⟨531⟩ THIAMINE ASSAY

ASSAY

• **CHEMICAL METHODS, PROCEDURE 1**

The following procedure is provided for the determination of thiamine as an ingredient of pharmacopeial preparations containing other active constituents. The procedure involves thiamine reacting with potassium ferricyanide and subsequently being determined by fluorescence detection. Throughout the procedure, protect solutions containing and derived from the test specimen and the Reference Standard from the atmosphere and light, preferably by the use of low-actinic glassware.

Potassium ferricyanide solution: Dissolve 1.0 g of potassium ferricyanide in water to make 100 mL. Prepare fresh on the day of use.

Oxidizing reagent: Mix 4.0 mL of *Potassium ferricyanide solution* with a sufficient amount of 3.5 N sodium hydroxide to make 100 mL. Use this solution within 4 h.

Quinine sulfate stock solution: Dissolve 10 mg of quinine sulfate in 0.1 N sulfuric acid to make 1000 mL. Preserve this solution, protected from light, in a refrigerator.

Quinine sulfate standard solution: Dilute 0.1 N sulfuric acid with *Quinine sulfate stock solution* (39:1). This solution fluoresces to approximately the same degree as the thiochrome obtained from 1 µg of thiamine hydrochloride and is used to correct the fluorometer at frequent intervals for variation in sensitivity from reading to reading within an assay. Prepare this solution fresh on the day of use.

Standard thiamine hydrochloride stock solution: Transfer about 25 mg of USP Thiamine Hydrochloride RS, accurately weighed, to a 1000-mL volumetric flask. Dissolve the weighed Standard in about 300 mL of dilute alcohol solution (1 in 5) adjusted with 3 N hydrochloric acid to a pH of 4.0, and add the acidified, dilute alcohol to volume. Store in a refrigerator in a light-resistant container. Prepare this stock solution fresh each month.

Standard solution: Dilute a portion of *Standard thiamine hydrochloride stock solution* quantitatively and stepwise with 0.2 N hydrochloric acid to obtain the *Standard solution*, each mL of which represents 0.2 µg of USP Thiamine Hydrochloride RS.

Sample solution: Place in a suitable volumetric flask a sufficient amount of the material to be assayed, accurately weighed or measured by volume as directed, such that when diluted with 0.2 N hydrochloric acid to volume, the resulting solution will contain about 100 µg of thiamine hydrochloride (or mononitrate) per mL. If the sample is difficult to dissolve, the solution may be heated on a steam bath, and then cooled and diluted with the acid to volume. Dilute 5 mL of this solution, quantitatively and stepwise, using 0.2 N hydrochloric acid, to an estimated concentration of 0.2 µg of thiamine hydrochloride (or mononitrate) per mL.

Instrumental conditions

(See *Fluorescence Spectroscopy* ⟨853⟩.)

Mode: Fluorescence

Excitation wavelength: 365 nm

Emission wavelength: 435 nm

Analysis: To each of three or more tubes (or other suitable vessels) of about 40-mL capacity, pipet 5 mL of *Standard solution*. To each of two of these tubes, add rapidly (within 1 to 2 s), with mixing, 3.0 mL of *Oxidizing reagent*, and within 30 s add 20.0 mL of isobutyl alcohol, then mix vigorously for 90 s by shaking the capped tubes manually, or by bubbling a stream of air through the mixture. Prepare a blank in the remaining tube of the standard by substituting for the *Oxidizing reagent* with an equal volume of 3.5 N sodium hydroxide and proceed in the same manner. Into each of three or more similar tubes, pipet 5 mL of the *Sample solution*. Treat these tubes in the same manner as directed for the tubes containing the *Standard solution*. Into each of the six tubes, pipet 2 mL of dehydrated alcohol, swirl for a few seconds, allow the phases to separate, and decant or draw off about 10 mL of the clear, supernatant isobutyl alcohol solution into the standardized cells; then measure the fluorescence in a suitable fluorometer, having an input filter of narrow transmittance range with a maximum at about 365 nm and an output filter of narrow transmittance range with a maximum at about 435 nm.

Calculate the quantity, in µg, of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$) in each 5 mL of the *Sample solution*:

$$\text{Result} = (A - b)/(S - d)$$

A = average fluorometer readings of the portions of the *Sample solution* treated with the *Oxidizing reagent*
b = reading for the blank of the *Sample solution*
S = average fluorometer readings of the portions of the *Standard solution* treated with the *Oxidizing reagent*
d = reading for the blank of the *Standard solution*

Calculate the quantity, in mg, of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$) in the assay material on the basis of the aliquots taken. Where indicated, the quantity, in mg, of thiamine mononitrate ($C_{12}H_{17}N_5O_4S$) may be calculated by multiplying the quantity of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$) found by 0.9706.

The following liquid chromatographic procedures are provided for the determination of thiamine as an active pharmaceutical ingredient, a dietary supplement ingredient, or a component in dietary supplements or pharmaceutical dosage forms.

Throughout these procedures, protect solutions containing and derived from the test specimen and the Reference Standards from the atmosphere and light, preferably by the use of low-actinic glassware.

• **CHROMATOGRAPHIC METHODS, PROCEDURE 1**

This procedure can be used to determine thiamine in:

- *Oil- and Water-Soluble Vitamins Capsules*
- *Oil- and Water-Soluble Vitamins Tablets*

- *Oil- and Water-Soluble Vitamins with Minerals Capsules*
- *Oil- and Water-Soluble Vitamins with Minerals Tablets*
- *Water-Soluble Vitamins Capsules*
- *Water-Soluble Vitamins Tablets*
- *Water-Soluble Vitamins with Minerals Capsules*
- *Water-Soluble Vitamins with Minerals Tablets*

This is the procedure that involves the extraction of thiamine from the formulation by the *Diluent*, heat, and mechanical shaking.

Unless specified in the individual monographs, the *Standard solution*, *Sample solutions*, and reagent solutions are prepared as follows.

Diluent: Acetonitrile, glacial acetic acid, and water (5:1:94)

Mobile phase: A mixture of methanol, glacial acetic acid, and water (27:1:73) containing 140 mg of sodium 1-hexanesulfonate per 100 mL

Standard solution: Transfer 20 mg of USP Thiamine Hydrochloride RS to a 200-mL volumetric flask, and add 180 mL of *Diluent*. Immerse the flask in a hot water bath maintained at 65°–70° for 10 min with regular shaking or using a vortex mixer until all of the solid materials are dissolved. Chill rapidly in a cold water bath for 10 min to room temperature, and dilute with *Diluent* to volume.

Sample solution for capsules: Weigh NLT 20 capsules in a tared weighing bottle. Open the capsules, without loss of shell material, and transfer the contents to a 100-mL beaker. Remove any contents adhering to the shells by washing with several portions of ether. Discard the washings, and dry the capsule shells with the aid of a current of dry air until the odor of ether is no longer perceptible. Weigh the empty capsule shells in the tared weighing bottle, and calculate the average net weight per capsule. Transfer a portion of the capsule contents, equivalent to 2.5 mg of thiamine hydrochloride, to a 50-mL centrifuge tube. Add 25.0 mL of *Diluent*, and mix using a vortex mixer for 30 s to completely suspend the powder. Immerse the centrifuge tube in a hot water bath maintained at 65°–70°, heat for 5 min, and mix on a vortex mixer for 30 s. Return the tube to the hot water bath, heat for another 5 min, and mix on a vortex mixer for 30 s. Filter a portion of the solution, cool to room temperature, and use the clear filtrate. [NOTE—Use the filtrate within 3 h of filtration.]

Sample solution for tablets: Finely powder NLT 30 tablets. Transfer a portion of the powder, equivalent to 2.5 mg of thiamine hydrochloride, to a 50-mL centrifuge tube. Add 25.0 mL of *Diluent*, and mix using a vortex mixer for 30 s to completely suspend the powder. Immerse the centrifuge tube in a hot water bath maintained at 65°–70°, heat for 5 min, and mix on a vortex mixer for 30 s. Return the tube to the hot water bath, heat for another 5 min, and mix on a vortex mixer for 30 s. Filter a portion of the solution, cool to room temperature, and use the clear filtrate. [NOTE—Use the filtrate within 3 h of filtration.]

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and appropriate *Sample solution*

For products containing thiamine hydrochloride, calculate the percentage of the labeled amount of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$) in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of thiamine from the appropriate *Sample solution*
r_S = peak response of thiamine from the *Standard solution*
C_S = concentration of USP Thiamine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of thiamine hydrochloride in the appropriate *Sample solution* (mg/mL)

For products containing thiamine mononitrate, calculate the percentage of the labeled amount of thiamine mononitrate ($C_{12}H_{17}N_5O_4S$) in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of thiamine from the appropriate *Sample solution*
r_S = peak response of thiamine from the *Standard solution*
C_S = concentration of USP Thiamine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of thiamine mononitrate in the appropriate *Sample solution* (mg/mL)
M_{r1} = molecular weight of thiamine mononitrate, 327.36
M_{r2} = molecular weight of thiamine hydrochloride, 337.27

- **CHROMATOGRAPHIC METHODS, PROCEDURE 2**

This procedure can be used to determine thiamine in:

- *Oil- and Water-Soluble Vitamins Capsules*
- *Oil- and Water-Soluble Vitamins Tablets*
- *Oil- and Water-Soluble Vitamins with Minerals Capsules*
- *Oil- and Water-Soluble Vitamins with Minerals Tablets*
- *Water-Soluble Vitamins Capsules*
- *Water-Soluble Vitamins Tablets*
- *Water-Soluble Vitamins with Minerals Capsules*
- *Water-Soluble Vitamins with Minerals Tablets*

This is the procedure that involves the extraction of thiamine from the formulation by dilute hydrochloric acid, heat, and mechanical shaking.

Unless specified in the individual monographs, the *Standard solution*, *Sample solutions*, and reagent solutions are prepared as follows.

Solution A: 1.88 g/L of sodium 1-hexanesulfonate in 0.1% phosphoric acid

Mobile phase: *Solution A* and acetonitrile (46:9)

Standard stock solution: 0.1 mg/mL of USP Thiamine Hydrochloride RS in 0.2 N hydrochloric acid

Standard solution: 0.02 mg/mL of USP Thiamine Hydrochloride RS from *Standard stock solution* diluted with 0.2 N hydrochloric acid

Sample solution for capsules: Weigh NLT 20 capsules in a tared weighing bottle. Open the capsules, without the loss of shell material, and transfer the contents to a 100-mL beaker. Remove any contents adhering to the empty shells by washing, if necessary, with several portions of ether. Discard the washings, and dry the capsule shells with the aid of a current of dry air until the odor of ether is no longer perceptible. Weigh the empty capsule shells in the tared weighing bottle, and calculate the average net weight per capsule. Mix a portion of the capsule contents with a volume of 0.2 N hydrochloric acid to obtain a concentration of 0.02 mg/mL of thiamine. Shake the solution for 15 min with a wrist-action shaker, and heat to boiling for 30 min. Cool to room temperature, and filter. Use the clear filtrate.

Sample solution for tablets: Weigh and finely powder NLT 20 tablets. Mix a portion of the powder with a volume of 0.2 N hydrochloric acid to obtain a concentration of 0.02 mg/mL of thiamine. Shake for 15 min with a wrist-action shaker, and heat to boiling for 30 min. Cool to room temperature, and filter. Use the clear filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and appropriate *Sample solution*

For products containing thiamine hydrochloride, calculate the percentage of the labeled amount of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$) in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of thiamine from the appropriate *Sample solution*
r_S = peak response of thiamine from the *Standard solution*
C_S = concentration of USP Thiamine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of thiamine hydrochloride in the appropriate *Sample solution* (mg/mL)

For products containing thiamine mononitrate, calculate the percentage of the labeled amount of thiamine mononitrate ($C_{12}H_{17}N_5O_4S$) in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of thiamine from the appropriate *Sample solution*
r_S = peak response of thiamine from the *Standard solution*
C_S = concentration of USP Thiamine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of thiamine mononitrate in the appropriate *Sample solution* (mg/mL)
M_{r1} = molecular weight of thiamine mononitrate, 327.36
M_{r2} = molecular weight of thiamine hydrochloride, 337.27

- **CHROMATOGRAPHIC METHODS, PROCEDURE 3**

This procedure can be used to determine thiamine in:

- *Oil- and Water-Soluble Vitamins Capsules*

- *Oil- and Water-Soluble Vitamins Oral Solution*
- *Oil- and Water-Soluble Vitamins Tablets*
- *Oil- and Water-Soluble Vitamins with Minerals Capsules*
- *Oil- and Water-Soluble Vitamins with Minerals Oral Solution*
- *Oil- and Water-Soluble Vitamins with Minerals Tablets*
- *Water-Soluble Vitamins Capsules*
- *Water-Soluble Vitamins Tablets*
- *Water-Soluble Vitamins with Minerals Capsules*
- *Water-Soluble Vitamins with Minerals Oral Solution*
- *Water-Soluble Vitamins with Minerals Tablets*

This is the procedure that involves the extraction of thiamine from the formulation by mixtures of organic solvents, heat, and mechanical shaking.

Unless specified in the individual monographs, the *Standard solutions*, *Sample solutions*, and reagent solutions are prepared as follows.

Diluent: 25 mg/mL of edetate disodium in water

Mobile phase: Transfer 0.4 mL of triethylamine, 15.0 mL of glacial acetic acid, and 350 mL of methanol to a 2000-mL volumetric flask. Dilute with 0.008 M sodium 1-hexanesulfonate to volume.

Standard stock solution: 0.24 mg/mL of USP Thiamine Hydrochloride RS in *Diluent*, with heating if necessary

Standard solution for capsules/tablets: Transfer 5.0 mL of *Standard stock solution* to a stoppered 125-mL flask. Add 10.0 mL of a mixture of methanol and glacial acetic acid (9:1) and 30.0 mL of a mixture of methanol and ethylene glycol (1:1). Insert the stopper, shake for 15 min in a water bath maintained at 60°, and cool. Filter, and discard the first few mL of the filtrate.

Standard solution for oral solution: 24 µg/mL of USP Thiamine Hydrochloride RS, diluted from the *Standard stock solution* with *Diluent*

Sample solution for capsules: Weigh NLT 20 capsules in a tared weighing bottle. Open the capsules, without the loss of shell material, and transfer the contents to a 100-mL beaker. Remove any contents adhering to the empty shells by washing, if necessary, with several portions of ether. Discard the washings, and dry the capsule shells with the aid of a current of dry air until the odor of ether is no longer perceptible. Weigh the empty capsule shells in the tared weighing bottle, and calculate the average net weight per capsule. Transfer a portion of the capsule contents, equivalent to 1.2 mg of thiamine hydrochloride, to a stoppered 125-mL flask. Add 10.0 mL of a mixture of methanol and glacial acetic acid (9:1) and 30.0 mL of a mixture of methanol and ethylene glycol (1:1). Insert the stopper, shake for 15 min in a water bath maintained at 60°, and cool. Filter, and discard the first few mL of the filtrate.

Sample solution for oral solution: Equivalent to 24 µg/mL of thiamine hydrochloride or thiamine mononitrate from oral solution in *Diluent*

Sample solution for tablets: Weigh and finely powder NLT 20 tablets. Transfer a portion of the powder, equivalent to 1.2 mg of thiamine, to a stoppered 125-mL flask. Add 10.0 mL of a mixture of methanol and glacial acetic acid (9:1) and 30.0 mL of a mixture of methanol and ethylene glycol (1:1). Insert the stopper, shake for 15 min in a water bath maintained at 60°, and cool. Filter, and discard the first few mL of the filtrate.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 270 nm

Column: 4.6-mm × 25-cm; packing L7

Column temperature: 50°

Flow rate: 2 mL/min

Injection volume: 5 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: Appropriate *Standard solution* and appropriate *Sample solution*

For products containing thiamine hydrochloride, calculate the percentage of the labeled amount of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$) in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of thiamine from the appropriate *Sample solution*
r_S = peak response of thiamine from the appropriate *Standard solution*
C_S = concentration of USP Thiamine Hydrochloride RS in the appropriate *Standard solution* (mg/mL)
C_U = nominal concentration of thiamine in the appropriate *Sample solution* (mg/mL)

For products containing thiamine mononitrate, calculate the percentage of the labeled amount of thiamine mononitrate ($C_{12}H_{17}N_5O_4S$) in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of thiamine from the appropriate *Sample solution*
r_S = peak response of thiamine from the appropriate *Standard solution*
C_S = concentration of USP Thiamine Hydrochloride RS in the appropriate *Standard solution* (mg/mL)
C_U = nominal concentration of thiamine mononitrate in the appropriate *Sample solution* (mg/mL)
M_{r1} = molecular weight of thiamine mononitrate, 327.36
M_{r2} = molecular weight of thiamine hydrochloride, 337.27

- **CHROMATOGRAPHIC METHODS, PROCEDURE 4**

This procedure can be used to determine thiamine in:

- An active pharmaceutical ingredient
- A dietary ingredient

This is the procedure that involves the dissolution of the sample directly into the *Mobile phase*. The procedure is applicable for the determination of thiamine hydrochloride or thiamine mononitrate as active pharmaceutical or dietary supplement ingredients.

Unless specified in the individual monographs, the *Standard solution*, *Sample solution*, and reagent solutions are prepared as follows.

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 A* and *Solution B* (60:40)
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 solution of thiamine hydrochloride or thiamine mononitrate 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: 4.6-mm × 25-cm; packing L1
Flow rate: 1 mL/min. [NOTE—The flow rate may be adjusted to obtain a retention time of about 12 min for thiamine.]
Injection volume: 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 thiamine
Relative standard deviation: NMT 2.0% for the ratios of thiamine peak response to the internal standard peak response
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 sample taken:

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

R_U = peak response ratio of thiamine to methylbenzoate from the *Sample solution*
R_S = peak response ratio of thiamine to methylbenzoate 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)

If the active pharmaceutical or dietary supplement ingredient is thiamine mononitrate, calculate the percentage of thiamine mononitrate ($C_{12}H_{17}N_5O_4S$) in the portion of sample taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

R_U = peak response ratio of thiamine to methylbenzoate from the *Sample solution*
R_S = peak response ratio of thiamine to methylbenzoate from the *Standard solution*
C_S = concentration of USP Thiamine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of thiamine mononitrate in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of thiamine mononitrate, 327.36
M_{r2} = molecular weight of thiamine hydrochloride, 337.27

- **CHROMATOGRAPHIC METHODS, PROCEDURE 5**

This procedure can be used to determine thiamine in:

- *Thiamine Hydrochloride Injection*

- *Thiamine Hydrochloride Oral Solution*
- *Thiamine Mononitrate Oral Solution*

This is the procedure that involves the dissolution of the sample directly into the *Mobile phase*. The procedure is applicable for the determination of thiamine hydrochloride or thiamine mononitrate as an active ingredient in the formulations such as those used in *Thiamine Hydrochloride Injection*, *Thiamine Hydrochloride Oral Solution*, and *Thiamine Mononitrate Oral Solution*.

Unless specified in the individual monographs, the *Standard solution*, *Internal standard solution*, and *Sample solution* are prepared as follows.

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 or thiamine mononitrate in *Mobile phase* from an accurately measured volume of oral solution or injection
Sample solution: Dilute a mixture of equal volumes of the *Internal standard solution* and *Sample stock solution* with *Mobile phase* to obtain a concentration of thiamine hydrochloride or thiamine mononitrate of about 50 µg/mL.
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 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
Column efficiency: NLT 1500 theoretical plates for thiamine
Relative standard deviation: NMT 2.0% for the ratios of thiamine peak response to the methylparaben peak response
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 sample taken:

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

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

If the products contain thiamine mononitrate, calculate the percentage of the labeled amount of thiamine mononitrate ($C_{12}H_{17}N_5O_4S$) in the portion of sample taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

R_U = peak response ratio of thiamine to methylparaben from the *Sample solution*
R_S = peak response ratio of thiamine to methylparaben from the *Standard solution*
C_S = concentration of USP Thiamine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = concentration of thiamine mononitrate in the *Sample solution* (mg/mL)
M_{r1} = molecular weight of thiamine mononitrate, 327.36
M_{r2} = molecular weight of thiamine hydrochloride, 337.27

- **CHROMATOGRAPHIC METHODS, PROCEDURE 6**

This procedure can be used to determine thiamine in the following:

- *Thiamine Hydrochloride Tablets*

This is the procedure that involves the dissolution of the sample directly into water. The procedure is applicable to the performance (dissolution) test for *Thiamine Hydrochloride Tablets*.

Unless specified in the individual monographs, the *Standard solution* and *Sample solution* are prepared as follows.
Medium: Water, 900 mL
Apparatus 2: 50 rpm
Time: 45 min
Mobile phase: A mixture of methanol, glacial acetic acid, and water (27:1:73) containing 140 mg of sodium 1-hexanesulfonate per 100 mL
Standard solution: A known concentration of USP Thiamine Hydrochloride RS in *Medium* (water)
Sample solution: Filtered portion of the solution under test, suitably diluted with *Medium* (water) if necessary

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 280 nm
Column: 4.6-mm × 25-cm; packing L1
Flow rate: 1 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 3.0%
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 sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S \times D \times V/L) \times 100$$

r_U = peak response of thiamine from the *Sample solution*
r_S = peak response of thiamine from the *Standard solution*
C_S = concentration of USP Thiamine Hydrochloride RS in the *Standard solution* (mg/mL)
D = dilution factor for the *Sample solution*
V = volume of *Medium*, 900 mL
L = labeled amount of thiamine hydrochloride (mg/Tablet)

• **CHROMATOGRAPHIC METHODS, PROCEDURE 7**
This procedure can be used to determine thiamine in:
- *Oil- and Water-Soluble Vitamins Capsules*
- *Oil- and Water-Soluble Vitamins Oral Solution*
- *Oil- and Water-Soluble Vitamins Tablets*
- *Oil- and Water-Soluble Vitamins with Minerals Capsules*
- *Oil- and Water-Soluble Vitamins with Minerals Oral Solution*
- *Oil- and Water-Soluble Vitamins with Minerals Tablets*
- *Water-Soluble Vitamins Capsules*
- *Water-Soluble Vitamins Tablets*
- *Water-Soluble Vitamins with Minerals Capsules*
- *Water-Soluble Vitamins with Minerals Oral Solution*
- *Water-Soluble Vitamins with Minerals Tablets*

This is a newly added procedure as part of the USP monograph modernization efforts. The procedure uses hydrophilic interaction liquid chromatography (HILIC), and the sample preparation involves the extraction of thiamine from the formulation by the *Diluent*, heat, and mechanical shaking.
Unless specified in the individual monographs, the *Standard solution*, *Sample solutions*, and reagent solutions are prepared as follows.
Diluent: Methanol, glacial acetic acid, and water (50:1:49)
Solution A: 50 mM ammonium formate. Adjust with ammonium hydroxide to a pH of 9.0.
Solution B: Acetonitrile
Mobile phase: Gradient elution. See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	11	89
8	17	83
15	23	77
20	30	70
21	50	50
24	50	50
25	11	89
30	11	89

Standard solution: Transfer 20 mg of USP Thiamine Hydrochloride RS to a 200-mL volumetric flask, and add 160 mL of *Diluent*. Immerse the flask in a hot water bath maintained at 65°–70° for 10 min with regular shaking or using a vortex

mixer, until all of the solid materials are dissolved. Chill rapidly in a cold water bath for 10 min to room temperature, and dilute with *Diluent* to volume.

Sample solution for capsules: Weigh NLT 20 capsules in a tared weighing bottle. Open the capsules, without the loss of shell material, and transfer the contents to a 100-mL beaker. Remove any contents adhering to the empty shells by washing, if necessary, with several portions of ether. Discard the washings, and dry the capsule shells with the aid of a current of dry air until the odor of ether is no longer perceptible. Weigh the empty capsule shells in the tared weighing bottle, and calculate the average net weight per capsule. Transfer a portion of the capsule contents, equivalent to 2.5 mg of thiamine hydrochloride, to a 50-mL centrifuge tube. Add 25.0 mL of *Diluent*, and mix using a vortex mixer for 30 s to completely suspend the powder. Immerse the centrifuge tube in a hot water bath maintained at 68°, heat for 10 min, and mix on a vortex mixer for 30 s. Return the tube to the hot water bath, heat for another 10 min, and mix on a vortex mixer for 30 s. Filter a portion of the solution, cool to room temperature, and use the clear filtrate.

Sample solution for oral solution: Equivalent to 0.1 mg/mL of thiamine hydrochloride in *Diluent*, from an accurately measured volume of oral solution. In an appropriate volumetric flask, dissolve the sample with *Diluent* to about 80% of the total volume, immerse the flask in a water bath maintained at 68° for 10 min, chill rapidly in a cold water bath to room temperature, and dilute with *Diluent*. Mix well, and filter a portion of the solution; finally, use the clear filtrate.

Sample solution for tablets: Finely powder NLT 30 tablets. Transfer a portion of the powder, equivalent to 2.5 mg of thiamine hydrochloride, to a 50-mL centrifuge tube. Add 25.0 mL of *Diluent*, and mix using a vortex mixer for 30 s to completely suspend the powder. Immerse the centrifuge tube in a hot water bath maintained at 65°–70°, heat for 10 min, and mix on a vortex mixer for 30 s. Return the tube to the hot water bath, heat for another 10 min, and mix on a vortex mixer for 30 s. Filter a portion of the solution, cool to room temperature, and use the clear filtrate. [NOTE—Use the filtrate within 3 h of filtration.]

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: LC

Detector: UV 267 nm

Column: 4.6-mm × 15-cm; 3.5-µm packing L68

Column temperature: 40°

Flow rate: 1.2 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and appropriate *Sample solution*

For products containing thiamine hydrochloride, calculate the percentage of the labeled amount of thiamine hydrochloride ($C_{12}H_{17}ClN_4OS \cdot HCl$) in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of thiamine from the appropriate *Sample solution*
r_S = peak response of thiamine from the *Standard solution*
C_S = concentration of USP Thiamine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of thiamine in the appropriate *Sample solution* (mg/mL)

For products containing thiamine mononitrate, calculate the percentage of the labeled amount of thiamine mononitrate ($C_{12}H_{17}N_5O_4S$) in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of thiamine from the appropriate *Sample solution*
r_S = peak response of thiamine from the *Standard solution*
C_S = concentration of USP Thiamine Hydrochloride RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of thiamine mononitrate in the appropriate *Sample solution* (mg/mL)
M_{r1} = molecular weight of thiamine mononitrate, 327.36
M_{r2} = molecular weight of thiamine hydrochloride, 337.27

ADDITIONAL REQUIREMENTS

- **USP REFERENCE STANDARDS** ⟨11⟩

USP Thiamine Hydrochloride RS