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

Filgrastim Injection

General Notices

(Ph. Eur. monograph 2848)

Action and use

Recombinant methionyl human granulocyte colony-stimulating factor.

Ph Eur

DEFINITION

Sterile solution for injection of a protein having the primary structure of the 174-amino-acid isoform of human granulocyte colony-stimulating factor (huG-CSF) plus 1 additional amino acid, an *N*-terminal methionine. In contrast to its natural counterpart, the protein is not glycosylated.

Content

90.0 per cent to 110.0 per cent of the amount of filgrastim stated on the label.

Potency

80 per cent to 125 per cent of the potency stated on the label when determined using the conditions described under Assay.

PRODUCTION

Filgrastim injection is prepared from *Filgrastim concentrated solution (2206)*.

The concentrated solution is diluted with a formulation buffer (acetate or glutamate) containing sodium hydroxide, sorbitol, and polysorbate 80 as excipients. This preparation is passed through a bacteria-retentive filter and distributed aseptically into the final containers. Where excipients or formulation buffer other than those mentioned above are used, their compatibility with the tests described hereafter must be confirmed.

CHARACTERS

Appearance

Clear, colourless or slightly yellowish liquid.

IDENTIFICATION

A. It shows the expected biological activity (see Assay).
B. Examine the chromatograms obtained in the test for related proteins.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and shape to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

pH (2.2.3)

As approved by the competent authority.

Impurities with molecular masses higher than that of filgrastim

Size-exclusion chromatography (2.2.30): use the normalisation procedure.

Solution A Dissolve 4.1 g of *sodium acetate R* in 400 mL of *water R*, adjust to pH 4.0 with *acetic acid R* and dilute to 500 mL with *water R*.

Test solution Dilute the preparation to be examined with solution A to obtain a concentration of 0.4 mg/mL.

Reference solution Dilute *filgrastim CRS* with solution A to obtain a concentration of 0.4 mg/mL.

Resolution solution Mix a sample of the reference solution for about 30 s using a vortex mixer.

Column:

— *size*: *l* = 0.3 m, Ø = 7.8 mm;

— *stationary phase*: *hydrophilic silica gel for chromatography R* (5 µm) of a grade suitable for fractionation of globular proteins in the relative molecular mass range of 10 000 to 500 000;

— *temperature*: 30 °C.

Mobile phase Dissolve 7.9 g of *ammonium hydrogen carbonate R* in 1000 mL of *water for chromatography R* and adjust to pH 7.0 with *phosphoric acid R*; dilute to 2000 mL with *water for chromatography R*.

Flow rate 0.5 mL/min.

Detection Spectrophotometer at 215 nm.

Injection 20 µL.

Relative retention With reference to the filgrastim monomer (retention time = about 19 min): aggregates = about 0.60; filgrastim oligomer 1 = about 0.75; filgrastim oligomer 2 = about 0.80; filgrastim dimer = about 0.85.

System suitability Resolution solution:

— *retention time*: filgrastim monomer = 17 min to 20 min;

— *resolution*: minimum 3.0 between the peaks due to the filgrastim dimer and the filgrastim monomer.

Calculate the percentage content of the dimer, oligomers and aggregates.

Limits:

— *impurities with molecular masses higher than that of filgrastim, other than the dimer*: maximum 0.5 per cent;

— *total of impurities with molecular masses higher than that of filgrastim*: maximum 1.0 per cent.

Impurities with charges differing from that of filgrastim

Isoelectric focusing (2.2.54) with the following modifications. *Prepare reference solutions (a), (b) and (c) immediately before loading on the gel.*

Gel concentration 5 per cent; commercially available gels may be used.

Test solution Dilute the preparation to be examined with *water R* to obtain a concentration of 0.6 mg/mL.

Reference solution (a) Dilute *filgrastim CRS* with *water R* to obtain a concentration of 0.6 mg/mL.

Reference solution (b) To 10 µL of reference solution (a) add 190 µL of *water R* to obtain a concentration of 0.03 mg/mL.

Reference solution (c) To 10 µL of reference solution (a) add 490 µL of *water R* to obtain a concentration of 0.012 mg/mL.

Reference solution (d) Use an isoelectric point (pI) calibration solution, in the pI range of 3-10, prepared according to the manufacturer's instructions.

Focusing:

— *pH gradient*: a combination of ampholytes giving a functional separation in the pI range of 5.0-7.0;

— *catholyte*: 20 g/L solution of *sodium hydroxide R*;

— *anolyte*: 30 g/L solution of *glacial acetic acid R*;

— *application*: 15 µL.

Proceed with the isoelectric focusing by applying the electrical parameters according to the manufacturer's instructions. The following parameters have been found suitable for gels of dimensions 250 mm × 110 mm × 0.43 mm:

— *pre-focusing*: 700 V, 12 mA, 8 W, 20 min;

— *post-application*: 500 V, 8 mA, 8 W, 20 min;

— *focusing*: 2000 V, 14 mA, 14 W, 90 min;

— *final focusing*: 2500 V, 14 mA, 14 W, 10 min.

Detection Silver staining.

Fixing solution A solution containing 36 g/L of *sulfosalicylic acid R* and 116 g/L of *trichloroacetic acid R*.

Sensitising solution Dissolve 17 g of *sodium acetate R* in about 100 mL of *water R*. Add 75 mL of *ethanol (96 per cent) R*, 10 mL of a 50 g/L solution of *anhydrous sodium thiosulfate R* and 1.25 mL of a 250 g/L solution of *glutaraldehyde R* and dilute to 250 mL with *water R*.

Silver solution Mix 100 µL of *formaldehyde solution R1* and 25 mL of a 25 g/L solution of *silver nitrate R* and dilute to 250 mL with *water R*.

Developing solution Dissolve 6.25 g of *sodium carbonate R* in about 200 mL of *water R*, add 50 µL of *formaldehyde solution R1* and dilute to 250 mL with *water R*.

Stopping solution Dissolve 3.65 g of *sodium edetate R* in 250 mL of *water R*.

Alternatively, commercially available staining kits may be used.

System suitability:

— in the electropherogram obtained with reference solution (d), the relevant isoelectric point markers are distributed along the entire length of the gel;

— in the electropherogram obtained with reference solution (c), the principal band is clearly visible.

Limits:

— maximum 7 bands of an intensity between that of the principal band in the electropherogram obtained with reference solution (c) (2 per cent) and that of the principal band in the electropherogram obtained with reference solution (b) (5 per cent) are present;

— no band has an intensity greater than that of the principal band in the electropherogram obtained with reference solution (b) (5 per cent).

Related proteins

Liquid chromatography (*2.2.29*): use the normalisation procedure.

Test solution Dilute the preparation to be examined with *water R* to obtain a concentration of 0.5 mg/mL.

Reference solution (a) Dilute the contents of a vial of *filgrastim CRS* with *water R* to obtain a concentration of 0.5 mg/mL.

Reference solution (b) To 250 µL of reference solution (a), add 2.5 µL of a 4.5 g/L solution of hydrogen peroxide. Mix and incubate at 25 ± 2 °C for 30 min, then add 1.9 mg of *L-methionine R*.

Reference solution (c) To 250 µL of reference solution (a), add 0.25 mg of *dithiothreitol R*. Mix and incubate at 35 ± 2 °C for 60 min.

Column:

— *size*: *l* = 0.15 m, *Ø* = 4.6 mm;

— *stationary phase*: *end-capped butylsilyl silica gel for chromatography R* (5 µm) with a pore size of 30 nm;

— *temperature*: 60 °C.

Mobile phase:

— *mobile phase A*: dilute 1 mL of *trifluoroacetic acid R* in 1000 mL of *water for chromatography R*;

— *mobile phase B*: dilute 1 mL of *trifluoroacetic acid R* in 100 mL of *water for chromatography R*, then add 900 mL of *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 30	60 → 20	40 → 80
30 - 35	20	80
35 - 45	20 → 60	80 → 40

Flow rate 0.8 mL/min.

Detection Spectrophotometer at 215 nm.

Injection 50 µL of the test solution and reference solutions (b) and (c).

Relative retention With reference to filgrastim (retention time = about 23 min): oxidised filgrastim (form 1) = about 0.84; oxidised filgrastim (form 2) = about 0.98; reduced filgrastim = about 1.04.

System suitability Reference solution (b):

— *symmetry factor*: maximum 1.8 for the peak due to filgrastim;

— *peak-to-valley ratio*: minimum 2.0, where H_p = height above the baseline of the peak due to oxidised filgrastim (form 2) and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to filgrastim.

System suitability Reference solution (c):

— *resolution*: minimum 1.5 between the peaks due to filgrastim and reduced filgrastim;

— *symmetry factor*: maximum 1.8 for the peak due to filgrastim.

Limits:

— *any impurity*: for each impurity, maximum 3.0 per cent;

— *total*: maximum 6.5 per cent.

Osmolality (*2.2.35*)

As approved by the competent authority.

Bacterial endotoxins (*2.6.14*)

Less than 10 IU in the volume that contains 1.0 mg of protein.

ASSAY

Protein

Liquid chromatography (2.2.29) as described in the test for related proteins with the following modification.

Injection Test solution and reference solution (a).

Calculate the content of filgrastim ($C_{845}H_{1339}N_{223}O_{243}S_9$) taking into account the assigned content of $C_{845}H_{1339}N_{223}O_{243}S_9$ in *filgrastim CRS*.

Potency

The potency of the preparation to be examined is determined by comparison of the dilutions of the test preparation with the dilutions of the International Standard of filgrastim or with 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 using a suitable method such as the following, which uses the conversion of a tetrazolium salt (MTS) as a staining method. Alternative methods of quantifying cell proliferation, such as measurement of intracellular ATP by luciferase bioluminescence, have also been found suitable, and may be used as the assay readout, subject to appropriate validation. The assay conditions (for example, cell concentration, incubation time and dilution steps) are then adapted accordingly.

Use an established cell line responsive to filgrastim. M-NFS-60 cells (ATCC No. CRL-1838) that have been made sensitive to G-CSF have been found suitable. Incubate with varying dilutions of test and reference preparations of filgrastim. Then incubate with a solution of *tetrazolium salt R*. This cytochemical stain is converted by cellular dehydrogenases to a coloured formazan product. The formazan is then measured spectrophotometrically.

Add 50 µL of dilution medium to each well of a 96-well microplate. Add an additional 50 µL of this solution to the wells designed for the blanks. Add 50 µL of each solution to be tested in triplicate (test preparation and reference preparation at a concentration of about 800 IU/mL, plus a series of 10 twofold dilutions to obtain a standard curve). Prepare a suspension of M-NFS-60 cells containing 7×10^5 cells per millilitre. Immediately before use, add 2-mercaptoethanol to a final concentration of 0.1 mM, and add 50 µL of the prepared cell suspension to each well, maintaining the cells in a uniform suspension during addition.

Incubate the plate at 36.0-38.0 °C for 44-48 h in a humidified incubator using 6 ± 1 per cent CO_2. Add 20 µL of a 5.0 g/L sterile solution of *tetrazolium salt R* to each well and reincubate for 4 h. Estimate the quantity of formazan produced using a microplate reader at 490 nm.

Calculate the potency of the preparation to be examined using a suitable statistical method, for example the parallel line assay (5.3).

The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The confidence limits (P = 0.95) are not less than 74 per cent and not more than 136 per cent of the estimated potency.

STORAGE

In a sterile, airtight, tamper-evident container, protected from light, at a temperature of 2 °C to 8 °C.

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