Aciclovir for injection (Acicloviri ad injectionem)

2014-01

Description. A white powder or loose lumps.

Category. Antiviral (Purine nucleoside analogue).

Storage. Preserve in tightly-closed containers. Protect from light and moisture.

Labelling. The label should state that the active ingredient is Aciclovir.

Additional information. Strength in the current WHO Model list of essential medicines: 250 mg (as sodium salt) in vial. Strength in the current WHO Model list of essential medicines for children: 250 mg (as sodium salt) in vial.

Requirements

The powder for injections and the reconstituted solution for injection comply with the monograph on *Parenteral preparations.*

Definition: Aciclovir for injection is a sterile powder prepared from Aciclovir with the aid of a suitable alkali. The container of Aciclovir for injection contains not less than 95.0% and not more than 105.0% of the labeled amount of aciclovir ($C_8H_{11}N_5O_3$).

Identity tests

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

A. To a quantity of the test substance, containing the equivalent of about 100 mg of aciclovir, add 10 mL water R, adjust to pH 4–7 with hydrochloric acid (0.1 mol/L) TS and allow to stand for 30 minutes. Filter, use 20 mL of water R to wash the precipitate and dry it at 105 °C for 3 hours. Carry out the test with the precipitate as described under *1.7 Spectrophotometry in the infrared region*. The infrared absorption spectrum is concordant with the spectrum obtained from aciclovir RS or with the reference spectrum of aciclovir. If the spectra thus obtained are not concordant repeat the test using the residues obtained by separately dissolving the precipitate obtained following the procedure above and aiclovir RS in a small amount of hot water R and evaporating on a water bath to dryness. Dry the residues at 100–105 °C for 3 h. The infrared absorption spectrum is concordant with the spectrum obtained from aciclovir RS.

B. Carry out the test as described under *1.14.1 Chromatography*, Thin-layer chromatography using the conditions given under Guanine and related substances test A. The principal spot in the chromatogram obtained with solution (B) corresponds in position, appearance and intensity to the spot due to aciclovir in the chromatogram obtained with solution (C).

C. Carry out the test as described under *1.14.1 Chromatography*, High-performance liquid chromatography using the conditions given under “Assay”, test A. The retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to the retention time of the aciclovir peak in the chromatogram obtained with solution (2).

D. The absorption spectrum (*1.6*) of the solution, prepared as described under “Assay”, test B, when observed between 230 nm and 350 nm, exhibits one maximum at about 255 nm.

Clarity and colour of solution. A solution, containing the equivalent to 0.10 g of aciclovir in 10 mL of water R, is clear and not more intensely coloured than standard colour solution Yw1 when compared as described under *1.11.1 Colour of liquids*.

Water. Determine as described under *2.8 Determination of water by the Karl Fischer method*, *Method A*. Use 0.25 g of the test substance. The water content is not more than 55 mg/g.

pH value. pH of a solution containing the equivalent to 25 mg of aciclovir per mL of water R, 10.7–11.7.

Guanine and related substances

-Either test A or test B may be applied.

A. **Guanine.** Carry out the test as described under *1.14.1 Chromatography*, Thin-layer chromatography using cellulose R1 as the coating substance and a mixture of 10 volumes of propan-1-ol, 30 volumes of ammonia (260 g/L) TS and 60 volumes of ammonium sulfate (50 g/L) TS as the mobile phase. Apply separately to the plate 10 µL of each of the following four, freshly prepared solutions in sodium hydroxide (0.1 mol/L) TS. For solution (A) dissolve a quantity of the powder to obtain a solution containing 5 mg of Aciclovir per mL. For solution (B) dilute 1 volume of solution (A) to 10 volumes. For solution (C) use a solution of 0.5 mg of aciclovir RS and 0.5 mg of guanine R per mL. For solution (D) use 35 µg of guanine R per mL. After removing the plate from the chromatographic chamber allow it to dry exhaustively in air and examine the chromatogram under ultraviolet light (254 nm). In the chromatogram obtained with solution (C) guanine is eluted with a R_f value of 0.5 and aciclovir

with a R_f value of 0.7. The test is not valid unless this chromatogram shows two clearly separated spots. Any secondary spot corresponding to guanine in the chromatogram obtained with solution (A) is not more intense than the principal spot in the chromatogram obtained with solution (D) (0.7%).

B.Carry out the test as described under *1.14.1 Chromatography*, High-performance liquid chromatography using a stainless steel column (25 cm x 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl group (5 µm).

Use the following conditions for gradient elution:

Mobile phase A: 1 volume of acetonitrile R and 99 volumes of phosphate buffer, pH 3.1, TS.

Mobile phase B: 50 volumes of acetonitrile R and 50 volumes of phosphate buffer, pH 2.5, TS.

Time (min)	Mobile phase A (%v/v)	Mobile phase B (%v/v)	Comments
0–5	100	0	Isocratic
5–27	100→80	0→20	Linear gradient
27–40	80	20	Isocratic
40–42	80→100	20→0	Return to initial composition
42–52	100	0	Re-equilibration

Operate with a flow rate of 1.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 254 nm. Maintain the column at 30 °C.

Prepare the following solutions. For solution (1) dissolve a quantity of the powder for injection, equivalent to 25 mg of aciclovir, in 5.0 mL of sodium hydroxide (0.1 mol/L) TS and dilute to 25.0 mL with water R. For solution (2) dilute 1.0 mL of solution (1) to 100.0 mL with water. Dilute 1.0 mL of this solution to 10.0 mL with water. For solution (3) dissolve 10 mg of guanine R in 10 mL of sodium hydroxide (0.1 mol/L) TS and dilute to 100.0 mL with water R. Dilute 5.0 mL of this solution to 50.0 mL with water R. For solution (4) dissolve 5 mg of aciclovir RS, 5 mg of guanine R and 10 mg of aciclovir impurity C RS in 10 mL of sodium hydroxide (0.1 mol/L) TS and dilute to 100 mL with water R.

Inject separately 20 µL each of solutions (1), (2), (3) and (4).

In the chromatogram obtained with solution (4) the peak of aciclovir impurity C is eluted with a relative retention time of 0.94 with reference to the peak of aciclovir (retention time about 13 min). The test is not valid unless the resolution factor between the peak due to aciclovir impurity C and the peak due to aciclovir is at least 1.5.

In the chromatogram obtained with solution (1):

· the area of any peak corresponding to guanine is not greater than 0.7 times the area of the principal peak in the chromatogram obtained with solution (3) (0.7 %);

· the area of any other peak, other than the principal peak, is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

· the sum of all other areas, other than the principal peak and the peak due to guanine, is not greater than 10 times the area of the principal peak obtained with solution (2) (1.0%). Disregard any peak with an area less than 0.3 times the area of the principal peak in the chromatogram obtained with solution (2) (0.03%).

Assay

-Either test A or test B may be applied.

A.Carry out the test as described *under* *1.14.1 Chromatography*, High-performance liquid chromatography using a stainless steel column (25 cm x 4.6 mm), packed with particles of silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl group (5 µm).

As the mobile phase use a mixture of 90 volumes of Mobile phase A, as described under “Guanine and related substances”, test B and 10 volumes of acetonitrile R.

Operate with a flow rate of 1.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a

wavelength of 254 nm. Maintain the column at 30 °C.

Prepare the following solutions. For solution (1) dissolve a quantity of the powder for injection, equivalent to about 20 mg of aciclovir, accurately weighed, in 10 mL of sodium hydroxide (0.1 mol/L) TS and dilute to 100 mL with water. Dilute 5.0 mL of this solution to 50 mL with water. For solution (2) dissolve 20 mg of aciclovir RS in 10 mL of sodium hydroxide (0.1 mol/L) TS and dilute to 100 mL with water. Dilute 5.0 mL of this solution to 50 mL with water.

Inject separately 20 µL each of solution (1) and (2). Record the chromatograms for about 20 min.

Measure the areas of the peak responses obtained in the chromatograms of solutions (1) and (2) and calculate the percentage content of aciclovir ($C_8H_{11}N_5O_3$) per sealed container using the declared content of $C_8H_{11}N_5O_3$ in aciclovir RS.

B. Mix the contents of 5 containers. Transfer a quantity of the powder for injection, equivalent to 150 mg of Aciclovir, accurately weighed, to a 100 mL volumetric flask and dilute to volume with hydrochloric acid (0.1 mol/L) TS, mix and filter. Dilute 1.0 mL of the resulting solution to 100.0 mL with hydrochloric acid (0.1 mol/L) TS. Measure the absorbance of this solution in a 1 cm layer at 255 nm using hydrochloric acid (0.1 mol/L) TS as the blank. Calculate the percentage content of aciclovir ($C_8H_{11}N_5O_3$) per sealed container using an absorptivity value of 56.0 ($A_{1cm}^{1\%}$ = 560).

Bacterial endotoxins. Carry out the test as described under *3.4 Test for bacterial endotoxins*. Contains not more than 0.17 IU of endotoxin per mg of aciclovir.