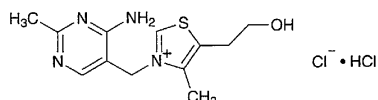


# Thiamine Hydrochloride

## Vitamin B<sub>1</sub> Hydrochloride

塩酸チアミン



$C_{12}H_{17}ClN_4OS \cdot HCl$ : 337.27

3-(4-Amino-2-methylpyrimidin-5-ylmethyl)-5-(2-hydroxyethyl)-4-methylthiazolium chloride monohydrochloride [67-03-8]

Thiamine Hydrochloride contains not less than 98.5% of  $C_{12}H_{17}ClN_4OS \cdot HCl$ , calculated on the anhydrous basis.

**Description** Thiamine Hydrochloride occurs as white crystals or crystalline powder. It is odorless or has a slight, characteristic odor.

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

Melting point: about 245°C (with decomposition).

**Identification (1)** To 5 mL of a solution of Thiamine Hydrochloride (1 in 500) add 2.5 mL of sodium hydroxide TS and 0.5 mL of potassium hexacyanoferrate (III) TS. Then add 5 mL of 2-methyl-1-propanol, shake the mixture vigorously for 2 minutes, allow to stand, and examine under ultraviolet light (main wavelength: 365 nm): the 2-methyl-1-propanol layer shows a blue-purple fluorescence. This fluorescence disappears when the mixture is acidified, but reappears when it is again made alkaline.

(2) Determine the absorption spectrum of a solution of Thiamine Hydrochloride (1 in 100,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Thiamine Hydrochloride Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Thiamine Hydrochloride, previously dried at 105°C for 2 hours, as directed in the potassium bromide disk method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum, or the spectrum of Thiamine Hydrochloride Reference Standard previously dried at 105°C for 2 hours: both spectra exhibit similar intensities of absorption at the same wave numbers. In case when some differences are found between the spectra, repeat the test with residues obtained by dissolving these substances in water, evaporating to dryness, and drying at 105°C for 2 hours.

(4) A solution of Thiamine Hydrochloride (1 in 500) responds to the Qualitative Tests for chloride.

**pH** Dissolve 1.0 g of Thiamine Hydrochloride in 100 mL of water: the pH of this solution is between 2.7 and 3.4.

**Purity (1)** Clarity and color of solution—Dissolve 1.0 g

of Thiamine Hydrochloride in 10 mL of water: the solution is clear, and has no more color than the following control solution.

Control solution: To 1.5 mL of  $\frac{1}{60}$  mol/L potassium dichromate VS add water to make 1000 mL.

(2) Sulfate—Weigh 1.5 g of Thiamine Hydrochloride, and perform the test. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.011%).

(3) Nitrate—Dissolve 0.5 g of Thiamine Hydrochloride in 25 mL of water. Add 2 mL of sulfuric acid to 2 mL of this solution, shake, cool, and superimpose iron (II) sulfate TS: no dark brown ring is produced at the junction of the two layers.

(4) Heavy metals—Proceed with 1.0 g of Thiamine Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(5) Related substances—Dissolve 0.10 g of Thiamine Hydrochloride in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution, as the standard solution. Perform the test with 10  $\mu$ L each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions, and determine the area of each peak from these solutions by the automatic integration method: total area of the peaks other than thiamine is not larger than the peak area of thiamine from the standard solution.

**Operating conditions—**

Detector, column, column temperature, mobile phase, flow rate, and selection of column: Proceed as directed in the operating conditions in the Assay.

Detection sensitivity: Adjust the sensitivity so that the peak height of thiamine obtained by 10  $\mu$ L of the standard solution is 30–60 mm.

Time span of measurement: About 3 times of the retention time of thiamine.

**Water** Not more than 5.0% (0.5 g, direct titration).

**Residue on ignition** Not more than 0.20% (1 g).

**Assay** Weigh accurately about 0.1 g each of Thiamine Hydrochloride and Thiamine Hydrochloride Reference Standard (previously determine its water content), and dissolve them in the mobile phase to make exactly 50 mL. To 10 mL each of the solutions, accurately measured, add exactly 5 mL each of the internal standard solution, add the mobile phase to make 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 10  $\mu$ L each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following operating conditions, and calculate the ratios,  $Q_T$  and  $Q_S$ , of the peak area of thiamine to that of the internal standard.

Amount (mg) of  $(C_{12}H_{17}ClN_4OS \cdot HCl)$

$$= \text{amount (mg) of Thiamine Hydrochloride Reference Standard, calculated on the anhydrous basis} \\ \times \frac{Q_T}{Q_S}$$

**Internal standard solution—**A solution of methyl benzoate in methanol (1 in 50).

**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  $\mu$ m in particle diameter).

**Column temperature:** A constant temperature of about 25°C.

**Mobile phase:** Dissolve 1.1 g of sodium 1-octanesulfonate in 1000 mL of diluted acetic acid (100) (1 in 100). To 600 mL of this solution add 400 mL of a mixture of methanol and acetonitrile (3:2).

**Flow rate:** Adjust the flow rate so that the retention time of thiamine is about 12 minutes.

**Selection of column:** Proceed with 10  $\mu$ L 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 6.

**Containers and storage** Containers—Tight containers.

Storage—Light-resistant.

**Thiamine Hydrochloride Injection****Vitamin B<sub>1</sub> Hydrochloride Injection**

塩酸チアミン注射液

Thiamine Hydrochloride Injection is an aqueous solution for injection. It contains not less than 95% and not more than 115% of the labeled amount of thiamine hydrochloride ( $C_{12}H_{17}ClN_4OS.HCl$ : 337.27).

**Method of preparation** Prepare as directed under Injections, with Thiamine Hydrochloride.

**Description** Thiamine Hydrochloride Injection is a clear, colorless liquid.

pH: 2.5 – 4.5

**Identification** To a volume of Thiamine Hydrochloride Injection, equivalent to 0.05 g of Thiamine Hydrochloride according to the labeled amount, add water to make 25 mL. Proceed with 5 mL of this solution as directed in the Identification (1) under Thiamine Hydrochloride.

**Assay** Dilute with 0.001 mol/L hydrochloric acid TS if necessary, then measure exactly a volume of Thiamine Hydrochloride Injection, equivalent to about 0.02 g of thiamine hydrochloride ( $C_{12}H_{17}ClN_4OS.HCl$ ), and add 20 mL of methanol and 0.001 mol/L hydrochloric acid TS to make 100 mL. To 25 mL of this solution, exactly measured, add exactly 5 mL of the internal standard solution, add 0.001 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of Thiamine Hydrochloride Reference Standard (determine previously its water content), and dissolve in 0.001 mol/L hydrochloric acid TS to make exactly 50 mL. To 10 mL of this solution, exactly measured, add 20 mL of methanol and 0.001 mol/L hydrochloric acid TS to make exactly 100 mL. To 25 mL of this solution, exactly measured,

add exactly 5 mL of the internal standard solution, add 0.001 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Thiamine Hydrochloride.

Amount (mg) of thiamine hydrochloride ( $C_{12}H_{17}ClN_4OS.HCl$ )

= amount (mg) of Thiamine Hydrochloride Reference Standard, calculated on the anhydrous basis

$$\times \frac{Q_T}{Q_S} \times \frac{1}{5}$$

**Internal standard solution—**A solution of methyl benzoate in methanol (1 in 200).

**Containers and storage** Containers—Hermetic containers.

Storage—Light-resistant.

**Thiamine Hydrochloride Powder****Vitamin B<sub>1</sub> Hydrochloride Powder**

塩酸チアミン散

Thiamine Hydrochloride Powder contains not less than 95% and not more than 115% of the labeled amount of thiamine hydrochloride ( $C_{12}H_{17}ClN_4OS.HCl$ : 337.27).

**Method of preparation** Prepare as directed under Powders, with Thiamine Hydrochloride.

**Identification** To a portion of Thiamine Hydrochloride Powder, equivalent to 0.02 g of Thiamine Hydrochloride according to the labeled amount, add 50 mL of water and 10 mL of dilute acetic acid, shake, and filter. Proceed with 5 mL of the filtrate as directed in the Identification (1) under Thiamine Hydrochloride.

**Purity** Rancidity—Thiamine Hydrochloride Powder has no unpleasant or rancid odor. It is tasteless.

**Assay** Weigh accurately a quantity of Thiamine Hydrochloride Powder, equivalent to about 0.02 g of thiamine hydrochloride ( $C_{12}H_{17}ClN_4OS.HCl$ ), add 60 mL of 0.01 mol/L hydrochloric acid TS, and heat on a water bath for 30 minutes. Shake vigorously for 10 minutes, cool, add methanol to make exactly 100 mL, and centrifuge. Pipet 25 mL of the supernatant, add exactly 5 mL of the internal standard solution, add water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of Thiamine Hydrochloride Reference Standard (determine previously its water content), and dissolve in 0.01 mol/L hydrochloric acid TS to make exactly 50 mL. To 10 mL of this solution, exactly measured, add 50 mL of 0.01 mol/L hydrochloric acid TS, and add methanol to make exactly 100 mL. To 25 mL of this solution, exactly measured, add exactly 5 mL of the internal standard solution, add water to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Thiamine Hydrochloride.