Procedure—Inject a volume (about 10 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak areas. Calculate the per centage of total impurities in the portion of Bisoprolol Fumarate taken by the formula:

$$100(r_i / r_s)$$

in which r_i is the sum of areas for all the peaks, excluding the fumaric acid and bisoprolol peaks; and r_s is the sum of the areas of all the peaks in the chromatogram: not more than 0.5% of total impurities is found.

Content of fumaric acid—Transfer about 500 mg of Bisoprolol Fumarate, accurately weighed, to a beaker, and dissolve in 70 mL of dehydrated alcohol. Add 8.0 mL of 0.1 N tetrabutylammonium hydroxide VS, and stir for 2 minutes. T itrate with 0.1 N tetrabutylammonium hydroxide VS, determining the endpoint potentiometrically, using a glass-calomel electrode system. Per form a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩). Each mL of 0.1 N tetrabutylammonium hydroxide is equivalent to 5.804 mg of fumaric acid: not less than 14.8% and not more than 15.4% of fumaric acid is found, calculated on the anhydrous basis.

Assay—

Diluent—Prepare a mixture of water and acetonitrile (65:35).

Mobile phase—To a 1-L portion of *Diluent* add 5 mL of heptafluorobutyric acid, 5 mL of diethylamine, and 2.5 mL of formic acid. Mix, filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

System suitability solution—Prepare a solution in *Diluent* containing about 0.5 mg of propranolol hydrochloride and 1 mg of Bisoprolol Fumarate per mL.

Standard preparation—Quantitatively dissolve an accurately weighed quantity of USP Bisoprolol Fumarate RS in *Diluent* to obtain a solution having a known concentration of about 1 mg per mL.

Assay preparation—Transfer about 50 mg of Bisoprolol Fumarate, accurately weighed, to a 50-mL volumetric flask. Dissolve in and dilute with *Diluent* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 273-nm detector and a 4.6-mm × 12.5-cm column that contains packing L7. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution,* and record the peak areas as directed for *Procedure:* the resolution, *R,* between bisoprolol and propranolol is not less than 7.0. Chromatograph the *Standard preparation,* and record the peak areas as directed for *Procedure:* the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of $(C_{18}H_{31}NO_4)_2 \cdot C_4H_4O_4$ in the portion of Bisoprolol Fumarate taken by the formula:

$$50C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Bisoprolol Fumarate RS in the *Standard preparation;* and r_U and r_S are the peak areas obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Bisoprolol Fumarate Tablets

DEFINITION

Bisoprolol Fumarate Tablets contain NLT 90.0% and NMT 105.0% of the labeled amount of bisoprolol fumarate [$(C_{18}H_{31}NO_4)_2 \cdot C_4H_4O_4$].

IDENTIFICATION

• **Thin-Layer Chromatographic Identification Test** ⟨201⟩

Sample solution: Equivalent to 40 mg of bisoprolol fumarate, from powdered T ablets (NLT 5), in a 50-mL flask. Add about 20 mL of a mixture of dichloromethane and methanol (7:3), shake for 30 min, centrifuge, and use the clear solution.

Application volume: 20 µL

Developing solvent system: Dichloromethane, methanol, and ammonia TS, stronger (70: 10: 0.8)

Analysis

Sample: *Sample solution*

Proceed as directed in the chapter, except to develop the chromatogram until the solvent front has moved about two-thirds of the length of the plate and to dr y the plate in a current of cold air.

ASSAY

• **Procedure**

Diluent: Acetonitrile and water (7:13)

Mobile phase: A 1-L portion of *Diluent*. Add 5 mL of heptafluorobutyric acid, 5 mL of diethylamine, and 2.5 mL of formic acid.

System suitability solution: 0.5 mg/mL of propranolol hydrochloride and 1 mg/mL of bisoprolol fumarate in *Diluent*

Standard solution: 1 mg/mL of USP Bisoprolol Fumarate RS in *Diluent*

Sample solution: Transfer an equivalent of 25 mg of bisoprolol fumarate, from powdered T ablets (NLT 20), to a 25-mL volumetric flask. Add 10 mL of *Diluent*, and sonicate for 10 min. Cool, dilute with *Diluent* to volume, and mix. Centrifuge for 20 min, and use the clear supernatant.

Chromatographic system

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

Mode: LC

Detector: UV 273 nm

Column: 4.6-mm × 12.5-cm; packing L7

Flow rate: 1 mL/min

Injection size: 10 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 7.0 between bisoprolol and propranolol, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the per centage of $(C_{18}H_{31}NO_4)_2 \cdot C_4H_4O_4$ in the portion of T ablets taken:

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

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

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

C_U = nominal concentration of bisoprolol fumarate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–105.0%

PERFORMANCE TESTS

Change to read:

• **Dissolution** ⟨711⟩

Test 1

Medium: Water; 900 mL

Apparatus 2: 75 rpm

Time: 20 min

Determine the amount of $(C_{18}H_{31}NO_4)_2 \cdot C_4H_4O_4$ dissolved by using the following method.

Diluent: Methanol, triethylamine, phosphoric acid, and water (160: 5: 2.5: 35)

Mobile phase: Methanol, triethylamine, and water (34:1:50). Adjust with phosphoric acid to a pH of 4.0 ± 0.1.
Standard stock solution: USP Bisoprolol Fumarate RS in water to obtain a solution having a known concentration of about twice the concentration of bisoprolol fumarate in the *Sample solution*
Standard solution: *Standard stock solution* and *Diluent* (1:1)
Sample solution: Sample per *Dissolution* ⟨711⟩. Withdraw a portion of the solution under test, filter, and dilute with an equal volume of *Diluent.*
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 227 nm
Column: 4.6-mm × 33-▲mm;▲*USP35* packing L7
Flow rate: 1 mL/min
Injection size: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Tolerances: NLT 80% (*Q*) of the labeled amount of $(C_{18}H_{31}NO_4)_2 \cdot C_4H_4O_4$ is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.
Medium: 0.5 M sodium chloride; 900 mL
Apparatus 2: 75 rpm
Time: 20 min
Analysis: Proceed as directed for *Test 1* with the following modifications.
Diluent: Prepare a mixture of methanol, 0.1 N hydrochloric acid, triethylamine, and phosphoric acid (160: 35: 5: 2.5). The dimensions of the column are 4.6 mm × 25 cm.
Tolerances: NLT 80% (*Q*) of the labeled amount of $(C_{18}H_{31}NO_4)_2 \cdot C_4H_4O_4$ is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** ⟨**905**⟩**:** Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Bisoprolol Fumarate RS
2-Propanol, 1-[4-[[2-(1-methylethoxy)ethoxy]methyl] phenoxy]-3-[(1-methylethyl)amino]-, (±)-, (*E*)-2-butenedioate (2:1) (salt).
$(C_{18}H_{31}NO_4)_2 \cdot C_4H_4O_4$ 766.96

Bisoprolol Fumarate and Hydrochlorothiazide Tablets

» Bisoprolol Fumarate and Hydrochlorothiazide Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the labeled amounts of bisoprolol fumarate $(C_{18}H_{31}NO_4)_2 \cdot C_4H_4O_4$ and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$).

Packaging and storage—Preserve in tight, light-resistant containers. Store at controlled room temperature.

USP Reference standards ⟨11⟩**—**
USP Bisoprolol Fumarate RS
USP Chlorothiazide RS
USP Hydrochlorothiazide RS

Identification—

A: *Thin-Layer Chromatographic Identification Test* ⟨201⟩—

Test solution—Finely powder 1 Tablet, and transfer the powder to a 5-mL volumetric flask. Dilute with methanol to volume, sonicate for 5 minutes, centrifuge, and use the supernatant.

Standard solution 1—Dissolve a suitable quantity of USP Bisoprolol Fumarate RS in methanol to obtain a solution containing 1 mg per mL.

Standard solution 2—Dissolve a suitable quantity of USP Hydrochlorothiazide RS in methanol to obtain a solution containing 1 mg per mL.

Application volume: 25 µL.

Developing solvent system: a mixture of methylene chloride, methanol, and 14.5 M ammonium hydroxide solution (43:20:8).

Procedure—Locate the spots on the plate under short-wavelength UV light and by exposure to iodine vapors: the R_F values of the principal spots in the chromatogram obtained from the *Test solution* correspond to those of the principal spots in the chromatograms obtained from *Standard solution 1* and *Standard solution 2.*

B: The retention times of the major peaks in the chromatograms of the *Bisoprolol fumarate assay preparation* and the *Hydrochlorothiazide assay preparation* correspond to those in the chromatogram of the *Standard preparation,* as obtained in the *Assay.*

Dissolution ⟨711⟩**—**

Medium: 0.1 N hydrochloric acid; 900 mL.

Apparatus 2: 75 rpm.

Times: 20 minutes for bisoprolol fumarate; 30 minutes for hydrochlorothiazide.

Triethylamine solution—Mix 2 mL of triethylamine with 1000 mL of water, and adjust with phosphoric acid to a pH of 3.0.

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile and *Triethylamine solution* (1:4). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard stock solution 1—Quantitatively dissolve an accurately weighed quantity of USP Bisoprolol Fumarate RS in *Medium* to obtain a solution having a known concentration of about 0.5 mg per mL.

Standard stock solution 2—Transfer about 30 mg of USP Hydrochlorothiazide RS, accurately weighed, to a 50-mL volumetric flask, dissolve in 5 mL of methanol, dilute with *Medium* to volume, and mix.

Standard solution—Dilute accurately measured volumes of *Standard stock solution 1* and *Standard stock solution 2* with *Medium* to obtain a solution having known concentrations of bisoprolol fumarate and hydrochlorothiazide corresponding to those of the solution under test.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a UV detector capable of measuring peak responses at 227 nm and 272 nm, simultaneously, and a 3.9-mm × 15-cm column that contains packing L11. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard solution,* and record the peak areas as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the filtered portions of the solution under test into the chromatograph, record the chromatograms, and measure the peak areas for bisoprolol at 227 nm and for hydrochlorothiazide at 272 nm. Calculate the quantities, in mg, of bisoprolol fumarate $(C_{18}H_{31}NO_4)_2 \cdot C_4H_4O_4$ and hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) dissolved.

Tolerances—Not less than 80% *(Q)* of the labeled amount of $(C_{18}H_{31}NO_4)_2 \cdot C_4H_4O_4$ is dissolved in 20 minutes and not less than 80% *(Q)* of the labeled amount of $C_7H_8ClN_3O_4S_2$ is dissolved in 30 minutes.

Uniformity of dosage units ⟨905⟩**:** Meet the requirements with respect to bisoprolol fumarate and to hydrochlorothiazide.