

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.