solution having a known concentration of USP Pyridostigmine Bromide RS in the same medium.

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_9H_{13}BrN_2O_2$ is dissolved in 60 minutes.

Uniformity of dosage units ⟨905⟩: meet the requirements.

Assay—

Buffer solution—Mix 11.2 g of phosphoric acid with 500 mL of water, and adjust with a 50% solution of sodium hydroxide in water to a pH of 7.0. Dilute with water to 1000 mL.

Mobile phase—Dissolve 1 g of sodium 1-heptanesulfonate in 500 mL of water in a 1000-mL volumetric flask, and add 5.0 mL of triethylamine and 100 mL of acetonitrile. Dilute with water to volume, and mix. Adjust with phosphoric acid to a pH of 3.0. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—Dissolve an accurately weighed quantity of USP Pyridostigmine Bromide RS in *Buffer solution*, and dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of 0.25 mg per mL.

Assay preparation—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 50 mg of pyridostigmine bromide, to a 200-mL volumetric flask, add 100 mL of *Buffer solution*, and shake for 30 minutes. Dilute with *Buffer solution* to volume, mix, and centrifuge. Use a portion of the supernatant as the *Assay preparation*.

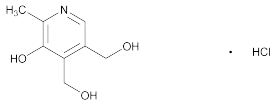
Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 270-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the tailing factor is not more than 1.5, and the relative standard deviation for replicate injections is not more than 1.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of $C_9H_{13}BrN_2O_2$ in the portion of Tablets taken by the formula:

$$200C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Pyridostigmine Bromide RS in the *Standard preparation*, and r_U and r_S are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

Pyridoxine Hydrochloride



$C_8H_{11}NO_3 \cdot HCl$ 205.64

3,4-Pyridinedimethanol, 5-hydroxy-6-methyl-, hydrochloride; Pyridoxol hydrochloride [58-56-0].

DEFINITION

Pyridoxine Hydrochloride contains NLT 98.0% and NMT 102.0% of pyridoxine hydrochloride ($C_8H_{11}NO_3 \cdot HCl$), calculated on the dried basis.

IDENTIFICATION

- **A. Infrared Absorption** ⟨197M⟩
- **B. Identification Tests—General,** *Chloride* ⟨191⟩**:** Meets the requirements

ASSAY

- **Procedure**

Mobile phase: Mix 10 mL of glacial acetic acid, 0.6 g of sodium 1-hexanesulfonate, and 700 mL of water in a 1000-mL volumetric flask. Adjust with glacial acetic acid or 1 N sodium hydroxide to a pH of 3.0. Add 235 mL of methanol, and dilute with water to volume.

Internal standard solution: 5 mg/mL of *p*-hydroxybenzoic acid in *Mobile phase*

Standard solution: Prepare a 0.5-mg/mL solution of USP Pyridoxine Hydrochloride RS in *Mobile phase*. Transfer 10.0 mL of this solution and 1.0 mL of *Internal standard solution* to a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.

Sample solution: Prepare a 0.5-mg/mL solution of Pyridoxine Hydrochloride in *Mobile phase*. Transfer 10.0 mL of this solution and 1.0 mL of *Internal standard solution* to a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.

Chromatographic system

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

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 1.5 mL/min

Injection size: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for pyridoxine and *p*-hydroxybenzoic acid are about 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.5 between pyridoxine and *p*-hydroxybenzoic acid

Relative standard deviation: NMT 3.0% for the ratios of the pyridoxine peak area response to the internal standard peak area response

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of pyridoxine hydrochloride ($C_8H_{11}NO_3 \cdot HCl$) in the portion of Pyridoxine Hydrochloride taken:

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

R_U = internal standard ratio (peak response of pyridoxine/peak response of the internal standard) from the *Sample solution*

R_S = internal standard ratio (peak response of pyridoxine/peak response of the internal standard) from the *Standard solution*

C_S = concentration of USP Pyridoxine Hydrochloride RS in the *Standard solution* (mg/mL)

C_U = concentration of Pyridoxine Hydrochloride in the *Sample solution* (mg/mL)

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

IMPURITIES

- **Residue on Ignition** ⟨281⟩**:** NMT 0.1%
- **Heavy Metals,** *Method II* ⟨231⟩**:** NMT 30 ppm

SPECIFIC TESTS

- **Content of Chloride**

Sample: 500 mg of Pyridoxine Hydrochloride

Blank: 50 mL of methanol

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Direct titration

Titrant: 0.1 N silver nitrate VS

Endpoint detection: Visual

Analysis: Dissolve the *Sample* in 50 mL of methanol. Add 5 mL of glacial acetic acid and 2–3 drops of eosin Y TS. Titrate with the *Titrant*. Perform a *Blank* determination.

Calculate the percentage of chloride (Cl) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)
V_B = *Titrant* volume consumed by the *Blank* (mL)
N = actual normalilty of the *Titrant* (mEq/mL)
F = equivalency factor, 35.45 mg/mEq
W = *Sample* weight (mg)

Acceptance criteria: 16.9%–17.6% on the dried basis

- **LOSS ON DRYING ⟨731⟩:** Dry a sample in vacuum over silica gel for 4 h: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS ⟨11⟩**
  USP Pyridoxine Hydrochloride RS

Pyridoxine Hydrochloride Injection

» Pyridoxine Hydrochloride Injection is a sterile solution of Pyridoxine Hydrochloride in Water for Injection. It contains not less than 95.0 percent and not more than 115.0 percent of the labeled amount of Pyridoxine Hydrochloride ($C_8H_{11}NO_3 \cdot HCl$).

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

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

Identification—Evaporate a volume of Injection, equivalent to about 50 mg of pyridoxine hydrochloride, on a steam bath to dryness. Add 5 mL of dehydrated alcohol, and again evaporate to dryness. Dry the residue at 105° for 3 hours: the residue so obtained responds to *Identification* tests *A* and *B* under *Pyridoxine Hydrochloride*.

Bacterial endotoxins ⟨85⟩**—**It contains not more than 0.4 USP Endotoxin Unit per mg of pyridoxine hydrochloride.

pH ⟨791⟩**:** between 2.0 and 3.8.

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

Assay—

Ammonium chloride–ammonium hydroxide buffer—Dissolve 16 g of ammonium chloride in 70 mL of water, add 16 mL of ammonium hydroxide, dilute with water to 100 mL, mix, and filter.

Chlorimide solution—Dissolve 40 mg of 2,6-dichloroquinonechlorimide in 100 mL of isopropyl alcohol. Store the solution in a refrigerator, and use within 1 month. Do not use the solution if it has become pink.

Standard stock solution—Dissolve a suitable quantity of USP Pyridoxine Hydrochloride RS, accurately weighed, in 0.1 N hydrochloric acid, quantitatively dilute with the same solvent to obtain a solution having a known concentration of about 0.1 mg per mL, and mix. Keep the solution in an amber bottle, in a cool place.

Standard preparation—In a 100-mL volumetric flask dilute 10.0 mL of the *Standard stock solution* with water to volume, and mix. Prepare this solution daily as needed.

Assay preparation—Dilute an accurately measured volume of Injection, equivalent to about 100 mg of pyridoxine hydrochloride, quantitatively and stepwise with water to a concentration of about 10 µg of pyridoxine hydrochloride per mL.

Procedure—

(a) Pipet 5 mL of the clear *Assay preparation* into a flask, add 25.0 mL of isopropyl alcohol, and mix. Pipet 5 mL of the isopropyl alcohol dilution into a glass-stoppered, 25-mL graduated cylinder or test tube; and add in succession, mixing after each addition, 1.0 mL of *Ammonium chloride–ammonium hydroxide buffer,* 1.0 mL of sodium acetate solution (1 in 5), and 1.0 mL of water. Cool to about 25°, then add 1.0 mL of *Chlorimide solution,* and shake vigorously for 10 seconds, accurately timed. Ninety seconds, accurately timed, after the addition of the *Chlorimide solution,* determine the absorbance at the wavelength of maximum absorbance at about 650 nm, with a suitable spectrophotometer, using water as the blank. [NOTE—Make the reading promptly to avoid errors due to fading of the color.] Designate the absorbance as A_U.

(b) Repeat procedure *(a),* but substitute 1.0 mL of boric acid solution (1 in 20) for the 1.0 mL of water. Designate the absorbance as A_U'.

(c) Repeat procedure *(a),* but substitute 5.0 mL of the *Standard preparation* for the 5.0 mL of the *Assay preparation.* Designate the absorbance as A_S.

(d) Repeat procedure *(c),* but substitute 1.0 mL of boric acid solution (1 in 20) for the 1.0 mL of water. Designate the absorbance as A_S'.

Calculate the quantity, in mg, of pyridoxine hydrochloride ($C_8H_{11}NO_3 \cdot HCl$) in each mL of the Injection taken by the formula:

$$10(C/V)(A_U - A_U') / (A_S - A_S')$$

in which C is the concentration, in µg per mL, of USP Pyridoxine Hydrochloride RS in the *Standard preparation; V* is the volume, in mL, of Injection taken; and the other terms are as defined above.

Pyridoxine Hydrochloride Tablets

DEFINITION

Pyridoxine Hydrochloride Tablets contain NLT 95.0% and NMT 115.0% of the labeled amount of pyridoxine hydrochloride ($C_8H_{11}NO_3 \cdot HCl$).

IDENTIFICATION

- **A. REACTION WITH FERRIC ION**
  Sample: Equivalent to 100 mg of pyridoxine hydrochloride from a quantity of powdered Tablets
  Analysis: Add 5 mL of water to the *Sample*. Shake the mixture, filter into a test tube, and add 2 or 3 drops of ferric chloride TS.
  Acceptance criteria: An orange to deep red color is produced.

ASSAY

- **PROCEDURE**
  Buffer: Dissolve 16 g of ammonium chloride in 70 mL of water, add 16 mL of ammonium hydroxide, dilute with water to 100 mL, and filter.
  Color reagent: 0.4 mg/mL of 2,6-dichloroquinone chlorimide in isopropyl alcohol. [NOTE—Store the solution in a refrigerator, and use within one month. Do not use the solution if it has become pink.]
  Standard stock solution: 0.1 mg/mL of USP Pyridoxine Hydrochloride RS in 0.1 N hydrochloric acid. [NOTE—Keep the solution in an amber bottle, in a cool place.]
  Standard solution: 10 µg/mL of USP Pyridoxine Hydrochloride RS from the *Standard stock solution* diluted with water. [NOTE—Prepare this solution daily as needed.]
  Sample solution: Weigh and finely powder NLT 20 Tablets. Transfer, with the aid of water, a portion of the powdered Tablets to a conical flask. Add 0.5 mL hydrochloric acid per each mg of the nominal amount of pyridoxine hydrochloride taken, then dilute with water to about 0.04 mg/mL, and heat on a steam bath until disintegration is complete. Cool, dilute with water to 10 µg/mL, and centrifuge a portion of the mixture.