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Cyclophosphamide Capsules

DEFINITION

Cyclophosphamide Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of anhydrous cyclophosphamide ($C_7H_{15}Cl_2N_2O_2P$).

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

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*.

Add the following:

▲• **B. Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*:** 197K

Sample: Transfer an appropriate amount of the Capsule contents containing nominally 100 mg of cyclophosphamide into a suitable beaker. Add 25 mL of chloroform and sonicate for NLT 20 min. Pass the solution through a suitable filter of 0.45-µm pore size. Transfer about 2 mL of the filtrate and mix the filtrate with 300 mg of potassium bromide. Evaporate the chloroform and prepare the pellet.

Acceptance criteria: Meet the requirements▲ (USP 1-Dec-2024)

ASSAY

• **Procedure**

Store the solutions containing cyclophosphamide at 2°–8° and protect from light.

Mobile phase: Acetonitrile and water (25:75)

Standard solution: 0.25 mg/mL of USP Cyclophosphamide RS in water

Sample solution: Nominally 0.25 mg/mL of anhydrous cyclophosphamide prepared as follows. Open and drop 10 Capsules (including Capsule shells) in a 1000-mL volumetric flask. Add about 500 mL of water and shake mechanically for NLT 20 min. Dilute with water to volume. Conduct a second dilution of the solution in water as needed. Pass a portion of the solution obtained through a suitable filter of 0.45-µm pore size and discard the first few milliliters.

Chromatographic system

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

Mode: LC

Detector: UV 195 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Temperatures

Autosampler: 4°

Column: 30°

Flow rate: 1.5 mL/min

Injection volume: 25 µL

Run time: NLT 1.5 times the retention time of cyclophosphamide

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 Capsules 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: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **Dissolution** ⟨711⟩

Store the solutions containing cyclophosphamide at 2°–8° and protect from light.

Dissolution procedure: Perform the test using the conditions under *Tier 1*. In the presence of cross-linking, repeat the test with new Capsules using the conditions under *Tier 2*; alternatively, the type and amount of enzymes as stated in *Dissolution ⟨711⟩, For Dosage Forms Containing or Coated with Gelatin* can be used.

Tier 1

Medium: Deaerated water; 900 mL

Apparatus 1: 100 rpm

Time: 15 min

Tier 2

Medium: Prepare a solution containing pepsin with pepsin activity of 125,000 units/L in deaerated water, mix well, and use within 4 h of preparation; 900 mL.

Apparatus 1: 100 rpm

Time: 15 min

Mobile phase: Acetonitrile and 0.1% (v/v) glacial acetic acid (20:80)

Standard solution: 0.05 mg/mL of USP Cyclophosphamide RS in the corresponding *Medium* that is used for the *Dissolution procedure*

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size, discard NLT 10 mL, and use the filtrate for analysis.

Chromatographic system

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

Mode: LC

Detector: UV 195 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Temperatures

Autosampler: 4°

Column: 30°

Flow rate: 1.3 mL/min

Injection volume: 100 µL

▲**Run time:** NLT 1.3 times the retention time of cyclophosphamide▲ (USP 1-Dec-2024)

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of anhydrous cyclophosphamide ($C_7H_{15}Cl_2N_2O_2P$) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

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)

V = volume of *Medium*, 900 mL

L = label claim (mg/Capsule)

Tolerances: NLT 80% (*Q*) of the labeled amount of anhydrous cyclophosphamide ($C_7H_{15}Cl_2N_2O_2P$) is dissolved

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

IMPURITIES

Change to read:

• **Organic Impurities**

Solution A: 1.7 g/L of potassium phosphate, dibasic in water. Adjust with phosphoric acid to a pH of 7.0.

Solution B: Acetonitrile and *Solution A* (75:25)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
7	100	0
20	65	35
35	65	35
40	40	60
41	100	0
50	100	0

System suitability solution: 5 mg/mL of USP Cyclophosphamide RS and 10 µg/mL of USP Cyclophosphamide Related Compound A RS in water

Sensitivity solution: 5 µg/mL of USP Cyclophosphamide RS in water

Standard solution: 10 µg/mL each of USP Cyclophosphamide RS, USP Cyclophosphamide Related Compound A RS, and USP Cyclophosphamide Related Compound B RS in water

Sample solution: Nominally 5 mg/mL of anhydrous cyclophosphamide prepared as follows. Transfer an appropriate amount of the composite sample from 20 Capsules into a suitable volumetric flask. Add 70% of the flask volume of water, and sonicate for NLT 10 min with intermittent swirling. Cool to room temperature and dilute with water to volume. Allow the solution to settle for about 5 min before passing the supernatant through a filter of 0.45-µm pore size. Discard the first few milliliters and use the filtrate for analysis. [Note—Inject sample solutions immediately after preparation.]

Chromatographic system

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

Mode: LC

Detector: UV 195 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Temperatures

Autosampler: 5°

Column: 40°

Flow rate: 1 mL/min

Injection volume: 100 µL

System suitability

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

▲[Note—The relative retention times in *Table 2* are provided as information that could aid in peak assignment.]

Table 2▲ (USP 1-Dec-2024)

Suitability requirements

Name	Relative Retention Time R
Cyclophosphamide related compound B	0.14
Cyclophosphamide related compound A	0.90
Cyclophosphamide	1.0

esolution: NLT 3.0 between cyclophosphamide related compound A and cyclophosphamide, *System suitability solution*

Relative standard deviation: NMT 5.0% for cyclophosphamide, cyclophosphamide related compound A, and cyclophosphamide related compound B, *Standard solution*

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

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of cyclophosphamide related compound A in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

r_U = peak response of cyclophosphamide related compound A from the *Sample solution*

r_S = peak response of cyclophosphamide related compound A from the *Standard solution*

C_S = concentration of USP Cyclophosphamide Related Compound A RS in the *Standard solution* (mg/mL)

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

M_{r1} = molecular weight of cyclophosphamide related compound A free base, 142.02

M_{r2} = molecular weight of cyclophosphamide related compound A hydrochloride, 178.49

Calculate the percentage of cyclophosphamide related compound B in the portion of Capsules taken:

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

r_U = peak response of cyclophosphamide related compound B from the *Sample solution*

r_S = peak response of cyclophosphamide related compound B from the *Standard solution*

C_S = concentration of USP Cyclophosphamide Related Compound B RS in the *Standard solution* (mg/mL)

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

Calculate the percentage of any unspecified degradation product in the portion of Capsules taken:

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

r_U = peak response of any unspecified degradation product 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: See *Table 3*. Disregard the peak at the relative retention time of 0.20 due to cyclophosphamide related compound D. The reporting threshold is 0.1%.

Table 3

Name	Acceptance Criteria, NMT (%)
Cyclophosphamide related compound B	0.2
Cyclophosphamide related compound A	0.2
▲▲ (USP 1-Dec-2024)	▲▲ (USP 1-Dec-2024)
Any unspecified degradation product	0.2
Total degradation products[a]	0.6

[a] Do not include cyclophosphamide related compound D, determined separately under the test for the *Limit of Cyclophosphamide Related Compound D*.

• **Limit of Cyclophosphamide Related Compound D**

Diluent: Methanol and water (50:50)

Standard solution: 0.05 mg/mL of USP Cyclophosphamide Related Compound D RS in *Diluent*, equivalent to 0.04 mg/mL of cyclophosphamide related compound D free base

Sample solution: Nominally 20 mg/mL of anhydrous cyclophosphamide prepared as follows. Transfer an appropriate amount of the composite sample from the contents of 20 Capsules into a suitable volumetric flask. Dissolve in *Diluent* with the aid of sonication. Dilute with *Diluent* to volume. Pass the solution through a suitable filter of 0.45-µm pore size and discard the first few milliliters.

Chromatographic system

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

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 20 µL

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

Reagent A: Dissolve 3.2 g of potassium permanganate in 1000 mL of 5% (v/v) hydrochloric acid in water. [Note—Operate in a fume hood. Mix in a small beaker at the time of use to generate chlorine gas, and immediately place the beaker with solution into a closed TLC chamber.]

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

Analysis

Samples: *Standard solution* and *Sample solution*

Develop the chromatogram in the solvent system until the solvent front has moved 10 cm from the point of spotting. Dry the plate at room temperature in a fume hood for NLT 15 min. Develop again in a fresh portion of the developing solvent system until the solvent front has moved another 10 cm from the previous point of spotting. Dry the plate at room temperature in a fume hood for NLT 15 min. Heat the plate at 50° under vacuum for NLT 20 min, or use a TLC heating plate at 50° for NLT 20 min in a fume hood. Cool the plate at room temperature for 5 min.

Place the plate in a closed chromatography tank containing *Reagent A* for NLT 15 min. Remove the plate and place it in the fume hood for 15 min to remove the excess chlorine. Spray the plate with *Reagent B*.

Acceptance criteria: The spot of cyclophosphamide related compound D in the *Sample solution* is not more intense than the spot of cyclophosphamide related compound D in the *Standard solution* (0.2%).

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers, and store at controlled room temperature.

• **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 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 CAPSULES	Documentary Standards Support	SM32020 Small Molecules 3

Chromatographic Database Information: Chromatographic Database

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