and 5 mL of glacial acetic acid, and mix. Adjust with 1 N sodium hydroxide to a pH of 4.4. Filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard preparation—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 0.4 mg per mL.

Assay preparation—Transfer about 20 mg of Cycloserine, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *pH 6.8 Phosphate buffer* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 219-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1 mL per minute. The column temperature is maintained at about 30°. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the tailing factor is not more than 1.8; 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 peak responses for cycloserine. Calculate the quantity, in µg, of $C_3H_6N_2O_2$ in each mg of Cycloserine taken by the formula:

$$50{,}000(C/W)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Cycloserine RS in the *Standard preparation; W* is the quantity, in mg, of Cycloserine taken to prepare the *Assay preparation;* and r_U and r_S are the peak responses for cycloserine obtained from the *Assay preparation* and the *Standard preparation,* respectively.

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.*

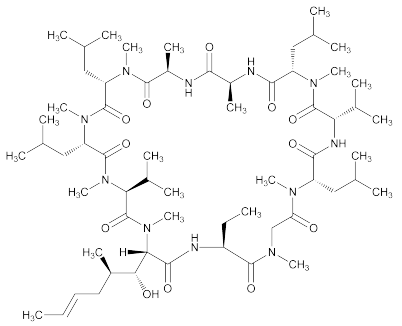
Assay preparation—Remove, as completely as possible, the contents of not fewer than 20 Capsules. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of cycloserine, to a 250-mL volumetric flask, dilute with *pH 6.8 Phosphate buffer* to volume, mix, and filter.

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].

DEFINITION

Cyclosporine contains NLT 97.0% and NMT 101.5% of cyclosporine A ($C_{62}H_{111}N_{11}O_{12}$), calculated on the dried basis.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: Acetonitrile, *tert*-butyl methyl ether, water, and phosphoric acid (430:50:520:1)

Diluent: Acetonitrile and water (1:1)

System suitability solution: 1.25 mg/mL of USP Cyclosporine Resolution Mixture RS in *Diluent*

Standard solution: 1.25 mg/mL of USP Cyclosporine RS in *Diluent*

Sample solution: 1.25 mg/mL of Cyclosporine in *Diluent*

Chromatographic system

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

Mode: LC

Detector: UV 210 nm

Column: 4-mm × 25-cm; 3- to 5-µm packing L1; with 0.25-mm × 1-m stainless steel tubing connected to the column inlet

Column temperature: 80°. The tubing and column are maintained at 80°, to ensure that the *Mobile phase* entering the column is heated to 80°.

Flow rate: 1.2 mL/min

Injection volume: 20 µL

System suitability

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

[NOTE—The relative retention times for cyclosporine U and cyclosporine are 0.95 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.0 between cyclosporine U and cyclosporine, *System suitability solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of cyclosporine ($C_{62}H_{111}N_{11}O_{12}$) in the portion of Cyclosporine taken:

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

r_U = peak area of cyclosporine from the *Sample solution*

r_S = peak area of cyclosporine from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

P = potency of cyclosporine in USP Cyclosporine RS (mg/mg)

Acceptance criteria: 97.0%–101.5% on the dried basis

IMPURITIES

Delete the following:

•• **HEAVY METALS,** *Method II* ⟨**231**⟩**:** NMT 20 ppm •• (Official 1-Jan-2018)

• **ORGANIC IMPURITIES**

Mobile phase, Diluent, System suitability solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard solution: 0.01 mg/mL of USP Cyclosporine RS in *Diluent*

System suitability

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

[NOTE—The relative retention times for cyclosporine U and cyclosporine are 0.95 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 1.0 between cyclosporine U and cyclosporine, *System suitability solution*

Relative standard deviation: NMT 10.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Cyclosporine taken:

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

r_U = peak area of an individual impurity from the *Sample solution*

r_S = peak area of cyclosporine from the *Standard solution*

C_S = concentration of the *Standard solution* (mg/mL)

C_U = concentration of the *Sample solution* (mg/mL)

P = potency of cyclosporine in USP Cyclosporine RS (mg/mg)

Acceptance criteria

Reporting threshold is 0.05%.

Any individual impurity: NMT 0.7%

Total impurities: NMT 1.5%

SPECIFIC TESTS

• **LOSS ON DRYING** ⟨**731**⟩

Sample: 100 mg

Analysis: Dry in a capillary-stoppered bottle under vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 h.

Acceptance criteria: NMT 2.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS** ⟨**11**⟩

USP Cyclosporine RS

USP Cyclosporine Resolution Mixture RS

This is a 100:1 (w/w) mixture of cyclosporine and cyclosporine U. The chemical name for cyclosporine U is given below.

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-L-leucyl-L-alanyl-D-alanyl-*N*-methyl-L-leucyl-*N*-methyl-L-leucyl-*N*-methyl-L-valyl].

$C_{61}H_{109}N_{11}O_{12}$ 1188.58

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