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Cyclosporine Injection

DEFINITION

Cyclosporine Injection is a sterile solution of Cyclosporine in a suitable vehicle. It contains NLT 90.0% and NMT 110.0% of the labeled amount of cyclosporine ($C_{62}H_{111}N_{11}O_{12}$).

IDENTIFICATION

• **A. Thin-Layer Chromatography**

Solution A: 17 mg/mL of bismuth subnitrate in 20% acetic acid

Solution B: 400 mg/mL of potassium iodide

Standard solution: 0.5 mg/mL of USP Cyclosporine RS in methanol

Sample solution: Nominally 0.5 mg/mL of cyclosporine from Injection in methanol

Chromatographic system

(See *Chromatography ⟨621⟩, Thin-Layer Chromatography*.)

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 µL

Developing solvent system 1: Ethyl ether

Developing solvent system 2: Ethyl acetate, methyl ethyl ketone, water, and formic acid (60:40:2:1)

Spray reagent 1: Mix 5 mL of *Solution A* with 5 mL of *Solution B* and 20 mL of glacial acetic acid, and dilute with water to 100 mL. Prepare freshly.

Spray reagent 2: Hydrogen peroxide TS

Analysis

Samples: *Standard solution* and *Sample solution*

Apply the *Standard solution* and the *Sample solution* to the plate. Allow the spots to dry in a current of air, place the plate in a suitable chromatographic chamber, and develop the chromatogram, using *Developing solvent system 1*, 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 *Developing solvent system 2* 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 *Spray reagent 1*. Immediately again spray the plate with *Spray reagent 2*. Cyclosporine appears as a brown spot having an R_F value of about 0.45.

Acceptance criteria: The R_F value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*. Disregard any spots at the origin.

• **B.** 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, methanol, water, and phosphoric acid (550:50:400:0.5)

Standard solution: 0.5 mg/mL of USP Cyclosporine RS in methanol. Use this solution promptly after preparation.

Sample solution 1 (where it is represented as being in a single-dose container): Nominally 0.5 mg/mL of cyclosporine from Injection in methanol, prepared as follows. Using a suitable hypodermic needle and syringe, withdraw all of the withdrawable contents from 1 container of Injection, and dilute with methanol. Use this solution promptly after preparation.

Sample solution 2 (where the label states the quantity of cyclosporine in a given volume): Nominally 0.5 mg/mL of cyclosporine from Injection in methanol, prepared as follows. Dilute a suitable aliquot of Injection with methanol. Use this solution promptly after preparation.

Chromatographic system

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

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; packing L16

Column temperature: 70°

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Capacity factor: NLT 3–NMT 10

Column efficiency: NLT 700 theoretical plates

Tailing factor: NMT 1.5

Relative standard deviation: NMT 1.5%

Analysis

Samples: *Standard solution* and *Sample solution 1* or *Sample solution 2*

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

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

r_U = peak response from *Sample solution 1* or *Sample solution 2*

r_S = peak response from the *Standard solution*

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

C_U = nominal concentration of *Sample solution 1* or *Sample solution 2* (mg/mL)

Acceptance criteria: 90.0%–110.0%

OTHER COMPONENTS

• **Content of Alcohol** (where present)

Internal standard solution: *n*-Propyl alcohol and butyl alcohol (3:50)

Standard stock solution: 64 mg/mL of dehydrated alcohol in butyl alcohol

Standard solution: 12.8 mg/mL of alcohol, prepared as follows. Transfer a suitable aliquot of *Standard stock solution* to a suitable volumetric flask. Add *Internal standard solution*, using 24% of the final volume, and dilute with butyl alcohol to volume.

Sample solution: Nominally 12.8 mg/mL of alcohol from Injection, prepared as follows. Transfer a suitable aliquot of Injection to a suitable volumetric flask. Add *Internal standard solution*, using 24% of the final volume, and dilute with butyl alcohol to volume.

Chromatographic system

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

Mode: GC

Detector: Flame ionization

Column: 2-mm × 2-m glass; packed with support S3

Temperatures

Injection port: 280°

Detector: 290°

Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
145	0	145	8
145	32	270	0

Carrier gas: Nitrogen

Flow rate: 35 mL/min

Injection volume: 1 µL. [Note—Make adjustments, if necessary, to obtain satisfactory chromatography.]

System suitability

Sample: *Standard solution*

Suitability requirements: [NOTE—The elution order is alcohol, *n*-propyl alcohol, and butyl alcohol.]

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of alcohol (C_2H_5OH) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

R_U = peak area ratio of alcohol to *n*-propyl alcohol from the *Sample solution*

R_S = peak area ratio of alcohol to *n*-propyl alcohol from the *Standard solution*

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

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

Acceptance criteria: 80.0%–120.0% of the labeled amount

SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST ⟨85⟩**

Sample solution: Make a 1:10 dilution of the Injection with Water for Injection.

Analysis: Add 0.1 mL of *Sample solution* and 0.1 mL of appropriately constituted LAL reagent to a suitable pyrogen-free test tube. Mix on a vortex mixer for about 5 s.

Acceptance criteria: NMT 0.84 USP Endotoxin Unit/mg of cyclosporine

- **STERILITY TESTS ⟨71⟩:** Meets the requirements

ADDITIONAL REQUIREMENTS

- **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 before intravenous infusion.
- **USP REFERENCE STANDARDS ⟨11⟩**

USP Cyclosporine RS

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
CYCLOSPORINE INJECTION	Documentary Standards Support	SM12020 Small Molecules 1

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