Cycloserine Capsules

» Cycloserine Capsules contain not less than 90.0 percent and not more than 120.0 percent of the labeled amount of cycloserine ($C_3H_6N_2O_2$).

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩**—**
USP Cycloserine RS

Identification—Shake a quantity of the contents of Capsules, equivalent to about 10 mg of cycloserine, with 100 mL of 0.1 N sodium hydroxide, and filter: 1 mL of the filtrate so obtained responds to the *Identification* test under *Cycloserine.*

Dissolution ⟨711⟩**—**

Medium: pH 6.8 Phosphate buffer (see *Buffer Solutions* under *Solutions* in the section *Reagents, Indicators, and Solutions*); 900 mL.

Apparatus 1: 100 rpm.

Time: 30 minutes.

Determine the amount of $C_3H_6N_2O_2$ dissolved by employing the following method.

pH 6.8 Phosphate buffer, *Mobile phase*, and *Chromatographic system*—Proceed as directed in the *Assay*.

Standard solution—Quantitatively dissolve an accurately weighed quantity of USP Cycloserine RS in *pH 6.8 Phosphate buffer* to obtain a solution having a known concentration of about 0.25 mg per mL.

Test solution—Use a filtered portion of the solution under test.

Procedure—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses for cycloserine. Calculate the quantity, in mg, of cycloserine ($C_3H_6N_2O_2$) dissolved by the formula:

$$900C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Cycloserine RS in the *Standard solution;* and r_U and r_S are the peak responses for cycloserine obtained from the *Test solution* and the *Standard solution*, respectively.

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_3H_6N_2O_2$ is dissolved in 30 minutes.

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

Loss on drying ⟨731⟩**—**Dry about 100 mg of the contents of Capsules in a capillary-stoppered bottle in vacuum at 60° for 3 hours: it loses not more than 1.0% of its weight.

Assay—

pH 6.8 Phosphate buffer, *Mobile phase*, *Standard preparation*, and *Chromatographic system*—Proceed as directed in the *Assay* under *Cycloserine.*

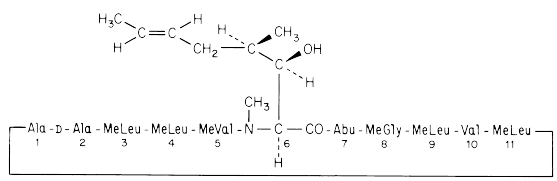
Assay preparation—Remove, as completely as possible, the contents of not fewer than 20 Capsules. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of cycloserine, to a 250-mL volumetric flask, dilute with *pH 6.8 Phosphate buffer* to volume, mix, and filter.

Procedure—Proceed as directed in the *Assay* under *Cycloserine.* Calculate the quantity, in mg, of cycloserine ($C_3H_6N_2O_2$) in the portion of Capsules taken by the formula:

$$250C(r_U / r_S)$$

in which the terms are as defined therein.

Cyclosporine



$C_{62}H_{111}N_{11}O_{12}$ 1202.61

Cyclo[[(*E*)-(2*S*,3*R*,4*R*)-3-hydroxy-4-methyl-2-(methylamino)-6-octenoyl]-L-2-aminobutyryl-*N*-methylglycyl-*N*-methyl-L-leucyl-L-valyl-*N*-methyl-L-leucyl-L-alanyl-D-alanyl-*N*-methyl-L-leucyl-*N*-methyl-L-leucyl-*N*-methyl-L-valyl].

[*R*-[*R**,*R**-(*E*)]]-Cyclic(L-alanyl-D-alanyl-*N*-methyl-L-leucyl-*N*-methyl-L-leucyl-*N*-methyl-L-valyl-3-hydroxy-*N*,4-dimethyl-L-2-amino-6-octenoyl-L-α-aminobutyryl-*N*-methylglycyl-*N*-methyl-L-leucyl-L-valyl-*N*-methyl-L-leucyl) [59865-13-3].

» Cyclosporine contains not less than 98.5 percent and not more than 101.5 percent of cyclosporine A ($C_{62}H_{111}N_{11}O_{12}$), calculated on the dried basis.

Packaging and storage—Preserve in tight, light-resistant containers.

USP Reference standards ⟨11⟩**—**
USP Cyclosporine RS
USP Cyclosporine Resolution Mixture RS
This material is a 100:1 mixture of cyclosporine and cyclosporine U.

Identification—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,* as obtained in the *Assay*.

Loss on drying ⟨731⟩**—**Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 2.0% of its weight.

Heavy metals, *Method II* ⟨231⟩: 0.002%.

Related compounds—Using the chromatograms obtained from *Standard preparation 2* and the *Assay preparation* in the *Assay*, calculate the percentage of each impurity by the formula:

$$2000(C / W)(r_i / r_{S2})$$

in which C is the concentration, in mg per mL, of USP Cyclosporine RS in *Standard preparation 2; W* is the weight, in mg, of Cyclosporine taken to prepare the *Assay preparation;* r_i is the response of an individual impurity observed in the chromatogram of the *Assay preparation;* and r_{S2} is the response of the main cyclosporine peak in the chromatogram obtained from *Standard preparation 2:* not more than 0.7% of any individual impurity is found, and the sum of all such impurities is not more than 1.5%, any impurities corresponding to less than 0.05% being disregarded.

Assay—

Mobile phase—Prepare a mixture of water, acetonitrile, *tert*-butyl methyl ether, and phosphoric acid (520:430:50:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

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

Standard preparation 1—Dissolve an accurately weighed quantity of USP Cyclosporine RS in *Diluent* to obtain a solution having a known concentration of about 1.25 mg per mL.

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-