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# Cyclophosphamide for Injection

## DEFINITION

Cyclophosphamide for Injection is a sterile mixture of Cyclophosphamide with or without a suitable excipient. The sterile powder formulation contains NLT 90.0% and NMT 105.0% of the labeled amount of anhydrous cyclophosphamide ( $C_7H_{15}Cl_2N_2O_2P$ ). The lyophilized formulation contains NLT 90.0% and NMT 110.0% of the labeled amount of anhydrous cyclophosphamide ( $C_7H_{15}Cl_2N_2O_2P$ ).

## IDENTIFICATION

**Change to read:**

- **A.** [▲ SPECTROSCOPIC IDENTIFICATION TESTS \(197\), Infrared Spectroscopy: 197K ▲](#) (CN 1-MAY-2020)

**If labeled as sterile powder formulation:** Proceed as directed in the chapter.

**If labeled as lyophilized formulation:** Prepare the *Sample* as follows.

**Sample:** Suspend 100 mg of the lyophilized formulation in 25 mL of methylene chloride, sonicate for 10 min and filter. Remove the solvent from the filtrate, and dissolve the resulting clear and colorless oil in 10 mL of diethylether that is saturated with water. Cyclophosphamide crystallizes from this solution after a few minutes. Remove diethylether by evaporation.

**Acceptance criteria:** Meets the requirements

- **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 and water (30:70)

**Standard solution:** 0.5 mg/mL of [USP Cyclophosphamide RS](#) in water

**Sample solution:** Nominally equivalent to 0.5 mg/mL of anhydrous cyclophosphamide in water

**Chromatographic system**

(See [Chromatography \(621\), System Suitability](#).)

**Mode:** LC

**Detector:** UV 195 nm

**Column:** 3.9-mm × 30-cm; packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 25 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 1.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of anhydrous cyclophosphamide ( $C_7H_{15}Cl_2N_2O_2P$ ) in the portion of Cyclophosphamide for Injection taken:

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

$r_U$  = peak response of cyclophosphamide from the *Sample solution*

$r_S$  = peak response of cyclophosphamide from the *Standard solution*

$C_S$  = concentration of [USP Cyclophosphamide RS](#) in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of anhydrous cyclophosphamide in the *Sample solution* (mg/mL)

### Acceptance criteria

**For the lyophilized formulation:** 90.0%–110.0%

**For the sterile powder formulation:** 90.0%–105.0%

### IMPURITIES

#### • ORGANIC IMPURITIES: PROCEDURE FOR THE STERILE POWDER FORMULATION

**Diluent:** Methanol and water (1:1)

**Standard solution A:** 30 µg/mL of [USP Cyclophosphamide Related Compound A RS](#) in *Diluent*

**Standard solution B:** 30 µg/mL of [USP Cyclophosphamide Related Compound B RS](#) in *Diluent*

**Standard solution C:** 30 µg/mL of [USP Cyclophosphamide Related Compound C RS](#) in *Diluent*

**Standard solution D:** 38.4 µg/mL of [USP Cyclophosphamide Related Compound D RS](#) in *Diluent*. [NOTE—Cyclophosphamide related compound D is free base (molecular weight = 260.66) and [USP Cyclophosphamide Related Compound D RS](#) is available as dihydrochloride salt (molecular weight = 333.60).]

**Standard solution E:** 22 µg/mL of [USP Cyclophosphamide RS](#) in *Diluent*

**Sample solution:** Nominally equivalent to 20 mg/mL of anhydrous cyclophosphamide in *Diluent*, from Cyclophosphamide for Injection

#### Chromatographic system

(See [Chromatography \(621\), General Procedures, Thin-Layer Chromatography](#).)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 20 µL

**Developing solvent system:** Methylene chloride, glacial acetic acid, methanol, and water (50:25:15:12)

**Reagent A:** 3.16 g/L solution of potassium permanganate in water and 10% hydrochloric acid (1:1). [NOTE—Mix in a small beaker at the time of use under a fume hood to generate chlorine gas, and immediately place the beaker with solution into a closed TLC chamber (placed in a fume hood).]

**Reagent B:** Dissolve 250 mg of tetramethylbenzidine in 50 mL of dehydrated alcohol, and dilute with cyclohexane to 200 mL.

#### Analysis

**Samples:** *Standard solution A, Standard solution B, Standard solution C, Standard solution D, Standard solution E, and Sample solution.* [NOTE—Apply *Standard solution E* after the plate development in the *Developing solvent system*. Proceed as directed in *Procedure* as follows.]

**Procedure:** Develop with *Developing solvent system* over a path of 10 cm followed by drying at room temperature for 15 min in a fume hood. Develop again in a fresh portion of the *Developing solvent system* over a path of 10 cm followed by drying at room temperature for 15 min in a fume hood. Apply *Standard solution E* at the starting point of the plate. Dry the plate in an oven at 50° under vacuum for 20 min or using a TLC heating plate at 50° for 20 min in a fume hood. Allow the plate to stand at room temperature for 5 min. Place the plate in a closed chromatography tank (placed in a fume hood) containing *Reagent A*, and leave the plate in the tank for at least 15 min. Remove the plate and place it in a fume hood for 15 min to remove the excess chlorine. Stain the plate by dipping it into *Reagent B* or spraying it with *Reagent B*. Examine the plate by visual evaluation.

**Acceptance criteria:** See [Table 1](#).

- The spot of cyclophosphamide related compound A in the *Sample solution* is not more intense than the spot of cyclophosphamide related compound A from *Standard solution A*.
- The spot of cyclophosphamide related compound B in the *Sample solution* is not more intense than the spot of cyclophosphamide related compound B from *Standard solution B*.
- The spot of cyclophosphamide related compound C in the *Sample solution* is not more intense than the spot of cyclophosphamide related compound C from *Standard solution C*.
- The spot of cyclophosphamide related compound D in the *Sample solution* is not more intense than the spot of cyclophosphamide related compound D from *Standard solution D*.
- The spot of any individual unspecified impurity in the *Sample solution* is not more intense than the spot of cyclophosphamide from *Standard solution E*.

**Table 1**

| Name                                             | Retardation Factor | Acceptance Criteria, NMT (%) |
|--------------------------------------------------|--------------------|------------------------------|
| Cyclophosphamide related compound D <sup>a</sup> | 0.15               | 0.15                         |

| Name                                             | Retardation Factor | Acceptance Criteria, NMT (%) |
|--------------------------------------------------|--------------------|------------------------------|
| Cyclophosphamide related compound C <sup>b</sup> | 0.20               | 0.15                         |
| Cyclophosphamide related compound B <sup>c</sup> | 0.43               | 0.15                         |
| Cyclophosphamide related compound A <sup>d</sup> | 0.90               | 0.15                         |
| Any unspecified impurity                         | —                  | 0.11                         |

<sup>a</sup> 3-[2-(2-Chloroethylamino)ethylamino]propyl dihydrogen phosphate.

<sup>b</sup> 3-Aminopropyl dihydrogen phosphate.

<sup>c</sup> 3-(2-Chloroethyl)-2-oxo-2-hydroxy-1,3,6,2-oxadiazaphosphonane.

<sup>d</sup> Bis(2-chloroethyl)amine hydrochloride.

• **ORGANIC IMPURITIES: PROCEDURE FOR THE LYOPHILIZED FORMULATION**

Use plastic containers to prepare the solutions containing cyclophosphamide and its related substances.

**Mobile phase:** 0.2 mL of 85% phosphoric acid in 1 L of water. Adjust to a pH of 2.6.

**Diluent:** 7.5 mg/mL of mannitol in water

**Standard stock solution A:** 0.36 mg/mL of [USP Cyclophosphamide Related Compound A RS](#) in water

**Standard stock solution B:** 0.28 mg/mL of [USP Cyclophosphamide Related Compound B RS](#) in water

**Standard stock solution D:** 0.44 mg/mL of [USP Cyclophosphamide Related Compound D RS](#) in water

**System suitability solution:** 0.036 mg/mL of [USP Cyclophosphamide Related Compound A RS](#), 0.028 mg/mL of [USP Cyclophosphamide Related Compound B RS](#), and 0.044 mg/mL of [USP Cyclophosphamide Related Compound D RS](#) in Diluent, from Standard stock solution A, Standard stock solution B, and Standard stock solution D, respectively

**Standard solution A:** 0.036 mg/mL of [USP Cyclophosphamide Related Compound A RS](#) in Diluent, from Standard stock solution A

**Standard solution B:** 0.028 mg/mL of [USP Cyclophosphamide Related Compound B RS](#) in Diluent, from Standard stock solution B

**Standard solution D:** 0.044 mg/mL of [USP Cyclophosphamide Related Compound D RS](#) in Diluent, from Standard stock solution D

**Sample solution:** Nominally equivalent to 10 mg/mL of anhydrous cyclophosphamide in water, from Cyclophosphamide for Injection

**Chromatographic system**

(See [Chromatography \(621\)](#), [System Suitability](#).)

**Mode:** LC

**Detectors:** UV at 200 nm and conductivity in series

**Polarity:** Negative

**Cell temperature:** 45°

**Column:** 4.6-mm × 12.5-cm; packing L76

**Autosampler temperature:** 5°

**Flow rate:** 1.2 mL/min

**Injection volume:** 10 µL

**Run time:** NLT 3 times the retention time of cyclophosphamide related compound D

**System suitability**

**Sample:** System suitability solution

**Suitability requirements**

**Resolution:** NLT 2 between cyclophosphamide related compound A and cyclophosphamide related compound D

**Relative standard deviation:** NMT 5% for each peak

**Analysis**

**Samples:** Standard solutions and Sample solution

Calculate the percentage of each impurity in the portion of Cyclophosphamide for Injection taken:

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

$r_U$  = peak response of each impurity from the Sample solution

$r_S$  = peak response of the corresponding USP Reference Standard from the Standard solution (see [Table 2](#))