• **Loss on Drying** ⟨731⟩
Analysis: Dry a sample at 105° for 3 h.
Acceptance criteria: NMT 1.0%

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in a tight container, and store at controlled room temperature.
• **USP Reference Standards** ⟨11⟩
USP Acetylcholine Chloride RS

Acetylcholine Chloride for Ophthalmic Solution

DEFINITION
Acetylcholine Chloride for Ophthalmic Solution is a sterile mixture of Acetylcholine Chloride with Mannitol or other suitable diluent, prepared by freeze-drying. Each container contains NLT 90.0% and NMT 115.0% of the labeled amount of acetylcholine chloride ($C_7H_{16}ClNO_2$).

IDENTIFICATION
• **A.**
Standard solution: 10 mg/mL of USP Acetylcholine Chloride RS
Sample solution: 10 mg/mL of acetylcholine chloride
Chromatographic system
(See *Chromatography* ⟨621⟩, *Thin-Layer Chromatography*.)
Adsorbent: 0.25-mm layer of aluminum oxide
Application volume: 2 µL
Developing solvent system: Mix butyl alcohol, glacial acetic acid, and water (40:10:50). Allow the layers to separate completely. Use the upper layer.
Spray reagent A: Freshly prepared solution of 5 mg/mL of cobaltous chloride prepared as follows. Dissolve the required amount of cobaltous chloride in 50% of the final volume of water, and dilute with 50% alcohol. [NOTE—This solution is freshly prepared.]
Spray reagent B: Freshly prepared potassium ferrocyanide solution prepared as follows. Dissolve 1.0 g of potassium ferrocyanide in 100 mL of water, and dilute with 50 mL of alcohol.
Analysis
Samples: *Standard solution* and *Sample solution*
Develop the chromatogram, without delay, in a vapor-saturated chamber containing the *Developing solvent system*. Allow the solvent front to move about 10 cm beyond the initial spotting line. Dry the plate with a current of warm air. Immediately spray the plate with *Spray reagent A*. Dry the plate as before, and immediately spray the plate with *Spray reagent B*. Dry the plate with a current of warm air.
Acceptance criteria: The R_F value and color of the principal spot from the *Sample solution* correspond to those from the *Standard solution*.
• **B.**
Sample solution: Nominally 10 mg/mL of acetylcholine chloride
Analysis: To 2 mL of *Sample solution* add 1 drop of nitric acid and 1 mL of silver nitrate TS.
Acceptance criteria: A curdy, white precipitate, soluble in an excess of 6 N ammonium hydroxide, is formed.

ASSAY
• **Procedure**
Mobile phase: Add 1.03 g of sodium 1-heptanesulfonate to a mixture of 900 mL of water and 10 mL of methanol. Adjust with ammonium hydroxide or glacial acetic acid to a pH of 4.0. Add 50 mL of acetonitrile. Dilute with water to 1 L. [NOTE—A slight variation of the amount of acetonitrile may be required to improve resolution or adjust retention time.]
Standard solution: A quantity of USP Acetylcholine Chloride RS in *Mobile phase*, to obtain a solution having a known concentration equal to that of the acetylcholine chloride in the *Sample solution*
Sample solution: Transfer the contents of 1 container of Acetylcholine Chloride for Ophthalmic Solution to a 10-mL volumetric flask with the aid of *Mobile phase*, and dilute with *Mobile phase* to volume.
System suitability solution: 0.2% each of acetylcholine chloride and choline chloride
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: Refractive index
Column: 3.9-mm × 30-cm; packing L1
Flow rate: 2 mL/min
Injection volume: 50 µL
System suitability
Samples: *Standard solution* and *System suitability solution*
Suitability requirements
Resolution: NLT 2.0 between acetylcholine chloride and choline chloride, *System suitability solution*
Relative standard deviation: NMT 3.5%, *Standard solution*
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of acetylcholine chloride ($C_7H_{16}ClNO_2$) in the container taken:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

r_U = peak response from the *Sample solution*
r_S = peak response from the *Standard solution*
C_S = concentration of USP Acetylcholine Chloride RS in the *Standard solution* (mg/mL)
V = volume of the *Sample solution*, 10 mL
L = label claim (mg/vial)
Acceptance criteria: 90.0%–115.0%

PERFORMANCE TESTS
• **Uniformity of Dosage Units** ⟨905⟩: Meets the requirements

SPECIFIC TESTS
• **Sterility Tests** ⟨71⟩: Meets the requirements
• **Acidity**
Analysis: Dissolve an amount of Acetylcholine Chloride for Ophthalmic Solution equivalent to 100 mg of acetylcholine chloride in 10 mL of recently boiled water. Add at once 1 drop of bromothymol blue TS.
Acceptance criteria: NMT 0.50 mL of 0.010 N sodium hydroxide is required to produce a color change.
• **Water Determination,** *Method I* ⟨921⟩
Analysis: Perform the titration in the original container, observing precautions against contact with water or moist atmosphere. Adjust the concentration of the reagent so that the titration volume approaches but does not exceed the capacity of the container. Titrate to an amber color that persists for 15 s after mixing.
Acceptance criteria: NMT 1.0%
• **Constituted Solution:** At the time of use, it meets the requirements for *Injections and Implanted Drug Products* ⟨1⟩, *Specific Tests, Completeness and clarity of solutions*.

ADDITIONAL REQUIREMENTS
• **Packaging and Storage:** Preserve in tight containers as described in *Packaging and Storage Requirements* ⟨659⟩, *Injection Packaging, Packaging for constitution*, and store at controlled room temperature.
• **USP Reference Standards** ⟨11⟩
USP Acetylcholine Chloride RS