Standard preparation 2—Transfer 2.0 mL of *Standard preparation 1* to a 250-mL volumetric flask, dilute with *Diluent* to volume, and mix. This solution contains about 0.01 mg of USP Cyclosporine RS per mL.

Assay preparation—Dissolve about 25 mg of Cyclosporine, accurately weighed, in *Diluent*, dilute with *Diluent* to 20.0 mL, and mix.

Resolution solution—Prepare a solution of USP Cyclosporine Resolution Mixture RS in *Diluent* having a concentration of about 1.25 mg per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 210-nm detector, a 0.25-mm × 1-m stainless steel tube connected to a 4-mm × 25-cm column that contains 3- to 5-µm packing L1. The tube and column are maintained at 80°. The flow rate is about 1.2 mL per minute. Chromatograph the *Resolution solution,* and record the responses as directed for *Procedure:* the cyclosporine U peak and the main cyclosporine peak are resolved from each other. Chromatograph *Standard preparation 1,* and record the responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 1.0%. Chromatograph *Standard preparation 2,* and record the responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 10%.

Procedure—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 20 µL) of *Standard preparation 1*, *Standard preparation 2*, and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of cyclosporine A ($C_{62}H_{111}N_{11}O_{12}$) in the Cyclosporine taken by the formula:

$$(CP/10U)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Cyclosporine RS in *Standard preparation 1; P* is the specified purity, in µg per mg, of USP Cyclosporine RS; U is the concentration, in mg per mL, of specimen in the *Assay preparation;* and r_U and r_S are the main cyclosporine peak responses obtained from the *Assay preparation* and *Standard preparation 1*, respectively.

Cyclosporine Capsules

» Cyclosporine Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of cyclosporine ($C_{62}H_{111}N_{11}O_{12}$).

Packaging and storage—Preserve in tight containers, and store at controlled room temperature.

Identification—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay*.

Dissolution ⟨711⟩**—**

WHERE CAPSULES CONTAIN LIQUID—

Medium: water; 500 mL.

Apparatus 2: 50 rpm.

Time: 15 minutes.

Procedure—Place 1 Capsule in each vessel, and allow the Capsule to sink to the bottom of the vessel before starting rotation of the blade. Observe the Capsules, and record the time taken for each Capsule shell to rupture.

Tolerances—The requirements are met if all of the Capsules tested rupture in not more than 15 minutes. If 1 or 2 of the Capsules rupture in more than 15 but not more than 30 minutes, repeat the test on 12 additional Capsules. Not more than 2 of the total of 18 Capsules tested rupture in more than 15 but not more than 30 minutes.

WHERE CAPSULES CONTAIN POWDER—

Medium: 0.1 N hydrochloric acid containing 0.5% of sodium lauryl sulfate; 1000 mL.

Apparatus 1: 150 rpm.

Time: 90 minutes.

Determine the amount of $C_{62}H_{111}N_{11}O_{12}$ dissolved by employing the following method.

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile, water, methanol, and phosphoric acid (900:450:50:0.5). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard solution—Quantitatively dissolve an accurately weighed quantity of USP Cyclosporine RS in *Dissolution Medium* to obtain a solution having a known concentration of about 0.001L mg per mL, L being the labeled quantity, in mg, of cyclosporine in each Capsule. Transfer 25.0 mL of this solution to a 50-mL volumetric flask, dilute with acetonitrile to volume, and mix. This solution contains about 0.0005L mg of USP Cyclosporine RS per mL.

Test solution—Filter a portion of the solution under test. Transfer 5.0 mL of the filtrate to a 10-mL volumetric flask, dilute with acetonitrile to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 210-nm detector and a 4.6-mm × 25-cm column that contains packing L1 and is maintained at a constant temperature of about 80°. The flow rate is about 2 mL per minute. Chromatograph the *Standard solution*, and record the peak areas as directed for *Procedure:* the column efficiency is not less than 700 theoretical plates; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 10 µL) of the solution estimated to contain 0.1 mg of cyclosporine per mL, or 40 µL of the solution estimated to contain 0.025 mg of cyclosporine per mL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of $C_{62}H_{111}N_{11}O_{12}$ dissolved by the formula:

$$2000C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Cyclosporine RS in the *Standard solution;* and r_U and r_S are the cyclosporine peak areas obtained from the *Test solution* and the *Standard solution*, respectively.

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{62}H_{111}N_{11}O_{12}$ is dissolved in 90 minutes.

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

Water, *Method I* ⟨921⟩**—**For Capsules that contain powder, not more than 3.5% is found, using finely ground Capsule contents.

Assay—

WHERE CAPSULES CONTAIN LIQUID—

Mobile phase and *Chromatographic system*—Proceed as directed in the *Assay* under *Cyclosporine Injection*.

Standard preparation—Dissolve an accurately weighed quantity of USP Cyclosporine RS in dehydrated alcohol to obtain a solution having a known concentration of about 1 mg per mL. Use this solution promptly after preparation.

Assay preparation—Using a sharp blade, carefully cut open not fewer than 20 Capsules, and with the aid of dehydrated alcohol transfer the contents of the Capsules to a suitable volumetric flask. Wash the blade with dehydrated alcohol, and transfer the washings to the volumetric flask. Dilute the contents of the volumetric flask with dehydrated alcohol to volume, and mix. Quantitatively dilute an accu-

rately measured volume of this solution with dehydrated alcohol to obtain a solution having a concentration of about 1 mg of cyclosporine per mL.

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 areas for the major peaks. Calculate the quantity, in mg, of cyclosporine ($C_{62}H_{111}N_{11}O_{12}$) in each Capsule taken by the formula:

$$(L/D)(CP/1000)(r_U / r_S)$$

in which L is the labeled quantity, in mg, of cyclosporine in each Capsule taken; D is the concentration, in mg per mL, of the *Assay preparation*, based on the labeled quantity of cyclosporine in the Capsules taken and the extent of dilution; C is the concentration, in mg per mL, of USP Cyclosporine RS in the *Standard preparation; P* is the purity, in µg per mg, of USP Cyclosporine RS; and r_U and r_S are the peak areas obtained from the *Assay preparation* and the *Standard preparation,* respectively.

WHERE CAPSULES CONTAIN POWDER—

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile, water, methanol, and phosphoric acid (605:400:50:0.5). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Diluting solvent—Prepare a mixture of acetonitrile, tetrahydrofuran, and dehydrated alcohol (9:5:4).

Standard preparation—Transfer about 25 mg of USP Cyclosporine RS, accurately weighed, to a 25-mL volumetric flask. Add 2.5 mL of water, and sonicate for 10 minutes. Add about 10 mL of *Diluting solvent*, sonicate for 5 minutes, dilute with *Diluting solvent* to volume, and mix.

Assay stock preparation—Transfer the contents of 20 Capsules to a volumetric flask of such capacity, V, in mL, to make a final concentration of 10 mg of cyclosporine per mL. Add $0.1V$ mL of water to the flask, and sonicate for 10 minutes. Add $0.4V$ mL of *Diluting solvent* to the flask, and sonicate for 5 minutes. Dilute with *Diluting solvent* to volume, and mix.

Assay preparation—Transfer 5.0 mL of *Assay stock preparation* to a 50-mL volumetric flask, add 5 mL of water, dilute with *Diluting solvent* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 210-nm detector and a 4.6-mm × 25-cm column that contains packing L13 and is maintained at a constant temperature of about 70°. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation,* and record the peak areas as directed for *Procedure:* the column efficiency is not less than 700 theoretical plates; the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

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 areas for the major peaks. Calculate the quantity, in mg, of cyclosporine ($C_{62}H_{111}N_{11}O_{12}$) in each Capsule taken by the formula:

$$10CV(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Cyclosporine RS in the *Standard preparation; V* is the volume, in mL, of the volumetric flask used to prepare the *Assay stock preparation;* and r_U and r_S are the cyclosporine peak areas obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Cyclosporine Injection

» Cyclosporine Injection is a sterile solution of Cyclosporine in a suitable vehicle. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of cyclosporine ($C_{62}H_{111}N_{11}O_{12}$).

Packaging and storage—Preserve in single-dose or multiple-dose containers.

Labeling—Label it to indicate that it is to be diluted with a suitable parenteral vehicle prior to intravenous infusion.

USP Reference standards ⟨11⟩**—**
USP Cyclosporine RS
USP Endotoxin RS

Identification—

A: Prepare a solution of it in methanol containing about 0.5 mg of cyclosporine per mL (test solution). Prepare a Standard solution containing 0.5 mg per mL of USP Cyclosporine RS in methanol. Separately apply 10-µL portions of the test solution and the Standard solution to a suitable thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry in a current of air, place the plate in a suitable chromatographic chamber, and develop the chromatogram, using ethyl ether as the developing solvent, until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow it to dry. Place the plate in a second chromatographic chamber, and develop the chromatogram in a solvent system consisting of a mixture of ethyl acetate, methyl ethyl ketone, water, and formic acid (60:40:2:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and allow it to dry. Spray the plate with a freshly prepared mixture of 5 mL of *Solution A* (340 mg of bismuth subnitrate dissolved in 20 mL of 20% acetic acid), 5 mL of *Solution B* (8 g of potassium iodide dissolved in 20 mL of water), 20 mL of glacial acetic acid, and water to make 100 mL. Immediately again spray the plate with hydrogen peroxide TS. Cyclosporine appears as a brown spot having an R_F value of about 0.45 on the chromatograms: the R_F value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution. [NOTE—Disregard any spots at the origin.]

B: The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for cyclosporine, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay*.

Bacterial endotoxins ⟨85⟩**—**Prepare the test specimen as follows, using USP Endotoxin RS. Make a 1:10 dilution of the Injection with Water for Injection. Add 0.1 mL of the resulting suspension and 0.1 mL of appropriately constituted LAL reagent to a suitable pyrogen-free test tube, and mix on a vortex mixer for about 5 seconds: the article under test contains not more than 0.84 USP Endotoxin Unit per mg of cyclosporine.

Sterility ⟨71⟩**:** meets the requirements.

Alcohol content (*where present*)—

Internal standard solution—Mix 3 mL of *n*-propyl alcohol and 50 mL of butyl alcohol.

Standard stock solution—Transfer about 1.6 g of dehydrated alcohol, accurately weighed, to a 25-mL volumetric flask, dilute with butyl alcohol to volume, and mix.

Standard preparation—Transfer 5.0 mL of *Standard stock solution* and 6.0 mL of *Internal standard solution* to a 25-mL