the quantity, in mg, of betaxolol hydrochloride \((C_{18}H_{29}NO_3 \cdot HC1)\) in the Tablet taken by the formula:

\[(CV)(A_v / A_t)\]

in which \(C\) is the concentration, in mg per mL, of USP Betaxolol Hydrochloride RS in the Standard solution; \(V\) is the volume, in mL, of 0.1 N hydrochloric acid used to dissolve the T ablet; and \(A_v\) and \(A_t\) are the absorbances of the solution from the Tablet and the Standard solution, respectively.

**Assay—**

**Mobile phase—**Prepare a filtered mixture of 0.025 M pH 6.0 ammonium phosphate buffer, acetonitrile, and methanol (35:35:30). Mix, and degas under vacuum while stirring. Make adjustments if necessary (see System Suitability under Chromatography (621)).

**Diluent—**Prepare a mixture of acetonitrile and water (1:1).

**Standard preparation—**Dissolve an accurately weighed quantity of USP Betaxolol Hydrochloride RS in Diluent to obtain a solution having a known concentration of about 2 mg per mL.

**Assay preparation—**Dissolve not fewer than 20 T ablets in an appropriate accurately measured volume of Diluent so that the final concentration, based on the labeled amount per T ablet, is about 2 mg of betaxolol hydrochloride per mL. Sonicate until the Tablets are disintegrated. Cool to room temperature, dilute with Diluent to volume, mix, and filter. Use the clear filtrate as the Assay preparation.

**Chromatographic system (see Chromatography (621))—**The liquid chromatograph is equipped with a 273-nm detector and a 4.6-mm x 15-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the Standard preparation, record the chromatogram, and measure the peak response as directed for Procedure: the tailing factor is not more than 1.0% and the resolution, \(R\), between betaxolol hydrochloride and betaxanechol is not less than 1.0 for betaxolol; the tailing factor is not more than 2.0%.

**Procedure—**Separately inject equal volumes (about 10 \(\mu\)L) of the Standard preparation and the Assay preparation, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of betaxolol hydrochloride \((C_{18}H_{29}NO_3 \cdot HC1)\) in each Tablet taken by the formula:

\[(CV/N)(r_v / r_s)\]

in which \(C\) is the concentration, in mg per mL, of USP Betaxolol Hydrochloride RS in the Standard preparation; \(V\) is the volume of Diluent used to dissolve the T ablets; \(N\) is the number of T ablets taken; and \(r_v\) and \(r_s\) are the betaxolol peak responses obtained from the Assay preparation and the Standard preparation, respectively.

**Bethanechol Chloride**

\[\text{C}_7\text{H}_7\text{ClN}_2\text{O}_2; \text{196.67}\]

1-Propanaminium, 2-(aminocarbonyloxy)-N,N,N-trimethyl-, chloride, (D)- (2-Hydroxypropyl)trimethylammonium chloride carbamate [590-63-6].

» Bethanechol Chloride contains not less than 98.0 percent and not more than 101.5 percent of \(\text{C}_7\text{H}_7\text{ClN}_2\text{O}_2\), calculated on the dried basis.

**Packaging and storage—**Preserve in tight containers.

**USP Reference standards (11)—**USP Bethanechol Chloride RS

**Identification—**

A: Infrared Absorption (197M).

B: Dissolve about 50 mg in 2 mL of water, add 0.1 mL of cobaltous chloride solution (1 in 1000), then add 0.1 mL of potassium ferrocyanide TS: an emerald-green color is produced, and almost entirely fades in 5 to 10 minutes (distinction from choline chloride, which gives the same reaction but the color does not fade).

C: To 1 mL of a solution (1 in 100) add 0.1 mL of iodine TS: a brown precipitate is formed, and it rapidly changes to a dark olive-green color.

D: A solution of it responds to the tests for Chloride (191).

pH (791): between 5.5 and 6.5, in a solution (1 in 100).

**Loss on drying (731):** Dry at 105 \(^\circ\)C for 2 hours; it loses not more than 1.0% of its weight.

**Residue on ignition (281):** not more than 0.1%.

**Heavy metals, Method I (231):** Dissolve 667 mg in 10 mL of water, add 2 mL of 1 N acetic acid, and dilute with water to 25 mL: the limit is 0.003%.

**Chloride content—**Dissolve about 400 mg, previously dried and accurately weighed, in 30 mL of water. Add 40.0 mL of 0.1 N silver nitrate VS, then add 3 mL of nitric acid and 5 mL of nitrobenzene, shake for a few minutes, add 2 mL of ferric ammonium sulfate TS, and titrate the excess silver nitrate with 0.1 N ammonium thiocyanate VS. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of Cl: the content of Cl is between 17.7% and 18.3%.

**Related compounds—**

Buffer solution—Transfer about 0.48 g of methanesulfonic acid to a 1000-mL volumetric flask. Dissolve in and dilute with water to volume.

Mobile phase—Prepare a filtered and degassed mixture of Buffer solution and acetonitrile (95:5). Make adjustments if necessary (see System Suitability under Chromatography (621)).

Standard solution—Dissolve an accurately weighed quantity of USP Bethanechol Chloride RS in Mobile phase, and dilute quantitatively, and stepwise if necessary, with Mobile phase to obtain a solution having a known concentration of about 1 \(\mu\)g of USP Bethanechol Chloride RS in 1 mL.

Test solution—Transfer about 25 mg of Bethanechol Chloride, accurately weighed, to a 250-mL volumetric flask. Dissolve in and dilute with Mobile phase to volume, and mix.

System suitability solution—Transfer about 25 mg of Bethanechol Chloride, accurately weighed, to a 250-mL volumetric flask. Add about 10 mL of 0.1 N sodium hydroxide, and allow to stand for about 15 minutes. Add 10 mL of 0.1 N hydrochloric acid. Dissolve in and dilute with Mobile phase to volume, and mix.

Chromatographic system (see Chromatography (621))—The liquid chromatograph is equipped with a conductivity detector and a 3.9 - x 150-mm column containing packing L55. The flow rate is about 1.0 mL per minute. The detector and column temperatures are maintained at 35 \(^\circ\)C and 30 \(^\circ\)C, respectively. Chromatograph the System suitability solution, and record the peak responses as directed for Procedure: the relative retention time is about 0.9 for 2-hydroxypropyltrimethyl ammonium chloride and 1.0 for betanechol; the resolution, \(R\), between 2-hydroxypropyltrimethyl ammonium chloride and betanechol is not less than 0.8. Chromatograph the Standard solution, and record the peak responses as directed for Procedure: the relative standard deviation for replicate injections is not more than 10.0% for betanechol chloride.

Procedure—Separately inject equal volumes (about 50 \(\mu\)L of the Mobile phase, the Standard solution, and the Test solution into the chromatograph, record the chromatograms, and measure the peak responses for all the peaks. Calculate the per-
age of each impurity in the portion of Bethanechol Chloride taken by the formula:

\[ 25,000C(f/W)(r_i / r) \]

in which \( C_i \) is the concentration, in mg per mL, of USP Bethanechol Chloride RS in the Standard solution; \( f \) is the relative response factor and is equal to 0.79 for 2-hydroxypropyltrimethyl ammonium chloride and 1.0 for any other impurity; \( W \) is the weight, in mg, of Bethanechol Chloride taken to prepare the Test solution; \( r_i \) is the peak response for any impurity in the Test solution; and \( r \) is the peak response of USP Bethanechol Chloride RS in the Standard solution. Not more than 1.0% of 2-hydroxypropyltrimethyl ammonium is found; not more than 0.1% of any other impurity is found; and the sum of all the impurities is not more than 1.5%.

**Assay—**

Buffer solution—Transfer about 29 mg of edetic acid to a 1000-mL volumetric flask, and dissolve in 500 mL of water. Add 300 \( \mu \)L of nitric acid to the volumetric flask, and dilute with water to volume. Pass through a 0.45- \( \mu \)m nylon membrane filter.

Mobile phase—Prepare a filtered and degassed mixture of Buffer solution and acetonitrile (95:5). Make adjustments if necessary (see System Suitability under Chromatography (621)).

System suitability solution—Transfer about 25 mg of Bethanechol Chloride, accurately weighed, to a 250-mL volumetric flask. Add 10 mL of 0.1 N sodium hydroxide, and allow to stand for about 15 minutes. Add 10 mL of 0.1 N hydrochloric acid. Dissolve in and dilute with Mobile phase to volume, and mix.

Standard preparation—Dissolve an accurately weighed quantity of USP Bethanechol Chloride RS in Mobile phase, and dilute quantitatively, and stepwise if necessary, with Mobile phase to obtain a solution having a known concentration of about 0.1 mg of USP Bethanechol Chloride RS per mL.

Assay preparation—Transfer about 25 mg of Bethanechol Chloride, accurately weighed, to a 250-mL volumetric flask. Dissolve in and dilute with Mobile phase to volume, and mix.

Chromatographic system (see Chromatography (621))—The liquid chromatograph is equipped with a conductivity detector and a 3.9- \( \times \) 150-mm column containing packing L5S. The flow rate is about 1.0 mL per minute. The detector and column temperatures are maintained at 35° and 30°, respectively. Chromatograph the System suitability solution, and record the peak responses as directed for Procedure: the relative retention times are about 0.9 for 2-hydroxypropyltrimethyl ammonium chloride and 1.0 for betahencol; and the resolution, \( R \), between 2-hydroxypropyltrimethyl ammonium chloride and betahencol is not less than 0.8. 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 3.0%.

Procedure—Separately inject equal volumes (about 25 \( \mu \)L) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of \( C_7H_{17}ClN_2O_2 \) in the portion of Bethanechol Chloride taken by the formula:

\[ 250C(r_0 / r) \]

in which \( C \) is the concentration, in mg per mL, of USP Bethanechol Chloride RS in the Standard preparation; and \( r_0 \) and \( r \) are the betahencol chloride peak responses obtained from the Assay preparation and the Standard preparation, respectively.

**Bethanechol Chloride Injection—**

Bethanechol Chloride Injection is a sterile solution of Bethanechol Chloride in W ater for Injec-

**Packaging and storage—**Preserve in single-dose containers, preferably of Type I glass.

**USP Reference standards** (11)—

USP Bethanechol Chloride RS
USP Endotoxin RS

**Identification—**It responds to Identification tests B, C, and D under Bethanechol Chloride.

**Bacterial endotoxins** (85)—It contains not more than 25.0 USP Endotoxin Units per mg of betahencol chloride.

**Limit of 2-hydroxypropyltrimethyl ammonium chloride—**

Diluent, Mobile phase, System suitability solution, and Chromatographic system—Prepare as directed in the Assay.

2-Hydroxypropyltrimethyl ammonium chloride solution—Transfer 50.0 mg of betahencol chloride into a 50-mL volumetric flask. Add about 40 mL of 0.1 N sodium hydroxide, and sonicate until fully dissolved. Dilute with 0.1 N sodium hydroxide to volume, and allow to stand for five days to allow adequate time for conversion from betahencol to 2-hydroxypropyltrimethyl ammonium chloride. Chromatograph as directed for Procedure to verify the presence and location of the peak for 2-hydroxypropyltrimethyl ammonium chloride.

Standard solution—Use the Standard preparation, prepared as directed in the Assay.

Test solution—Use the Assay preparation.

Procedure—Separately inject equal volumes (about 50 \( \mu \)L) of the 2-Hydroxypropyltrimethyl ammonium chloride solution, the Standard solution, and the Test solution into the chromatograph, record the chromatograms, and measure the responses for the betahencol and 2-hydroxypropyltrimethyl ammonium chloride peaks. Calculate the percentage of 2-hydroxypropyltrimethyl ammonium chloride in each mL of the Injection taken by the formula:

\[ 100(C_i / C)(r_i / r) \]

in which \( C_i \) is the concentration, in mg per mL, of USP Bethanechol Chloride RS in the Standard solution; \( C \) is the concentration, in mg per mL, of betahencol chloride in the Test solution; \( r_i \) is the peak response for 2-hydroxypropyltrimethyl ammonium chloride obtained from the Test solution; and \( r \) is the peak response for betahencol obtained from the Standard solution. Not more than 4.0% is found.

**Other requirements—**It meets the requirements under Injections (1).

**Assay—**

Diluent—Transfer 10 mg of calcium chloride and 10 mg of magnesium chloride to a 100-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

Mobile phase—Prepare a filtered and degassed solution of 20 mM methanesulfonic acid. Make adjustments if necessary (see System Suitability under Chromatography (621)).

System suitability solution—Transfer 25 mg of USP Bethanechol Chloride RS, accurately weighed, to a 25-mL volumetric flask, and add 15 mL of water, 2.0 mL of the Diluent, and 0.5 mL of 0.1 N sodium hydroxide. Dilute with water to volume, and mix.

Standard preparation—Dissolve an accurately weighed quantity of USP Bethanechol Chloride RS in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 1.0 mg per mL.

Assay preparation—Dilute an accurately measured volume of Injection, if necessary, with water to obtain a solution having a concentration of about 1.0 mg per mL.