⟨441⟩ NIACIN OR NIACINAMIDE ASSAY

ASSAY

Chemical Method

- **PROCEDURE**

The following photometric procedure involves the colorimetric reaction of niacin or niacinamide with cyanogen bromide in the presence of sulfanilic acid. It can be used for the determination of niacin or niacinamide in the monographs such as *Niacin Injection*, *Niacinamide Injection*, and *Niacinamide Tablets*.

[NOTE—Determine from the labeling if the vitamin in the assay specimen is niacin or niacinamide, and use the corresponding standard preparation (either *Standard niacin preparation* or *Standard niacinamide preparation*) as directed in the *Procedure*.]

Cyanogen bromide solution: Dissolve 5 g of cyanogen bromide in water to make 50 mL. [**CAUTION**—Prepare this solution under a hood, as cyanogen bromide volatilizes at room temperature, and the vapor is highly irritating and poisonous.]

Sulfanilic acid solution: To 2.5 g of sulfanilic acid add 15 mL of water and 3 mL of 6 N ammonium hydroxide. Mix, and add while stirring, more 6 N ammonium hydroxide, if necessary, until the acid dissolves. Adjust the solution with 3 N hydrochloric acid to a pH of about 4.5 using bromocresol green TS as an external indicator, and dilute with water to 25 mL.

Standard niacin stock solution: Transfer 25.0 mg of USP Niacin RS to a 500-mL volumetric flask, dissolve in alcohol solution (1 in 4), dilute with alcohol solution (1 in 4) to volume, and mix. Store in a refrigerator. Each mL of this solution contains 50 µg of USP Niacin RS.

Standard niacin preparation: Transfer 10.0 mL of *Standard niacin stock solution* to a 100-mL volumetric flask, dilute with water to volume, and mix. Each mL of this solution contains 5 µg of USP Niacin RS.

Standard niacinamide stock solution: Transfer 50.0 mg of USP Niacinamide RS to a 500-mL volumetric flask, dissolve in alcohol solution (1 in 4), dilute with alcohol solution (1 in 4) to volume, and mix. Store in a refrigerator. Each mL of this solution contains 100 µg of USP Niacinamide RS.

Standard niacinamide preparation: Transfer 10.0 mL of *Standard niacinamide stock solution* to a 100-mL volumetric flask, dilute with water to volume, and mix. Each mL of this solution contains 10 µg of USP Niacinamide RS.

Assay preparation: Prepare as directed in the individual monograph.

Procedure: Pipet into four marked tubes the quantities of the appropriate *Standard preparation*, *Assay preparation*, *Ammonia dilution*, and *Water* indicated in *Table 1*. Then add the other constituents, respectively, as listed in the table, according to the directions given herein.

Table 1. Reaction Mixtures for Niacin or Niacinamide Assay—Chemical Method

Constituent	Tube 1 (mL)	Tube 2 (mL)	Tube 3 (mL)	Tube 4 (mL)
Standard preparation	1.0	1.0	—	—
Assay preparation	—	—	1.0	1.0
Ammonia dilution (ammonium hydroxide, diluted to 1 in 50)	0.5	0.5	0.5	0.5
Water	6.5	1.5	6.5	1.5
Cyanogen bromide solution	—	5.0	—	5.0
Sulfanilic acid solution	2.0	2.0	2.0	2.0
Hydrochloric acid	1 drop	—	1 drop	—

To *Tube 1* add the *Sulfanilic acid solution*, shake well, and add the *Hydrochloric acid*. Mix, place in a suitable spectrophotometer, and adjust to zero absorbance at 450 nm. To *Tube 2* add the *Cyanogen bromide solution*, mix, and 30 s accurately timed after completion of the addition of the cyanogen bromide, add the *Sulfanilic acid solution*, with swirling. Close the tube, place it in the spectrophotometer, and after 2 min measure its absorbance at 450 nm against *Tube 1* as a blank, designating the absorbance as A_S. Repeat the procedure with *Tube 3* (as blank) and *Tube 4*, designating the absorbance of *Tube 4* as A_U. Calculate the quantity of niacin or niacinamide in the sample as directed in the individual monograph.

Chromatographic Methods

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

Use the appropriate USP Reference Standard for the niacin or niacinamide present in the formulation.

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.

- **PROCEDURE 1**

- This procedure can be used to determine niacin or niacinamide in:
  - Oil- and water-soluble vitamins with minerals tablets
- This procedure involves the extraction of analytes from the formulation by hot water or mechanical shaking
- Unless specified in the individual monographs, the *Standard solution*, *Sample solution*, 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 80 mg of either USP Niacin RS or USP Niacinamide 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 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 tablets, finely powder NLT 30 tablets. Transfer a portion of the powder, equivalent to 10 mg of niacinamide and 2.5 mg each of pyridoxine hydrochloride, riboflavin, and 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: 3.9-mm × 30-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*
Measure the peak areas for niacin or niacinamide.
Calculate the percentage of the labeled amount of niacin ($C_6H_5NO_2$) or niacinamide ($C_6H_6N_2O$) in the portion of the *Sample* taken:

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

r_U = peak response of niacin or niacinamide from the *Sample solution*
r_S = peak response of niacin or niacinamide from the *Standard solution*
C_S = concentration of USP Niacin RS or USP Niacinamide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of niacin or niacinamide in the *Sample solution* (mg/mL)

- **PROCEDURE 2**
  - This procedure can be used to determine niacin or niacinamide in:
    - Oil- and water-soluble vitamins with minerals tablets
  - This procedure involves the extraction of analytes from the formulation by the *Extraction solvent*, heat, and mechanical shaking
  - Unless specified in the individual monographs, the *Standard solution*, *Sample solution*, and reagent solutions are prepared as follows.

Solution A: Transfer 1 mL of glacial acetic acid and 2.5 g of edetate disodium to a 100-mL volumetric flask. Dissolve in and dilute with water to volume.
Extraction solvent: *Solution A* and methanol (3:1)
Mobile phase: 0.1 M sodium acetate solution (13.6 mg/mL of sodium acetate in water). Adjust with acetic acid to a pH of 5.4. [NOTE—A small amount of methanol (up to 1%) may be added to the *Mobile phase* to improve resolution.]
Standard stock solution: 1 mg/mL of USP Niacin RS or USP Niacinamide RS in *Extraction solvent*
Standard solution: Transfer 5.0 mL of *Standard stock solution* to a 25-mL volumetric flask, and dilute with *Extraction solvent* to volume.
Sample solution: [NOTE—This preparation is suitable for the determination of niacin or niacinamide, pyridoxine, and riboflavin, when present in the formulation.] For tablets, finely powder NLT 20 tablets. Transfer a portion of the powder, equivalent to 2 mg of riboflavin, to a 200-mL volumetric flask. If riboflavin is not present in the formulation, transfer a portion of the powder, equivalent to 2 mg of pyridoxine. If pyridoxine is not present in the formulation, transfer a portion of the powder, equivalent to 20 mg of niacin or niacinamide. Add 100.0 mL of *Extraction solvent*, and mix for 20 min, using a wrist-action shaker. Immerse the flask in a water bath maintained at 70°–75°, and heat for 20 min. Mix on a vortex mixer for 30 s, 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: 1 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 3.0% [NOTE—If necessary, flush the column with methanol between injections.]

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of niacin ($C_6H_5NO_2$) or niacinamide ($C_6H_6N_2O$) in the portion of the *Sample* taken:

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

r_U = peak response of niacin or niacinamide from the *Sample solution*
r_S = peak response of niacin or niacinamide from the *Standard solution*
C_S = concentration of USP Niacin RS or USP Niacinamide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of niacin or niacinamide in the *Sample solution* (mg/mL)

- **PROCEDURE 3**
  - This procedure can be used to determine niacin or niacinamide in:
    - Oil- and water-soluble vitamins with minerals tablets
  - This procedure involves the extraction of analytes from the formulation by mixtures of organic solvents, heat, and mechanical shaking
  - Unless specified in the individual monographs, the *Standard solution*, *Sample solution*, and reagent solutions are prepared as follows.

Reagent: 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: 1.5 mg/mL of USP Niacin RS or USP Niacinamide RS in the *Reagent*, with heating if necessary

Standard solution: 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, discarding the first few mL of the filtrate.

Sample solution: For tablets, weigh and finely powder NLT 20 tablets. Transfer a portion of the powder, equivalent to 7.5 mg of niacin or niacinamide, 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, discarding 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: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of niacin ($C_6H_5NO_2$) or niacinamide ($C_6H_6N_2O$) in the portion of the *Sample* taken:

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

r_U = peak response of niacin or niacinamide from the *Sample solution*
r_S = peak response of niacin or niacinamide from the *Standard solution*
C_S = concentration of USP Niacin RS or USP Niacinamide RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of niacin or niacinamide in the *Sample solution* (mg/mL)

- **PROCEDURE 4**
  - This procedure can be used to describe niacin as:
    - An active pharmaceutical ingredient
    - A dietary supplement ingredient
    - An ingredient in the niacin extended-release tablets
  - This procedure involves either dissolving the analyte in the *Diluent*, a mixture of methanol and water (82:18), or extracting the analyte from the formulation with *Diluent* and mechanical shaking
  - Unless specified in the individual monographs, the *System suitability solution*, *Standard solution A*, *Standard solution B*, *Sample solution A*, *Sample solution B*, and reagent solutions are prepared as follows.

Diluent: Methanol and water (82:18)

Mobile phase: Methanol and water (82:18), adjusted with glacial acetic acid to a pH of 3.15 ± 0.05

System suitability solution: 0.25 mg/mL of USP Niacin RS, 0.050 mg/mL of USP 6-Hydroxynicotinic Acid RS, and 0.10 mg/mL of pyridine in *Diluent*
Standard solution A: For active pharmaceutical ingredient or dietary supplement ingredient, use 0.25 mg/mL of USP Niacin RS in *Diluent*.
Standard solution B: For extended-release tablets, use 0.25 mg/mL of USP Niacin RS, 0.050 mg/mL of USP 6-Hydroxynicotinic Acid RS, and 0.0978 mg/mL of pyridine in *Diluent*.
Sample solution A: For active pharmaceutical ingredient or dietary supplement ingredient, use 0.25 mg/mL of Niacin in *Diluent*.
Sample solution B: For extended-release tablets, transfer a quantity of powder, equivalent to 50 mg of niacin from NLT 20 finely powdered tablets, to a suitable flask. Add *Diluent*, and stir for 2 h. Dilute with *Diluent* to a final concentration of 0.25 mg/mL of niacin.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 260 nm
Column: 4.6-mm × 15-cm; 5-µm packing L8
Flow rate: 1 mL/min
Injection volume: 25 µL
System suitability
Samples: *System suitability solution* and *Standard solution A* or *Standard solution B*
[NOTE—The relative retention times for pyridine, 6-hydroxynicotinic acid, and niacin are 0.14, 0.64, and 1.0, respectively, *System suitability solution*.]
Suitability requirements
Resolution: NLT 1.5 between pyridine and 6-hydroxynicotinic acid, and NLT 1.5 between 6-hydroxynicotinic acid and niacin, *System suitability solution*
Relative standard deviation: NMT 2.0% for the niacin peak from replicate injections, *Standard solution A* or *Standard solution B*
Analysis
Samples: *Standard solution A* and *Sample solution A* or *Standard solution B* and *Sample solution B*
Calculate the percentage of niacin ($C_6H_5NO_2$) in the portion of the *Sample* taken:

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

r_U = peak response of niacin from *Sample solution A* or *Sample solution B*
r_S = peak response of niacin from *Standard solution A* or *Standard solution B*
C_S = concentration of USP Niacin RS in *Standard solution A* or *Standard solution B* (mg/mL)
C_U = concentration of Niacin in *Sample solution A* or nominal concentration of niacin in *Sample solution B* (mg/mL)

- **PROCEDURE 5**
  - This procedure can be used to determine niacinamide as:
    - An active pharmaceutical ingredient
    - A dietary supplement ingredient
  - This procedure involves dissolving the niacinamide as the active pharmaceutical ingredient or a dietary supplement ingredient in the *Mobile phase*
  - Unless specified in the individual monographs, the *System suitability solution*, *Standard solution*, *Niacin solution*, *Sample solution*, and reagent solutions are prepared as follows.

Mobile phase: Methanol and 0.005 M sodium 1-heptanesulfonate (30:70)
Standard solution: Transfer 50 mg of USP Niacinamide RS to a 100-mL volumetric flask. Add 3 mL of water to dissolve, and dilute with *Mobile phase* to volume. Dilute with *Mobile phase* to 0.04 mg/mL.
Niacin solution: Prepare as directed in the *Standard solution*, using USP Niacin RS instead of USP Niacinamide RS.
System suitability solution: Mix equal volumes of the *Standard solution* and *Niacin solution*.
Sample solution: Prepare as directed in the *Standard solution*, using Niacinamide instead of USP Niacinamide RS.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 2 mL/min
Injection volume: 20 µL
System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 3.0 between niacin and niacinamide, *System suitability solution*
Relative standard deviation: NMT 2.0% for the niacinamide peak from replicate injections, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of niacinamide ($C_6H_6N_2O$) in the portion of the *Sample* taken:

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

r_U = peak response of niacinamide from the *Sample solution*
r_S = peak response of niacinamide from the *Standard solution*
C_S = concentration of USP Niacinamide RS in the *Standard solution* (mg/mL)
C_U = concentration of Niacinamide in the *Sample solution* (mg/mL)

- **PROCEDURE 6**
  - This procedure can be used to determine niacin as:
    - An active ingredient in niacin tablets
  - This procedure involves the extraction of the niacin from the formulation by water, heat, and mechanical shaking
  - Unless specified in the individual monographs, the *Standard solution*, *Sample solution*, and reagent solutions are prepared as follows.

Solution A: 5-mM solution of sodium 1-hexanesulfonate in water
Mobile phase: Methanol, acetonitrile, glacial acetic acid, and *Solution A* (14:7:1:78)
Standard solution: 0.050 mg/mL of USP Niacin RS in water. Dissolve with the aid of heat in a steam bath.
Sample solution: For tablets, transfer an equivalent to 500 mg of niacin from NLT 20 finely powdered tablets to a suitable flask. Add 50 mL of water, and heat on a steam bath for 30 min. Sonicate for 2 min, shake by mechanical means for 15 min, and cool to room temperature. Dilute with water to 0.050 mg/mL, and filter.
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 262 nm
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 1.3 mL/min
Injection volume: 20 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Column efficiency: NLT 1000 theoretical plates for the niacin peak
Tailing factor: NMT 2.0 for the niacin peak
Relative standard deviation: NMT 2.0% for the niacin peak from replicate injections, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of niacin ($C_6H_5NO_2$) in the portion of the *Sample* taken:

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

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

ADDITIONAL REQUIREMENTS

- **USP REFERENCE STANDARDS** ⟨11⟩
  USP 6-Hydroxynicotinic Acid RS
  (see *Procedure 4*)
  USP Niacin RS
  USP Niacinamide RS

[NOTE—The previously dried Reference Standards may be stored in a desiccator over silica gel, protected from light.]