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.
- **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
  USP Endotoxin RS
  USP Propanolamine RS
  3-Aminopropan-1-ol.
  C_3H_9NO 75.11

Cyclophosphamide for Injection

» Cyclophosphamide for Injection is a sterile mixture of Cyclophosphamide with or without a suitable diluent. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous cyclophosphamide ($C_7H_{15}Cl_2N_2O_2P$).

Packaging and storage—Preserve in *Containers for Sterile Solids* as described under *Injections* ⟨1⟩. 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°.

USP Reference standards ⟨11⟩**—**
USP Cyclophosphamide RS
USP Endotoxin RS

Constituted solution—At the time of use, it meets the requirements for *Constituted Solutions* under *Injections* ⟨1⟩.

Identification—

A: It responds to the *Thin-layer Chromatographic Identification Test* ⟨201⟩, a solution of it in chloroform, equivalent to 20 mg of cyclophosphamide per mL, filtered if necessary,

being used as the test solution. Apply 5 µL of the test solution and the Standard solution, use a solvent system consisting of a mixture of chloroform, methanol, and ammonium hydroxide (75:20:5), and visualize the spots by placing the plate in an iodine chamber.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay*.

Bacterial endotoxins ⟨85⟩**—**It contains not more than 0.20 USP Endotoxin Unit per mg of cyclophosphamide.

pH ⟨791⟩: between 3.0 and 9.0, but the range does not exceed 3 pH units, in a solution containing the equivalent of 20 mg of anhydrous cyclophosphamide per mL, determined 30 minutes after its preparation.

Other requirements—It meets the requirements for *Sterility Tests* ⟨71⟩, *Uniformity of Dosage Units* ⟨905⟩, and *Labeling* under *Injections* ⟨1⟩.

Assay—

Mobile phase—Prepare a suitable, degassed solution of water and acetonitrile (70:30).

Internal standard solution—Dissolve 185 mg of ethylparaben in 250 mL of alcohol in a 1000-mL volumetric flask, dilute with water to volume, and mix.

Standard preparation—Transfer an accurately weighed quantity of USP Cyclophosphamide RS, equivalent to about 25 mg of anhydrous cyclophosphamide, to a 50-mL volumetric flask, add about 25 mL of water, and shake to dissolve the USP Reference Standard. Add 5.0 mL of *Internal standard solution*, dilute with water to volume, and mix to obtain a *Standard preparation* having a known concentration of about 0.5 mg of anhydrous cyclophosphamide per mL.

Assay preparation—Accurately weigh a portion of Cyclophosphamide for Injection, equivalent to about 200 mg of anhydrous cyclophosphamide, to a 200-mL volumetric flask, add about 50 mL of water, and shake for about 5 minutes, dilute with water to volume, and mix. Pipet 25 mL of this solution and 5 mL of *Internal standard solution* into a 50-mL volumetric flask, dilute with water to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatography is equipped with a 195-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph six replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation is not more than 2%, and the resolution factor between cyclophosphamide and ethylparaben is not less than 2.

Procedure—Separately inject equal volumes (about 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.7 for cyclophosphamide and 1.0 for ethylparaben. Calculate the quantity, in mg, of $C_7H_{15}Cl_2N_2O_2P$ in the portion of Cyclophosphamide for Injection taken by the formula:

$$400C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of anhydrous cyclophosphamide in the *Standard preparation,* as determined from the concentration of USP Cyclophosphamide RS corrected for moisture by a titrimetric water determination; and R_U and R_S are the ratios of the peak responses of cyclophosphamide to those of the internal standard in the *Assay preparation* and the *Standard preparation*, respectively.

Cyclophosphamide Tablets

» Cyclophosphamide Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous cyclophosphamide ($C_7H_{15}Cl_2N_2O_2P$).

Packaging and storage—Preserve in tight containers. Storage at a temperature not exceeding 25° is recommended. Tablets will withstand brief exposure to temperatures up to 30°, but are to be protected from temperatures above 30°.

USP Reference standards ⟨11⟩**—**
USP Cyclophosphamide RS

Identification—

A: Extract a portion of finely powdered Tablets, equivalent to about 50 mg of cyclophosphamide, with 25 mL of chloroform, filter about 2 mL of the chloroform solution, mix the filtrate with 500 mg of potassium bromide, evaporate the chloroform, carefully removing the last trace of solvent in a small vacuum flask, and use the residue to prepare a potassium bromide dispersion: the IR absorption spectrum of the potassium bromide dispersion so obtained exhibits maxima, between 6.5 and 14 µm, only at the same wavelengths as that of a similar preparation of USP Cyclophosphamide RS.

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation,* as obtained in the *Assay*.

Dissolution ⟨711⟩**—**

Medium: water; 900 mL, deaerated.

Apparatus: 1:100 rpm.

Time: 45 minutes.

Determine the amount of $C_7H_{15}Cl_2N_2O_2P$ dissolved by employing the following method.

Mobile phase—Prepare a suitable filtered and degassed mixture of water and acetonitrile (7:3). Make adjustments if necesary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard solution—Dissolve an accurately weighed quantity of USP Cyclophosphamide RS in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration corresponding to that of the solution under test.

Test solution—Use portions of the solution under test passed through a 0.8-µm filter.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 195-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the tailing factor is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 50 µL) of the *Standard solution* and *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peak. Calculate the amount of cyclophosphamide ($C_7H_{15}Cl_2N_2O_2P$) dissolved by the formula:

$$900C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Cyclophosphamide RS in the *Standard solution;* and r_U and r_S are the peak responses for cyclophosphamide obtained from the *Test solution* and *Standard solution,* respectively.