

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

= 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).

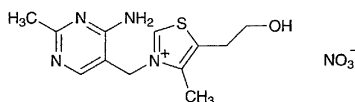
Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Thiamine Nitrate

Vitamin B₁ Nitrate

硝酸チアミン



$C_{12}H_{17}N_5O_4S$: 327.36

3-(4-Amino-2-methylpyrimidin-5-ylmethyl)-5-(2-hydroxyethyl)-4-methylthiazolium nitrate [532-43-4]

Thiamine Nitrate, when dried, contains not less than 98.0% and not more than 102.0% of $C_{12}H_{17}N_5O_4S$.

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

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

Melting point: about 193°C (with decomposition).

Identification (1) Take 2-mL portions of a solution of Thiamine Nitrate (1 in 500), and add 2 to 3 drops of iodine TS: a red-brown precipitate or turbidity is produced. Upon further addition of 1 mL of 2,4,6-trinitrophenol TS, a yellow precipitate or turbidity is produced.

(2) To 1 mL of a solution of Thiamine Nitrate (1 in 500) add 1 mL of lead (II) acetate TS and 1 mL of a solution of sodium hydroxide (1 in 10), and warm: the color of the solution changes through yellow to brown, and on standing, a black-brown precipitate is produced.

(3) To 5 mL of a solution of Thiamine Nitrate (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.

(4) A solution of Thiamine Nitrate (1 in 50) responds to the Qualitative Tests (1) and (2) for nitrate.

pH Dissolve 1.0 g of Thiamine Nitrate in 100 mL of water: the pH of this solution is between 6.5 and 8.0.

Purity (1) Chloride—Perform the test with 0.20 g of Thiamine Nitrate. Prepare the control solution with 0.30

mL of 0.01 mol/L hydrochloric acid VS (not more than 0.053%).

(2) **Sulfate**—Dissolve 1.5 g of Thiamine Nitrate in 30 mL of water and 2 mL of dilute hydrochloric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS and 2 mL of dilute hydrochloric acid, and add water to make 50 mL (not more than 0.011%).

(3) **Heavy metals**—Dissolve 1.0 g of Thiamine Nitrate in 30 mL of water by warming, cool, and add 12 mL of 6 mol/L acetic acid (31) TS and water to make 50 mL. Perform the test with this solution as the test solution. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Loss on drying Not more than 1.0% (0.5 g, 105°C, 2 hours).

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

Assay Weigh accurately about 0.1 g each of Thiamine Nitrate, previously dried, and Thiamine Hydrochloride Reference Standard (previously determine its water content in the same manner as directed under Thiamine Nitrate), 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 μ 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 $C_{12}H_{17}N_5O_4S$

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

$$\times \frac{Q_T}{Q_S} \times 0.9706$$

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

Operating conditions—

Detector: An ultraviolet spectrophotometer (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 μ m in particle diameter).

Column temperature: A constant temperature of 25–30°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 μ 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.