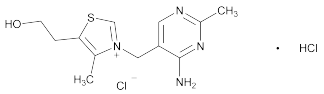
the peak responses obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Thiamine Hydrochloride



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

Thiazolium, 3-[(4-amino-2-methyl-5-pyrimidinyl)methyl]-5-(2-hydroxyethyl)-4-methyl-, chloride, monohydrochloride; Thiamine monohydrochloride [67-03-8].

DEFINITION

Thiamine Hydrochloride contains NLT 98.0% and NMT 102.0% of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$), calculated on the anhydrous basis.

IDENTIFICATION

- **A. Infrared Absorption** ⟨**197K**⟩
  Analysis: Dry specimens at 105° for 2 h.
  Acceptance criteria: Meets the requirements
- **B. Identification Tests—General,** *Chloride* ⟨**191**⟩**:** A 20-mg/mL solution meets the requirements.

ASSAY

- **Procedure**
  Solution A: 0.005 M sodium 1-octanesulfonate in dilute glacial acetic acid (1 in 100)
  Solution B: Methanol and acetonitrile (3:2)
  Mobile phase: *Solution B* and *Solution A* (40:60)
  Internal standard solution: 2% (v/v) of methylbenzoate in methanol
  Standard solution: Prepare a 1-mg/mL solution of USP Thiamine Hydrochloride RS in *Mobile phase*. Transfer 20.0 mL of this solution and 5.0 mL of *Internal standard solution* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume. The *Standard solution* contains 400 µg/mL of thiamine hydrochloride.
  Sample solution: Prepare a 2-mg/mL of Thiamine Hydrochloride in *Mobile phase*. Transfer 10.0 mL of this solution and 5.0 mL of *Internal standard solution* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.
  Chromatographic system
  (See *Chromatography* ⟨621⟩*, System Suitability*.)
  Mode: LC
  Detector: UV 254 nm
  Column: 4-mm × 30-cm; packing L1
  Flow rate: 1 mL/min
  [NOTE—The flow rate may be adjusted as needed to obtain a retention time of about 12 min for thiamine hydrochloride.]
  Injection size: 10 µL
  System suitability
  Sample: *Standard solution*
  Suitability requirements
  Resolution: NLT 4.0 between the thiamine and methylbenzoate peaks
  Tailing factor: NMT 2.0 for the thiamine peak
  Column efficiency: NLT 1500 theoretical plates for the thiamine peak
  Relative standard deviation: NMT 2.0% for the ratios of thiamine peak areas to the internal standard peak area
  Analysis
  Samples: *Standard solution* and *Sample solution*
  Calculate the percentage of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$) in the portion of Thiamine Hydrochloride taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = internal standard ratio (peak area of thiamine/peak area of the internal standard) from the *Sample solution*
R_S = internal standard ratio (peak area of thiamine/peak area of the internal standard) from the *Standard solution*
C_S = concentration of USP Thiamine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of Thiamine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **Residue on Ignition** ⟨**281**⟩**:** NMT 0.2%
- **Limit of Nitrate**
  Sample solution: 20 mg/mL of Thiamine Hydrochloride
  Analysis: To 2 mL of the *Sample solution* add 2 mL of sulfuric acid. Cool, and superimpose 2 mL of ferrous sulfate TS.
  Acceptance criteria: No brown ring is produced at the junction of the two layers.
- **Related Compounds**
  Solution A, Solution B, and **Mobile phase:** Proceed as directed in the *Assay*.
  Sample solution: 1.0 mg/mL of Thiamine Hydrochloride in *Mobile phase*
  Chromatographic system
  (See *Chromatography* ⟨621⟩*, System Suitability*.)
  Mode: LC
  Detector: UV 254 nm
  Column: 4.0-mm × 15-cm; packing L1
  Flow rate: 0.75 mL/min
  Injection size: 10 µL
  Analysis
  Sample: *Sample solution*
  Allow the *Sample solution* to elute for NLT three times the retention time of the main peak.
  Calculate the percentage of total secondary peaks in the portion of Thiamine Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = sum of the areas of all the peaks, except that of the thiamine peak
r_T = sum of the areas of all the peaks

Acceptance criteria: NMT 1.0%

SPECIFIC TESTS

- **pH** ⟨**791**⟩
  Sample solution: 10-mg/mL solution
  Acceptance criteria: 2.7–3.4
- **Water Determination,** *Method I* ⟨**921**⟩**:** NMT 5.0%
- **Absorbance of Solution**
  Sample solution: 100 mg/mL in water. Filter through a fine-porosity, sintered-glass funnel.
  Blank: Water
  Instrumental conditions
  (See *Spectrophotometry and Light-Scattering* ⟨851⟩.)
  Mode: UV-Vis
  Analytical wavelength: 400 nm
  Cell: 1 cm
  Analysis
  Samples: *Sample solution* and *Blank*
  Determine the absorbance of the *Sample solution* against that of the *Blank*.
  Acceptance criteria: NMT 0.025

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Thiamine Hydrochloride RS

Thiamine Hydrochloride Injection

» Thiamine Hydrochloride Injection is a sterile solution of Thiamine Hydrochloride in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$).

Packaging and storage—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light.

USP Reference standards ⟨11⟩**—**
USP Endotoxin RS
USP Thiamine Hydrochloride RS

Identification—

A: It yields a white precipitate with mercuric chloride TS, and a red-brown precipitate with iodine TS. It also yields a precipitate with mercuric-potassium iodide TS, and with trinitrophenol TS.

B: Dilute a portion of Injection with water to a concentration of about 10 mg of thiamine hydrochloride per mL. To 0.5 mL of this solution add 5 mL of 0.5 N sodium hydroxide, then add 0.5 mL of potassium ferricyanide TS and 5 mL of isobutyl alcohol, shake the mixture vigorously for 2 minutes, and allow the liquid layers to separate: when illuminated from above by a vertical beam of UV light and viewed at a right angle to this beam, the air-liquid meniscus shows a vivid blue fluorescence, which disappears when the mixture is slightly acidified, but reappears when it is again made alkaline.

C: It responds to the tests for *Chloride* ⟨191⟩.

Bacterial endotoxins ⟨85⟩**—**It contains not more than 3.5 USP Endotoxin Units per mg of thiamine hydrochloride.

pH ⟨791⟩: between 2.5 and 4.5.

Other requirements—It meets the requirements under *Injections* ⟨1⟩.

Assay—

Mobile phase, Internal standard solution, Standard preparation, and *Chromatographic system*—Prepare as directed in the *Assay* under *Thiamine Hydrochloride Oral Solution*.

Assay preparation—Quantitatively dilute an accurately measured volume of Injection with *Mobile phase* to obtain a solution containing about 500 µg of thiamine hydrochloride per mL. Pipet 10 mL of the resulting solution and 10 mL of *Internal standard solution* into a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

Procedure—Proceed as directed for *Procedure* in the *Assay* under *Thiamine Hydrochloride Oral Solution*. Calculate the quantity, in mg, of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$) in each mL of the Injection taken by the formula:

$$C(L/D)(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Thiamine Hydrochloride RS in the *Standard preparation; L* is the labeled quantity, in mg per mL, of thiamine hydrochloride in the Injection; D is the concentration, in mg per mL, of thisamine hydrochloride in the *Assay preparation* on the basis of the labeled quantity and the extent of dilution; and R_U and R_S are the ratios of the peak responses of thiamine to methylparaben obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Thiamine Hydrochloride Oral Solution

DEFINITION
Thiamine Hydrochloride Oral Solution contains NLT 95.0% and NMT 135.0% of the labeled quantity of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$).

IDENTIFICATION
• **A.**
Sample solution: Dilute a portion of Oral Solution with water to a concentration of 10 mg/mL of thiamine hydrochloride.
Analysis: To 0.5 mL of the *Sample solution* add 5 mL of 0.5 N sodium hydroxide, then add 0.5 mL of potassium ferricyanide TS and 5 mL of isobutyl alcohol. Shake the mixture vigorously for 2 min, and allow the liquid layers to separate. Illuminate from above by a vertical beam of UV light, and observe the air–liquid meniscus at a right angle to this beam.
Acceptance criteria: The air–liquid meniscus shows a vivid blue fluorescence, which disappears when the mixture is slightly acidified, but reappears when it is again made alkaline.

ASSAY
• **PROCEDURE**
Mobile phase: Methanol and 0.04 M aqueous monobasic potassium phosphate (45:55)
Internal standard solution: 100 µg/mL of methylparaben in *Mobile phase*
Standard stock solution: 500 µg/mL of USP Thiamine Hydrochloride RS in *Mobile phase*
Standard solution: Dilute a mixture of equal volumes of the *Standard stock solution* and *Internal standard solution* with *Mobile phase* to obtain a concentration of USP Thiamine Hydrochloride RS of about 50 µg/mL.
Sample stock solution: Equivalent to 500 µg/mL of thiamine hydrochloride in *Mobile phase* from an accurately measured volume of Oral Solution
Sample solution: Dilute a mixture of equal volumes of the *Sample stock solution* and *Internal standard solution* with *Mobile phase* to obtain a concentration of thiamine hydrochloride of about 50 µg/mL.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1 mL/min
Injection size: 25 µL
System suitability
Sample: *Standard solution*
[NOTE—The relative retention times for thiamine and methylparaben are about 0.35 and 1.0, respectively.]
Suitability requirements
Resolution: NLT 6.0 between thiamine and methylparaben
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$) in the portion of Oral Solution taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak area ratio of thiamine to methylparaben from the *Sample solution*
R_S = peak area ratio of thiamine to methylparaben from the *Standard solution*
C_S = concentration of USP Thiamine Hydrochloride RS in the *Standard stock solution* (µg/mL)
C_U = nominal concentration of thiamine hydrochloride in the *Sample stock solution* (µg/mL)