Cyclophosphamide for Injection

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Chemical Medicines Monographs 3

**Reason for Revision**  
Compliance

In accordance with the Rules and Procedures of the 2015–2020 Council of Experts, the Chemical Medicines Monographs 3 Expert Committee has revised the Cyclophosphamide for Injection monograph. The purpose for the revision is to widen the acceptance criteria of the sterile powder formulation of *Water Determination* from 6.0%–6.8% to 4.6%–7.0% to be consistent with the FDA-approved specification.

The Cyclophosphamide for Injection Revision Bulletin supersedes the currently official monograph.

Should you have any questions, please contact Jane Li, Associate Scientific Liaison (301-230-6345 or jane.li@usp.org).
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\textsubscript{10}H\textsubscript{7}Cl\textsubscript{2}N\textsubscript{2}O\textsubscript{3}P). The lyophilized formulation contains NLT 90.0% and NMT 110.0% of the labeled amount of anhydrous cyclophosphamide (C\textsubscript{10}H\textsubscript{7}Cl\textsubscript{2}N\textsubscript{2}O\textsubscript{3}P).

IDENTIFICATION
• A. INFRARED ABSORPTION (197K)
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 diethyl ether that is saturated with water. Cyclophosphamide crystallizes from this solution after a few minutes. Remove diethyl ether 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 x 30-cm; packing L1
Flow rate: 1.5 mL/min
Injection volume: 25 µL
System suitability
Sample: Standard solution
Suitability requirements
Tailing factor: NMT 1.0%
Relative standard deviation: NMT 1.0%
Analysis
Samples: Standard solution and Sample solution
Calculate the percentage of anhydrous cyclophosphamide (C\textsubscript{10}H\textsubscript{7}Cl\textsubscript{2}N\textsubscript{2}O\textsubscript{3}P) in the portion of Cyclophosphamide for Injection taken:

\[
\text{Result} = \left( \frac{r_d}{r_s} \right) \times \left( \frac{C_s}{C_d} \right) \times 100
\]

\( r_d \) = 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)
\( C_d \) = 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
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
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...
2 Cyclophosphamide

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

<table>
<thead>
<tr>
<th>Name</th>
<th>Retardation Factor</th>
<th>Acceptance Criteria, NMT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclophosphamide related compound D&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>Cyclophosphamide related compound C&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.20</td>
<td>0.15</td>
</tr>
<tr>
<td>Cyclophosphamide related compound B&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.43</td>
<td>0.15</td>
</tr>
<tr>
<td>Cyclophosphamide related compound A&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.90</td>
<td>0.15</td>
</tr>
<tr>
<td>Any unspecified impurity</td>
<td>—</td>
<td>0.11</td>
</tr>
</tbody>
</table>

<sup>a</sup> 3-[2-(2-Chloroethylamino)ethylamino]propyl dihydrogen phosphate.

<sup>b</sup> 3-Aminopropyl dihydrogen phosphate.

<sup>c</sup> 3-(2-Chloroethyl)-2-oxo-2-hydroxy-1,3,6,2-oxadiazaphosphonane.

<sup>d</sup> 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

**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:

\[ \text{Result} = \left( \frac{r_u}{r_s} \right) \times \left( \frac{C_i}{C_0} \right) \times 100 \]

- \( r_u \) = 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)
- \( C_i \) = concentration of the corresponding USP Reference Standard in the Standard solution (mg/mL)
- \( C_0 \) = 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

<table>
<thead>
<tr>
<th>Name</th>
<th>Relative Retention Time&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Detection Mode</th>
<th>External Reference Standard</th>
<th>Acceptance Criteria, NMT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Impurity 1</td>
<td>0.23</td>
<td>Conductivity</td>
<td>—</td>
<td>Disregard</td>
</tr>
<tr>
<td>Cyclophosphamide related compound B&lt;sup*b&lt;/sup&gt;</td>
<td>0.49</td>
<td>UV</td>
<td>USP Cyclophosphamide Related Compound B RS</td>
<td>0.25</td>
</tr>
<tr>
<td>Piperazinylpropyl pentahydroxyhexyl phosphat&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.60–0.72</td>
<td>Conductivity</td>
<td>USP Cyclophosphamide Related Compound D RS</td>
<td>0.50</td>
</tr>
<tr>
<td>Chlorodiazinonyl pentahydroxyhexyl phosphat&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.77–0.86</td>
<td>Conductivity</td>
<td>USP Cyclophosphamide Related Compound D RS</td>
<td>0.30</td>
</tr>
<tr>
<td>Dihydroxy cyclophosphamide&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.80–0.91</td>
<td>Conductivity</td>
<td>USP Cyclophosphamide Related Compound D RS</td>
<td>1.0</td>
</tr>
<tr>
<td>Impurity 2</td>
<td>0.84</td>
<td>Conductivity</td>
<td>—</td>
<td>Disregard</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>1.0</td>
<td>UV</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>
Mobile phase:
Solution A: Methanol with acetic acid to a pH of 4.6.
Solution B: Methanol with acetic acid to a pH of 4.6.

Column:
3.5 bar Nitrogen pressure:
1.5 mL/min Nitrogen gas flow:
Detector:
Evaporative light scattering

Sample solution:
Perform one injection for each Sample solution. Determine the concentration \( C_i \) 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:

\[
\text{Result} = \left( \frac{C_i}{C_{ij}} \right) \times 100
\]

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

\( C_{ij} \) = 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

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### Table 2 (continued)

<table>
<thead>
<tr>
<th>Name</th>
<th>Relative Retention Time</th>
<th>Detection Mode</th>
<th>External Reference Standard</th>
<th>Acceptance Criteria, NMT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclophosphamide related compound D&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.0</td>
<td>Conductivity</td>
<td>USP Cyclophosphamide Related Compound D RS</td>
<td>2.0</td>
</tr>
<tr>
<td>Chlorodiiazinonyl phosphamid&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.19–1.31</td>
<td>Conductivity</td>
<td>USP Cyclophosphamide Related Compound D RS</td>
<td>0.50</td>
</tr>
<tr>
<td>Cyclophosphamide related compound A&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.40–1.70</td>
<td>Conductivity</td>
<td>USP Cyclophosphamide Related Compound A RS</td>
<td>2.0</td>
</tr>
<tr>
<td>Cyclophosphamide pyrophosphate analog&lt;sup&gt;g&lt;/sup&gt;</td>
<td>1.46–1.67</td>
<td>Conductivity</td>
<td>USP Cyclophosphamide Related Compound D RS</td>
<td>0.50</td>
</tr>
<tr>
<td>Any individual unspecified impurity</td>
<td>—</td>
<td>Conductivity</td>
<td>USP Cyclophosphamide Related Compound D RS</td>
<td>0.20</td>
</tr>
<tr>
<td>Total impurities&lt;sup&gt;c&lt;/sup&gt;</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>5.0</td>
</tr>
</tbody>
</table>

<sup>a</sup>The relative retention times are measured with respect to cyclophosphamide for UV detection and to cyclophosphamide related compound D for conductivity detection.
<sup>b</sup>3-(2-Chloroethyl)-2-oxo-2-hydroxy-1,3,6,2-oxadiazaphosphonane.
<sup>c</sup>2,3,4,5,6-Pentahydroxyhexyl [3-(piperazin-1-yl)propyl] hydrogen phosphate.
<sup>d</sup>3-(2-Chloroethyl)amino)[3-(piperazin-1-yl)propyl] hydrogen phosphate.
<sup>e</sup>2-(3-Hydroxypropyl)amino)[3-(piperazin-1-yl)propyl] hydrogen phosphate.
<sup>f</sup>2-[2-Chloroethyl]amine hydrochloride.
<sup>g</sup>3-[2-Chloroethyl]amino)[3-(piperazin-1-yl)propyl] hydrogen phosphate.

### Table 3 (continued)

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Solution A (%)</th>
<th>Solution B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>7.5</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>8.2</td>
<td>85</td>
<td>15</td>
</tr>
<tr>
<td>10.0</td>
<td>85</td>
<td>15</td>
</tr>
<tr>
<td>12.0</td>
<td>15</td>
<td>85</td>
</tr>
<tr>
<td>20.0</td>
<td>15</td>
<td>85</td>
</tr>
<tr>
<td>22.5</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

**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°
- **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 \( C_i \) 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:

\[
\text{Result} = \left( \frac{C_i}{C_{ij}} \right) \times 100
\]

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

\( C_{ij} \) = 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.

Titrmetric system
(See Titrmetry (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:

\[
\text{Result} = \frac{\left( V_s - V_b \right) \times N_a \times F \times 100}{N_t \times W \times (100 - A)/100}
\]

- \( V_s \): 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

Change to read:

• 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: NMT 4.6%–7.0%.

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