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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[a]	0.15	0.15

Name	Retardation Factor	Acceptance Criteria, NMT (%)
Cyclophosphamide related compound C[b]	0.20	0.15
Cyclophosphamide related compound B[c]	0.43	0.15
Cyclophosphamide related compound A[d]	0.90	0.15
Any unspecified impurity	—	0.11

[a] 3-[2-(2-Chloroethylamino)ethylamino]propyl dihydrogen phosphate.

[b] 3-Aminopropyl dihydrogen phosphate.

[c] 3-(2-Chloroethyl)-2-oxo-2-hydroxy-1,3,6,2-oxadiazaphosphonane.

[d] 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*)

C_S = concentration of the corresponding USP Reference Standard in the *Standard solution* (mg/mL)

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

Acceptance criteria: See *Table 2*. Disregard any impurity peaks less than 0.02%.

Table 2

Name	Relative Retention Time[a]	Detection Mode	External Reference Standard	Acceptance Criteria, NMT (%)
Impurity 1	0.23	Conductivity	—	Disregard
Cyclophosphamide related compound B[b]	0.49	UV	USP Cyclophosphamide Related Compound B RS	0.25
Piperazinylpropyl pentahydroxyhexyl phosphate[c]	0.60–0.72	Conductivity	USP Cyclophosphamide Related Compound D RS	0.50
Chlorodiazinonyl pentahydroxyhexyl phosphate[d]	0.77–0.86	Conductivity	USP Cyclophosphamide Related Compound D RS	0.30
Dihydroxycyclophosphamide[e]	0.80–0.91	Conductivity	USP Cyclophosphamide Related Compound D RS	1.0
Impurity 2	0.84	Conductivity	—	Disregard
Cyclophosphamide	1.0	UV	—	—
Cyclophosphamide related compound D[f]	1.0	Conductivity	USP Cyclophosphamide Related Compound D RS	2.0
Chlorodiazinonyl phosphamide[g]	1.19–1.31	Conductivity	USP Cyclophosphamide Related Compound D RS	0.50
Cyclophosphamide related compound A[h]	1.40–1.70	Conductivity	USP Cyclophosphamide Related Compound A RS	2.0
Cyclophosphamide pyrophosphate analog[i]	1.46–1.67	Conductivity	USP Cyclophosphamide Related Compound D RS	0.50
Any individual unspecified impurity	—	Conductivity	USP Cyclophosphamide Related Compound D RS	0.20
Total impurities[j]	—	—	—	5.0

[a] The relative retention times are measured with respect to cyclophosphamide for UV detection and to cyclophosphamide related compound D for conductivity detection.

[b] 3-(2-Chloroethyl)-2-oxo-2-hydroxy-1,3,6,2-oxadiazaphosphonane.

[c] 2,3,4,5,6-Pentahydroxyhexyl [3-(piperazin-1-yl)propyl] hydrogen phosphate.

[d] 3-({2-[(2-Chloroethyl)amino]ethyl}amino)propyl (2,3,4,5,6-pentahydroxyhexyl) hydrogen phosphate.

[e] 2-[Bis(2-hydroxyethyl)amino]-1,3,2-oxazaphosphinane 2-oxide.

[f] 3-[2-(2-Chloroethylamino)ethylamino]propyl dihydrogen phosphate.

[g] (2-Chloroethyl){2-[(3-hydroxypropyl)amino]ethyl}phosphoramidic acid.

[h] Bis(2-chloroethyl)amine hydrochloride.

[i] 3-[2-(2-Chloroethylamino)ethylamino]propyl 3-aminopropyl dihydrogen diphosphate.

[j] The total impurities are the sum of the impurities in this table and cyclophosphamide related compound C in the *Limit of Cyclophosphamide Related Compound C: Procedure for the Lyophilized Formulation* test.

- **Limit of Cyclophosphamide Related Compound C: Procedure for the Lyophilized Formulation**

Solution A: 10 mM ammonium acetate in water. Adjust with acetic acid to a pH of 4.6.

Solution B: Methanol

Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	100	0
7.5	100	0
7.7	85	15
10.0	85	15
12.0	15	85
20.0	15	85
22.5	100	0
30.0	100	0

Diluent: 7.5 mg/mL of mannitol in water

Standard stock solution: 0.44 mg/mL of USP Cyclophosphamide Related Compound C RS in water

Sensitivity solution: 0.007 mg/mL of USP Cyclophosphamide Related Compound C RS in *Diluent*, from *Standard stock solution*

Standard solutions C1–C5: Prepare solutions at concentrations of 0.0088 mg/mL (C1), 0.022 mg/mL (C2), 0.044 mg/mL (C3), 0.088 mg/mL (C4), and 0.132 mg/mL (C5) in *Diluent*, from *Standard stock solution*.

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

Detector: Evaporative light scattering

Nitrogen gas flow: 1.5 mL/min

Nitrogen pressure: 3.5 bar

Column: 4.6-mm × 25-cm; 5-µm packing L109

Temperatures

Autosampler: 5°

Column: 20°

Detector: 55°

Flow rate: 0.8 mL/min

Injection volume: 20 µL

System suitability

Samples: *Sensitivity solution* and *Standard solution C3*

Suitability requirements

Relative standard deviation: NMT 10%, *Standard solution C3*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solutions C1–C5* and *Sample solution*

Calibration curve: Perform one injection for each concentration of the *Standard solutions*. Plot the logarithm of the peak areas of *Standard solutions C1–C5* versus the logarithm of their concentrations, in mg/mL. The linear regression coefficient is NLT 0.99.

Perform one injection for each *Sample solution*. Determine the concentration (C_S) of cyclophosphamide related compound C in the *Sample solution* from *Calibration curve*.

Calculate the percentage of cyclophosphamide related compound C in the portion of Cyclophosphamide for Injection taken:

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

C_S = concentration of cyclophosphamide related compound C in the *Sample solution* (mg/mL)

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

Acceptance criteria: NMT 1.0% for cyclophosphamide related compound C

PERFORMANCE TESTS

• **Uniformity of Dosage Units ⟨905⟩:** Meets the requirements

SPECIFIC TESTS

• **Limit of Chloride for the Lyophilized Formulation**

Sample solution: Dissolve an amount equivalent to 2.0 g of anhydrous cyclophosphamide, from Cyclophosphamide for Injection, in 30 mL of water. Add 80 mL of isopropyl alcohol and 5 mL of 10% nitric acid.

Titrimetric system

(See *Titrimetry ⟨541⟩*.)

Mode: Direct titration

Titrant: 0.01 N silver nitrate VS

Endpoint detection: Potentiometric

Analysis: Titrate potentiometrically with *Titrant*. Perform a blank determination, and make any necessary correction. Each 1.0 mL of 0.01 N silver nitrate equals 0.355 mg of chloride ion.

Calculate the percentage of chloride in the portion of Cyclophosphamide for Injection taken:

$$\text{Result} = [(V_S - V_B) \times N_A \times F \times 100]/[N_T \times W \times (100 - A)/100]$$

V_S = *Titrant* volume consumed by the sample (mL)

V_B = *Titrant* volume consumed by the blank (mL)

N_A = actual normality of the *Titrant*

F = equivalency factor, 0.355 mg of chloride ion/mL of N_T

N_T = theoretical normality of the *Titrant*, 0.01 N

W = sample weight (mg)

A = assay correction for water

Acceptance criteria: NMT 1.4%

• **pH ⟨791⟩**

Sample solution: Nominally 20 mg/mL of anhydrous cyclophosphamide, determined 30 min after preparation

Acceptance criteria

For the sterile powder formulation: 3.0–9.0, but the range does not exceed 3 pH units

For the lyophilized formulation: 3.0–6.4

• **Water Determination ⟨921⟩, *Method I***

Sample for sterile powder formulation: Proceed as directed in the chapter.

Sample solution for lyophilized formulation: 10 mg/mL of anhydrous cyclophosphamide prepared as follows. Transfer an appropriate amount of the drug product in anhydrous methanol. Shake or sonicate for 15 min, and allow the suspension to rest. Use 10 mL of the supernatant.

Acceptance criteria: 4.6%–7.0% for the sterile powder formulation; NLT 6.4% for the lyophilized formulation based on the anhydrous cyclophosphamide in the drug product.

• **Bacterial Endotoxins Test ⟨85⟩:** NMT 0.0625 USP Endotoxin Units/mg of cyclophosphamide

• **Sterility Tests ⟨71⟩:** Meets the requirements

• **Constituted Solution:** At the time of use, it meets the requirements for *Injections and Implanted Drug Products ⟨1⟩, Product Quality Tests Common to Parenteral Dosage Forms, Specific Tests, Completeness and clarity of solutions*.

• **Other Requirements:** It meets the requirements in *Labeling ⟨7⟩, Labels and Labeling for Injectable Products*.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve as described in *Packaging and Storage Requirements ⟨659⟩, Injection Packaging, Packaging for Constitution*. Storage at a temperature not exceeding 25° is recommended. It will withstand brief exposure to temperatures up to 30° but is to be protected from temperatures above 30°.

• **Labeling:** The labeling should indicate that it is either the sterile powder formulation or the lyophilized formulation.

• **USP Reference Standards ⟨11⟩**

USP Cyclophosphamide RS

USP Cyclophosphamide Related Compound A RS

Bis(2-chloroethyl)amine hydrochloride.

$C_4H_9Cl_2N \cdot HCl$ 178.49

USP Cyclophosphamide Related Compound B RS

3-(2-Chloroethyl)-2-oxo-2-hydroxy-1,3,6,2-oxadiazaphosphonane.

$C_7H_{16}ClN_2O_3P$ 242.64

USP Cyclophosphamide Related Compound C RS

3-Aminopropyl dihydrogen phosphate.

$C_3H_{10}NO_4P$ 155.09

USP Cyclophosphamide Related Compound D RS

3-[2-(2-Chloroethylamino)ethylamino]propyl dihydrogen phosphate dihydrochloride.

$C_7H_{18}ClN_2O_4P \cdot 2HCl$ 333.58

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

Topic/Question	Contact	Expert Committee
CYCLOPHOSPHAMIDE FOR INJECTION	Documentary Standards Support	SM32020 Small Molecules 3

Chromatographic Database Information: Chromatographic Database

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