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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