

## Ganciclovir for injection (Gancicloviri ad injectionem)

2018-01

**Description.** A white powder or loose lumps.

**Category.** Antiviral (Purine nucleoside analogue).

**Storage.** Ganciclovir for injection should be kept in a tightly closed container, protected from moisture and light.

Additional information. Ganciclovir for injection 500 mg is listed on the 12th invitation to manufacturers of medicinal products for HIV infection and related diseases to submit an Expression of Interest (EOI) for product evaluation to the WHO Prequalification of Medicines Team.

Handle Ganciclovir for injection with great care because it is a potent cytotoxic agent and suspected carcinogen.

Ganciclovir for injection is hygroscopic.

### Requirements

The powder for injection and the reconstituted solution for injection complies with the monograph for [Parenteral preparations](#).

**Definition.** Ganciclovir for injection is a freeze-dried powder prepared by the neutralization of Ganciclovir with the aid of sodium hydroxide. Ganciclovir for injection contains not less than 90.0% and not more than 110.0% of the labelled amount of ganciclovir ( $C_9H_{13}N_5O_4$ ).

### Identity tests

-Either test A alone, or tests B and D or tests C and D may be applied.

A. Dilute a quantity of the test substance, containing the equivalent of 0.2 g of Ganciclovir with 10 mL water R. Adjust the suspension to pH 6–7 with hydrochloric acid (0.1 mol/L) TS and allow to stand for 30 minutes. Filter the suspension, wash the filtrate with 20 mL water R and dry it at 105 °C for 3 hours. Carry out the test as described under [1.7 Spectrophotometry in the infrared region](#). The infrared absorption spectrum is concordant with the reference spectrum of ganciclovir or with the spectrum obtained from ganciclovir RS treated similarly.

If the spectra thus obtained are not concordant repeat the test using the residues obtained by separately dissolving the dried filtrate and ganciclovir RS in a small amount of hot water R (80 °C), allowing to cool in an ice-bath, filtering and drying the precipitate at 105 °C for 3 hours. The infrared absorption spectrum is concordant with the spectrum obtained from ganciclovir RS.

B. Carry out test B.1 or, where UV detection is not available, test B.2.

B.1 Carry out the test as described under [1.14.1 Chromatography, Thin-layer chromatography](#) using silica gel R6 as the coating substance and a mixture of 4 volumes of ammonia (260 g/L) TS, 40 volumes of methanol R and 60 volumes of dichloromethane R as the mobile phase. Apply separately to the plate 5 µL of each of the following three solutions. For solution (A) dissolve a quantity of the test substance, containing the equivalent of 10 mg of ganciclovir in 2 mL water R and dilute to 10 mL with methanol R. For solution (B) dissolve 10 mg of ganciclovir RS in 2 mL of sodium hydroxide (0.8 g/L) TS and dilute to 10 mL with methanol R. After removing the plate from the chromatographic chamber allow it to dry exhaustively in air and examine the chromatogram under ultraviolet light (254 nm).

The principal spot in the chromatogram obtained with solution (A) corresponds in position, appearance and intensity with the spot due to ganciclovir in the chromatogram obtained with solution (B).

B.2 Carry out the test as described under [1.14.1 Chromatography, Thin-layer chromatography](#) using the conditions described above under test B.1 but using silica gel R5 as the coating substance. After removing the plate from the chromatographic chamber allow it to dry exhaustively in air or heat the plate for five minutes at 120 °C. Spray the plate with Dragendorff reagent TS and allow it to dry exhaustively in air. Then spray the plate with a mixture of sulfuric acid (~1760 g/L) TS and dehydrated ethanol R (1:1). Examine the chromatogram in daylight. The test is not valid unless the chromatogram obtained with solution (C) shows two clearly separated spots.

The principal spot in the chromatogram obtained with solution (A) corresponds in position, appearance and intensity with the spot due to ganciclovir in the chromatogram obtained with solution (B).

C. Carry out the test as described under [1.14.1 Chromatography, High-performance liquid chromatography](#) using the conditions given under "Assay". The retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to the retention time of the peak due to ganciclovir in the chromatogram obtained with solution (2).

D. Dissolve a quantity of the powder for injection equivalent to 20 mg of ganciclovir in 2 mL hydrochloric acid (~420 g/L) TS, evaporate the solution to dryness on a hot water-bath, add 1 mL hydrochloric acid (~420 g/L) TS and about 30 mg

potassium chlorate R. Then evaporate the solution to dryness on a hot water-bath and add drops of ammonia (~100 g/L) TS to the residues; a violet-red colour is produced. Add drops of sodium hydroxide (~40 g/L) TS and the violet-red colour disappears.

**pH value (1.13).** pH of a solution containing the equivalent to 12.5 mg of ganciclovir per mL of water R, 10.5–11.5.

**Clarity and colour of solution.** A solution, containing the equivalent to 0.10 g of ganciclovir in 10 mL of water R, is clear and not more intensely coloured than reference solution Y<sub>5</sub>, when compared as described under [1.11.2 Degree of coloration of liquids](#), Method II.

**Water.** Determine as described under [2.8 Determination of water by the Karl Fischer method](#), Method A, using 0.300 g of the substance and methanol as solvent. The substance to be examined has a limited solubility in methanol and will appear as a slurry. Replace the solvent after each titration. The water content is not more than 30 mg/g.

**Related substances.** Carry out the test as described under [1.14.4 High performance liquid chromatography](#) using the conditions given under "Assay".

Prepare the following solutions using mobile phase as a diluent. For test solution (1) dissolve a quantity of the powder for injection, containing the equivalent of about 30 mg ganciclovir, using sonication, and dilute to 50.0 mL. For solution (2) dilute 1 volume of solution (1) to 1000 volumes. For solution (3) dissolve using sonication 3.0 mg of ganciclovir RS and dilute to 5.0 mL. For solution (4) dissolve the content of a vial of ganciclovir for system suitability RS (containing the impurities A, B, C, D, E and F) in 1.0 mL of solution (3).

Inject alternately 20 µL of each of solutions (1), (2), (3) and (4). Record the chromatograms for 2.5 times of the retention time of ganciclovir (retention time 14 minutes).

Use the chromatogram supplied with ganciclovir for system suitability RS and the chromatogram obtained with reference solution (4) to identify the peaks due to ganciclovir and the impurities A, B, C, D, E and F. The following peaks are eluted at the following relative retention with reference to the peak of ganciclovir: impurity A = about 0.6; impurity B = about 0.67; impurity C = about 0.71; impurity D = about 0.8; impurity E = about 0.9; impurity F = about 2.0.

The test is not valid unless in the chromatogram obtained with solution (4) the peak-to-valley ratio ( $H_p/H_v$ ) is at least 5, where  $H_p$  is the height above the baseline of the peak due to impurity E and  $H_v$  is the height above the baseline of the lowest point of the curve separating this peak from the peak due to ganciclovir.

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity F, when multiplied by a correction factor of 0.7, is not greater than 4 times the area of the peak due to ganciclovir in the chromatogram obtained with solution (2) (0.4%);

**Assay.** Carry out the test as described under [1.14.1 Chromatography, High-performance liquid chromatography](#) using a stainless steel column (25 cm × 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically-bonded strong acidic cation-exchange groups (3–10 µm).

Use the following mobile phase: Dilute 0.5 mL of trifluoroacetic acid R to 1000 mL with water R. Mix 500 volumes of this solution with 500 volumes of acetonitrile R.

Operate with a flow rate of 1.5 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 254 nm. Maintain the column at 40 °C.

Weigh and mix the contents of 10 containers. Prepare the following solutions in mobile phase. For solution (1) dissolve a quantity of the powders for injection, equivalent to 30.0 mg of ganciclovir and dilute to 50.0 mL. Dilute 10.0 mL of this solution to 100.0 mL. For solution (2) dissolve 15.0 mg of ganciclovir RS, and dilute to 25.0 mL. Dilute 10.0 mL of this solution to 100.0 mL.

Inject alternately 20 µL each of solution (1) and (2).

Measure the areas of the peaks corresponding to ganciclovir in the chromatograms of solution (1) and (2) and calculate the percentage content of ganciclovir (C<sub>9</sub>H<sub>13</sub>N<sub>5</sub>O<sub>4</sub>) per container, using the declared content of C<sub>9</sub>H<sub>13</sub>N<sub>5</sub>O<sub>4</sub> in ganciclovir RS.

**Bacterial endotoxins.** Carry out the test as described under [3.4 Test for bacterial endotoxins](#); contains not more than 0.84 IU of endotoxin per mg of ganciclovir.

#### Impurities

- The impurities limited by the requirements of this monograph include impurity F listed in the monograph on Ganciclovir.