Each mL of 0.2 mol/L sodium hydroxide VS
= 30.018 mg of $C_4H_6O_6$

Containers and storage Containers—Tight containers.

Dried Yeast

乾燥酵母

Dried Yeast is dried and powdered cells of yeast belonging to *Saccharomyces*.

Dried Yeast contains not less than 400 mg of protein and not less than 100 μg of thiamine compounds [as thiamine hydrochloride ($C_{12}H_{17}ClN_4OS.HCl$: 337.27)] in each 1 g.

Description Dried Yeast occurs as a light yellowish white to brown powder. It has a characteristic odor and taste.

Identification Dried Yeast, when examined under a microscope, shows isolated cells, spheroidal or oval in shape, and 6 to 12 μm in length.

Purity **(1)** Rancidity—Dried Yeast is free from any unpleasant or rancid odor or taste.

(2) Starch—Add iodine TS to Dried Yeast, and examine microscopically: no or only a few granules are tinted blackish purple.

Loss on drying Not more than 8.0% (1 g, 100°C, 8 hours).

Total ash Not more than 9.0% (1 g, proceed as directed in the Total ash under the Crude Drugs).

Assay **(1)** Protein—Weigh accurately about 0.05 g of Dried Yeast and perform the test as directed under the Nitrogen Determination.

$$\text{Amount (mg) of protein in 1 g of Dried Yeast} = \text{amount (mg) of nitrogen (N)} \times 6.25 \times \frac{1}{\text{amount (g) of sample}}$$

(2) Thiamine—Weigh accurately about 1 g of Dried Yeast, add 1 mL of dilute hydrochloric acid and 80 mL of water, and heat in a water bath at 80°C to 85°C for 30 minutes with occasional shaking. After cooling, add water to make exactly 100 mL, and centrifuge for 10 minutes. Pipet 4 mL of the supernatant liquid, add exactly 5 mL of acetic acid-sodium acetate TS and exactly 1 mL of enzyme TS, and allow to stand at 45°C to 50°C for 3 hours. Place exactly 2 mL of this solution onto a chromatographic column prepared by pouring 2.5 mL of a weakly acidic CM-bridged cellulose cation exchanger (H type) (40 to 110 μm in particle diameter) into a chromatographic tube about 1 cm in inside diameter and about 17 cm in length, and elute at the flow rate of about 0.5 mL per minute. Wash the upper part of the column with a small amount of water, and wash the column with two 10-mL portions of water at the flow rate of about 1 mL per minute. Elute the column with two 2.5-mL portions of diluted phosphoric acid (1 in 50) at the flow rate of about 0.5 mL per minute, and combine the eluate. To the eluate add exactly 1 mL of the internal standard solution and 0.01 g of sodium 1-octanesulfonate, and after dissolving, use this solution as the sample solution. Separately, weigh accurately about 0.015 g of the Thiamine Hydrochloride Reference Standard (determine the water content in the same manner as for Thiamine Hydrochloride), dissolve in 0.001 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 1 mL of this solution, and add the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution, add exactly 1 mL of the internal standard solution and 3 mL of the mobile phase, and use this solution as the standard solution. Perform the test with 200 μL each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S, of the peak area of thiamine to that of the internal standard.

$$\text{Amount (μg) of thiamine in 1 g of Dried Yeast} = \text{amount (mg) of Thiamine Hydrochloride Reference Standard, calculated on the anhydrous basis} \times \frac{Q_T}{Q_S} \times \frac{1}{\text{amount (g) of the sample}} \times 12.5$$

Internal standard solution—Dissolve 0.01 g of phenacetin in acetonitrile to make 100 mL, and to 1 mL of this solution add diluted acetonitrile (1 in 5) to make 100 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4 mm in inside diameter and 15 to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 2.7 g of potassium dihydrogenphosphate in 1000 mL of water, and adjust the pH to 3.5 with diluted phosphoric acid (1 in 10). Dissolve 1.6 g of sodium 1-octanesulfonate in 800 mL of this solution, and add 200 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of thiamine is about 8 minutes.

Selection of column: Proceed with 200 μL of the standard solution under the above operating conditions, and calculate the resolution. Use a column giving elution of thiamine and the internal standard in this order with the resolution between these peaks being not less than 8.

Containers and storage Containers—Tight containers.

Zanthoxylum Fruit

Zanthoxyli Fructus

サンショウ

Zanthoxylum Fruit is the pericarps of the ripe fruit of *Zanthoxylum piperitum* De Candolle (*Rutaceae*), from which the seeds separated from the pericarps have been mostly removed.

Description Capsules of 2 or 3 flattened spheroidal mericarps, which are dehiscent in 2 pieces about 5 mm in diameter; the outer surface of pericarp, dark yellow-red to