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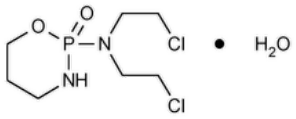
Cyclophosphamide



$C_7H_{15}Cl_2N_2O_2P \cdot H_2O$ 279.10
$C_7H_{15}Cl_2N_2O_2P$ 261.09
2*H*-1,3,2-Oxazaphosphorin-2-amine, *N*,*N*-bis(2-chloroethyl)tetrahydro-, 2-oxide, monohydrate, (±);
(±)-2-[Bis(2-chloroethyl)amino]tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-oxide monohydrate CAS RN®: 6055-19-2; UNII: 8N3DW7272P.
Anhydrous CAS RN®: 50-18-0; UNII: 6UXW23996M.

DEFINITION

Cyclophosphamide contains NLT 97.0% and NMT 103.0% of $C_7H_{15}Cl_2N_2O_2P$, calculated on the anhydrous basis.

[**Caution—**Great care should be taken in handling Cyclophosphamide, as it is a potent cytotoxic agent.]

IDENTIFICATION

Change to read:

• **A.** ▲**Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*: 197K**▲ (CN 1-May-2020)

• **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

Change to read:

• **Procedure**

Mobile phase: Acetonitrile and water (3:7)

Ethylparaben solution: Dissolve 185 mg of ethylparaben in 250 mL of alcohol in a 1000-mL volumetric flask, and dilute with water to volume.

System suitability solution: Transfer USP Cyclophosphamide RS, equivalent to 25 mg of anhydrous cyclophosphamide, to a 50-mL volumetric flask, add 25 mL of water, and shake to dissolve the USP Reference Standard. Add 5.0 mL of *Ethylparaben solution*, and dilute with water to volume.

Standard solution: 0.5 mg/mL of USP Cyclophosphamide RS in water

Sample solution: 0.5 mg/mL of 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 size: 25 µL

System suitability

Sample: *System suitability solution*

[Note—The relative retention times for cyclophosphamide and ethylparaben are about 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2 between cyclophosphamide and ethylparaben

Relative standard deviation: NMT 2% from six replicate injections, cyclophosphamide peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_7H_{15}Cl_2N_2O_2P$ in the Cyclophosphamide taken:

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

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Cyclophosphamide RS in the *Standard solution* (mg/mL) ▲▲ (ERR 1-Oct-2018)

C_U = concentration of Cyclophosphamide in the *Sample solution* (mg/mL) ▲▲ (ERR 1-Oct-2018)

Acceptance criteria: 97.0%–103.0% on the anhydrous basis

IMPURITIES

Inorganic Impurities

Organic Impurities

- **Procedure 1: Limit of Propanolamine**

Diluent: Methylene chloride and dehydrated alcohol (17:3)

Standard solution: 12.5 µg/mL of USP Propanolamine RS in *Diluent*. [Note—Propanolamine in the *Standard solution* is 0.025% of Cyclophosphamide in the *Sample solution*.]

Sample solution: 50 mg/mL of Cyclophosphamide in *Diluent*

Chromatographic system

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

Mode: TLC

Adsorbent: 0.1-mm layer of chromatographic silica gel

Application volume: 2 µL

Developing solvent system A: Toluene, methylene chloride, and methanol (5:5:1). Prepare at time of use.

Developing solvent system B: Methanol and glacial acetic acid (9:1)

Solution A: Hydrochloric acid and water (7:18)

Solution B: 5 g/L of potassium permanganate in water

Reagent A: *Solution A* and *Solution B* (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 closed TLC chamber (placed in a fume hood).]

Reagent B: 100 mg of tetramethylbenzidine in 2.5 mL of methylene chloride, and diluted with cyclohexane to 100 mL

Analysis

Samples: *Standard solution* and *Sample solution*

Develop with *Developing solvent system A* over a path of 7 cm followed by air drying for 15 min. Develop again in *Developing solvent system B* over a path of 2 cm followed by air drying for NLT 10 min. [Note—Transfer *Developing solvent system B* to the chamber 15 min before development.] Dry the plate at 45° under a vacuum for 50 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 10 min. Remove the plate and place it in a fume hood for 10 min to remove the excess chlorine. Stain the plate by dipping it into *Reagent B*. Remove it from *Reagent B* and wait for 15 min, evaluate it with a suitable densitometer, equipped with a filter having its maximum transmittance at 375 nm, and locate and scan the spot produced by propanolamine from the *Standard solution* and any spot from the *Sample solution* having the same R_F as that produced by propanolamine from the *Standard solution*.

Acceptance criteria

Propanolamine: The spot of propanolamine from the *Sample solution* is not more intense than the spot of propanolamine from the *Standard solution* (0.025%).

- **Procedure 2: Limit of Degradation Products**

Diluent: Methanol and water (1:1)

Standard solution A: 12 µg/mL of USP Cyclophosphamide Related Compound A RS in *Diluent*

Standard solution B: 12 µg/mL of USP Cyclophosphamide Related Compound B RS in *Diluent*

Standard solution C: 12 µg/mL of USP Cyclophosphamide Related Compound C RS in *Diluent*

Standard solution D: 15 µg/mL of USP Cyclophosphamide Related Compound D RS in *Diluent*. [Note—Cyclophosphamide related compound D is free base (M_r = 260.66) and USP Cyclophosphamide Related Compound D RS is available as dihydrochloride salt (M_r = 333.58).]

Standard solution E: 12 µg/mL of USP Cyclophosphamide RS in *Diluent*

Sample solution: 20 mg/mL of Cyclophosphamide in *Diluent*

Chromatographic system

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

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture containing a fluorescent indicator

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 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 the *Analysis* below.]

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 the 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 a 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

The spot of cyclophosphamide related compound A from the *Sample solution* is not more intense than the spot of cyclophosphamide related compound A from *Standard solution A* (0.06%).

The spot of cyclophosphamide related compound B from the *Sample solution* is not more intense than the spot of cyclophosphamide related compound B from *Standard solution B* (0.06%).

The spot of cyclophosphamide related compound C from the *Sample solution* is not more intense than the spot of cyclophosphamide related compound C from *Standard solution C* (0.06%).

The spot of cyclophosphamide related compound D from the *Sample solution* is not more intense than the spot of cyclophosphamide related compound D from *Standard solution D* (0.06%).

The spot of any individual unspecified impurity in the *Sample solution* is not more intense than the spot of cyclophosphamide from *Standard solution E* (0.06%).

Individual impurities: See *Impurity Table 1*.

Impurity Table 1

Name	Retardation Factor	Acceptance Criteria, NMT (%)
Cyclophosphamide related compound D[a]	0.15	0.06
Cyclophosphamide related compound C[b]	0.20	0.06
Cyclophosphamide related compound B[c]	0.43	0.06
Cyclophosphamide related compound A[d]	0.90	0.06
Any unspecified impurity	—	0.06

[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.

SPECIFIC TESTS

• **Limit of Chloride**

Sample solution: Dissolve 2.0 g of Cyclophosphamide in 30 mL of water, and add 80 mL of isopropyl alcohol and 5 mL of 10% nitric acid.

Analysis: Titrate potentiometrically with 0.01 N silver nitrate VS. Perform a blank determination, and make any necessary correction (see *Titrimetry* ⟨541⟩). 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 taken:

$$\text{Result} = [(V - B) \times N \times F \times 100]/[TN \times W \times (100 - A)/100]$$

V = sample titrant volume (mL)

B = blank titrant volume (mL)

N = titrant normality

F = equivalence factor, 0.355 mg of chloride ion/mL of TN

TN = theoretical normality, 0.01 N

W = sample weight (mg)

A = assay correction for water

Acceptance criteria: NMT 0.033%

• **Limit of Phosphate**

Diluent: 0.2 g/mL of hydrochloric acid in water

Solution A: Heat 20 g of tin with 85 mL of hydrochloric acid until no more hydrogen is released. Allow to cool. Transfer 1.0 mL of this solution into a 10-mL volumetric flask, and dilute with *Diluent* to volume.

Standard stock solution: 0.72 g/L of monobasic potassium phosphate. Transfer 1.0 mL of this solution into a 100-mL volumetric flask, and dilute with water to volume. Prepare immediately before use.

Standard solution: *Standard stock solution* and water (1:49). Prepare immediately before use. [Note—This solution contains 100 µg/L of PO_4.]

Sample solution: Dissolve 100 mg of Cyclophosphamide in water, and dilute to 100 mL.

Analysis: To the *Sample solution* add 4 mL of sulfomolybdic acid TS. Shake and add 0.1 mL of *Solution A*. Prepare a standard in the same manner using the *Standard solution*. After 10 min, compare the colors using 20 mL of each solution in color comparison tubes in diffused daylight, viewing vertically against a white background.

Acceptance criteria: Any color from the *Sample solution* is not more intense than that from the *Standard solution* (NMT 0.01%).

• **Bacterial Endotoxins Test** ⟨85⟩**:** Where the label states that Cyclophosphamide is sterile, it contains NMT 0.0625 USP Endotoxin Unit/mg of cyclophosphamide.

• **Sterility Tests** ⟨71⟩**:** Where the label states that Cyclophosphamide is sterile, it meets the requirements.

• **pH** ⟨791⟩**:** 3.9–7.1, in a solution (1 in 100), determined 30 min after its preparation

• **Water Determination,** *Method I* ⟨921⟩**:** 5.7%–6.8%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers at a temperature between 2° and 30°.

• **Labeling:** Where the label states that Cyclophosphamide is sterile, the tests for *Bacterial Endotoxins Test* ⟨85⟩ and *Sterility Tests* ⟨71⟩ should be performed.

Change to read:

• **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

▲▲ (CN 1-May-2018)

USP Propanolamine RS

3-Aminopropan-1-ol.

C_3H_9NO 75.11

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

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

Chromatographic Database Information: Chromatographic Database

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