Edition: BP 2025 (Ph. Eur. 11.6 update)

Interferon Alfa-2 Injection

General Notices

Action and use

Cytokine.

DEFINITION

Interferon Alfa-2 Injection is a dilution of Interferon Alfa-2 Concentrated Solution in a suitable liquid. It is supplied as a ready-to-use solution.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Potency

The estimated potency is not less than 80% and not more than 125% of the stated potency.

CHARACTERISTICS

A clear, colourless or slightly yellowish solution virtually free from particles.

IDENTIFICATION

A. It gives the appropriate response when examined using the conditions under the Assay.
B. In the test for Related proteins, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that of the principal peak in the chromatogram obtained with solution (2).
C. In the test for Impurities of molecular weights differing from that of Interferon Alfa-2a, the principal band in the gel obtained with solution (1) corresponds to that in the gel obtained with solution (3).

TESTS

Acidity

pH 4.7 to 5.3, Appendix V L.

Impurities of molecular weights differing from that of Interferon Alfa-2

Carry out the method for *polyacrylamide gel electrophoresis*, Appendix III F, under reducing and non-reducing conditions, using slab gels 1.0 mm thick and 14% w/v *acrylamide* as the resolving gel.

For the test under non-reducing conditions, the sample buffer consists of equal volumes of *water* and *SDS PAGE sample buffer (concentrated)*. For the test under reducing conditions, the sample buffer consists of equal volumes of *water* and

SDS PAGE sample buffer solution for reducing conditions (concentrated) containing *2-mercaptoethanol* as the reducing agent, using the following solutions.

(1) Dilute, if necessary, the injection being examined in sample buffer to a concentration of 5.5 MIU of Interferon Alfa-2 per mL.
(2) Dilute 0.20 mL of solution (1) to 1 mL with sample buffer. For solution (3) prepare a 25 µg per mL solution of the *interferon alfa-2b EPCRS* in sample buffer.
(3) Prepare a 25 µg/mL solution of the *interferon alfa-2b EPCRS* in sample buffer.
(4) Dilute 0.2 mL of solution (3) to 1 mL with sample buffer.
(5) Dilute 0.2 mL of solution (4) to 1 mL with sample buffer.
(6) Dilute 0.2 mL of solution (5) to 1 mL with sample buffer.
(7) Dilute 0.4 mL of solution (6) to 1 mL with sample buffer.
(8) Prepare a solution of molecular weight markers suitable for calibrating SDS-polyacrylamide gels in the range of 15 kDa to 67 kDa.

Place solutions (1) to (8), contained in covered test-tubes, on a water-bath for 2 minutes prior to application. Apply 10 µL of solution (8) and 50 µL of each of the other solutions to the stacking gel wells. Perform the electrophoresis under the conditions recommended by the manufacturer of the equipment. Proteins in the gel are visualised by silver staining.

SYSTEM SUITABILITY

The test is not valid unless:

there is compliance with the validation criteria;

in the electrophoretogram obtained with solution (7), a band is seen;

in the electrophoretograms obtained, respectively, with solutions (1) and (2) and with solutions (3) to (7), a gradation of intensity of staining is seen;

LIMITS

The electrophoretogram obtained with solution (1) under reducing conditions may show, in addition to the principal band, less intense bands with molecular weights lower than the principal band. No such band is more intense than the principal band in the electrophoretogram obtained with solution (6) (1%) and not more than three such bands are more intense than the principal band in the electrophoretogram obtained with solution (7) (0.4%).

The electrophoretogram obtained with solution (1) under non-reducing conditions may show, in addition to the principal band, less intense bands with molecular weights higher than the principal band. No such band is more intense than the principal band in the electrophoretogram obtained with solution (6) (1%) and not more than three such bands are more intense than the principal band in the electrophoretogram obtained with solution (7) (0.4%).

Related proteins

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions and the *normalisation procedure*. Store the solutions for not longer than 24 hours at a temperature of 2° to 8°.

(1) Dilute the injection, if necessary, with *water* to produce a solution containing 5.5 MIU of Interferon Alfa-2 per mL.
(2) Add a suitable volume of 0.25% w/w *Hydrogen Peroxide Solution (3 per cent)* to a volume of the solution (1) to give a final hydrogen peroxide concentration of 0.005% w/w and allow to stand at room temperature for 1 hour or for the length of time that will generate about 5% oxidised interferon. Add 12.5 mg of *L-methionine* per mL of solution. Allow to stand at room temperature for 1 hour.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) (Bakerbond C18 is suitable).
(b) Use gradient elution and the mobile phase described below.
(c) Use a flow rate of 1 mL per minute.
(d) Use an ambient column temperature.
(e) Use a detection wavelength of 210 nm.
(f) Inject 100 µL of each solution.

MOBILE PHASE

Mobile phase A 0.2 volumes of *trifluoroacetic acid*, 30 volumes of *acetonitrile* and 70 volumes of *water*.

Mobile phase B 0.2 volumes of *trifluoroacetic acid*, 20 volumes of *water* and 80 volumes of *acetonitrile*.

Equilibrate the column with the mobile phases in the initial gradient ratio for at least 15 minutes.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-1	72	28	isocratic
1-5	72→67	28→33	linear gradient
5-20	67→63	33→37	linear gradient
20-30	63→57	37→43	linear gradient
30-40	57→40	43→60	linear gradient
40-42	40	60	isocratic
42-50	40→72	60→28	linear gradient
50-60	72	28	re-equilibration

When the chromatograms are recorded using the prescribed conditions the retention time of the peak due to interferon alfa-2 is about 20 minutes. The relative retention times with reference to interferon alfa-2 are: impurity A , about 0.7; impurity B, about 1.1; impurity C, about 1.3.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution* between the peaks corresponding to oxidised interferon (impurity A) and interferon is at least 1.0.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than 5.0%;

the area of any peak corresponding to impurity B is not greater than 3.0%;

the sum of the areas of any other *secondary peaks* is not greater than 5.0%;

the sum of the areas of any *secondary peaks* is not greater than 12.0%.

Disregard any peak with a relative retention time less than 0.7 times, or greater than 1.4 times, of that of the principal peak in the chromatogram obtained with solution (2).

Bacterial endotoxins

Carry out the *test for Bacterial endotoxins*, Appendix XIV C. The endotoxin limit is not more than 2 IU in a volume containing 1 MIU of Interferon Alfa-2.

ASSAY

The potency of interferon alfa-2 is calculated by comparing its effect to protect cells against a viral cytopathic effect with the same effect of the appropriate International Standard of human recombinant interferon alfa-2 or of a reference preparation calibrated in International Units.

The International Unit is the activity contained in a stated amount of the appropriate International Standard. The equivalence in International Units of the International Standard is stated by the World Health Organization.

Carry out the assay by a suitable method, based on the following design.

Use, in standard culture conditions, an established cell line sensitive to the cytopathic effect of a suitable virus (a human diploid fibroblast cell line, free of microbial contamination, responsive to interferon and sensitive to encephalomyocarditis virus, is suitable).

The following cell cultures and virus have shown to be suitable: MDBK cells (ATCC No. CCL22), or Mouse L cells (NCTC clone 929; ATCC No. CCL 1) as the cell culture and vesicular stomatitis virus VSV, Indiana strain (ATCC No. VR-158) as

the infective agent; or A-549 cells (ATCC No. CCL-185) responsive to interferon as the cell culture, and encephalomyocarditis virus (ATCC No. VR-129B) as the infective agent.

Incubate in at least four series, cells with three or more different concentrations of the preparation being examined and the reference preparation in a microtitre plate and include in each series appropriate controls of untreated cells. Choose the concentrations of the preparations such that the lowest concentration produces some protection and the largest concentration produces less than maximal protection against the viral cytopathic effect. Add at a suitable time the cytopathic virus to all wells with the exception of a sufficient number of wells in all series, which are left with uninfected control cells. Determine the cytopathic effect of virus quantitatively with a suitable method. Calculate the potency of the preparation being examined by the standard statistical methods for a parallel line assay.

The fiducial limits of error are not less than 64% and not more than 156% of the stated potency.

STORAGE

Interferon Alfa-2 Injection should be stored at a temperature of 2° to 8°. It should not be allowed to freeze.

LABELLING

The label of the sealed container states the number of MIU (Million International Units) contained in it.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under *Interferon Alfa-2 Concentrated Solution* and:

impurity A (oxidised interferon alfa-2a);
impurity B (N -acetyl interferon);
impurity C (interferon with broken disulfide bridge).