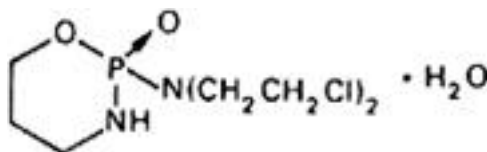


Cyclophosphamide (Cyclophosphamidum)**Molecular formula.** $C_7H_{15}Cl_2N_2O_2P \cdot H_2O$ **Relative molecular mass.** 279.1**Graphic formula.****Chemical name.** 2-[Bis(2-chloroethyl)amino]tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-oxide monohydrate; *N,N*-bis(2-chloroethyl)tetrahydro-2*H*-1,3,2-oxazaphosphorin-2-amine 2-oxide monohydrate; CAS Reg. No. 6055-19-2 (monohydrate).**Other name.** Cyclophosphanum.**Description.** A white, crystalline powder.**Solubility.** Soluble in water; freely soluble in ethanol (~750 g/l) TS; slightly soluble in ether R.**Category.** Cytotoxic drug.**Storage.** Cyclophosphamide should be kept in a tightly closed container and stored at a temperature between 2 and 30 °C.**Additional information.** CAUTION: Cyclophosphamide must be handled with care, avoiding contact with the skin and inhalation of airborne particles.**Requirements****Definition.** Cyclophosphamide contains not less than 98.0% and not more than 101.0% of $C_7H_{15}Cl_2N_2O_2P$, calculated with reference to the anhydrous substance.**Identity tests**

A. Dissolve 0.1 g in 10 mL of water and add 5 mL of silver nitrate (40 g/l) TS; no precipitate is produced. Boil; a white precipitate is produced, which is insoluble in nitric acid (~130 g/l) TS but soluble in ammonia (~100 g/l) TS from which it is reprecipitated by the addition of nitric acid (~130 g/l) TS.

B. Dissolve 20 mg in 1 mL of sulfuric acid (~100 g/l) TS and heat until white fumes are evolved. After cooling, add 5 mL of water and shake. Neutralize with ammonia (~100 g/l) TS, then acidify with nitric acid (~130 g/l) TS; this test yields reaction A described under [2.1 General identification tests](#) as characteristic of orthophosphates.

Melting range. 49-53 °C, determined without previous drying.**Clarity and colour of solution.** A solution of 0.20 g in 10 mL of water is clear and colourless.**Water.** Determine as described under [2.8 Determination of water by the Karl Fischer method](#), Method A, using about 0.3 g of the substance; the water content is not less than 55 mg/g and not more than 70 mg/g.**pH value.** pH of a 20 mg/mL solution in carbon-dioxide-free water R, determined 30 minutes after its preparation, 4.0-7.0.**Related substances.** Carry out the test as described under [1.14.1 Chromatography, Thin-layer chromatography](#), using silica gel R1 as the coating substance and a mixture of 50 volumes of benzene R, 25 volumes of chloroform R, and 25 volumes of methanol R as the mobile phase. Apply separately to the plate 10 µl of each of 2 solutions: (A) 25 mg of the test substance per mL of chloroform R and (B) 0.125 g of the test substance dissolved in 5.0 mL of water, boiled under a reflux condenser for 30 minutes, and then cooled to 20 °C. After removing the plate from the chromatographic chamber, allow it to dry in air and spray it with triketohydrindene/methanol TS. Examine the chromatogram in daylight. With solution B, a pale violet spot is obtained at an R_f value between 0.10 and 0.25; other spots could also appear. With solution A, a brown-violet spot is obtained with an R_f value between 3.50 and 5.50, and no other spot is obtained above this spot.**Assay.** To about 0.2 g, accurately weighed, add 20 mL of potassium hydroxide/ethanol (0.5 mol/l) VS. Boil under a reflux condenser for 1 hour, cool, then add 30 mL of water, 3 mL of nitric acid (~1000 g/l) TS and 20.0 mL of silver nitrate (0.1 mol/l) VS. Shake the flask, add 5 mL of diethyl phthalate R and titrate the excess of silver nitrate with ammonium thiocyanate (0.1 mol/l) VS, using 5 mL of ferric ammonium sulfate (45 g/l) TS as indicator. Repeat the operation without the substance being examined and make any necessary corrections. Each mL of silver nitrate (0.1 mol/l) VS is equivalent to 13.05 mg of $C_7H_{15}Cl_2N_2O_2P$.