously, shake the tube constantly, and determine the time for the first appearance of clot. Calculate the average values of five determinations for the four kinds of standard solutions, respectively. If the deviation between the maximum and the minimum values of five determinations is more than 10% of the average value, reject the whole run, and try the experiment again. The concentration of the standard solution may be changed appropriately within the range between 14 and 60 seconds of the clotting time. The determination proceeds at the same temperature described above. Next, weigh accurately the whole contents of a single container of Thrombin, dissolve it in isotonic sodium chloride solution to provide a solution which is presumed to contain about 5 Units in each mL, treat 0.10 mL of the solution with the same reagents in the same manner five times, determine the clotting times, and calculate the average value. Plot the average values of the clotting times of the four kinds of the standard solutions on a logarithmic graph, using Units as the abscissa and clotting times as the ordinate, and draw a calibration line which best fits the four plotted points. Using this line, read the Units U from the average value of the clotting times of the sample solution.

$$\text{Units of a single container of Thrombin} = U \times 10 \times V$$

V : The number of mL of the volume in which the contents of a single container of Thrombin has been dissolved.

Calculate the units for 1 mg of the contents.

Containers and storage Containers—Hermetic containers.

Storage—Not exceeding 10°C.

Expiration date Use within 36 months after the date of manufacture.