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 Organisation.

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) 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 all wells of a 96-well microtitre plate. 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 microtitre well plate 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.

LABELLING

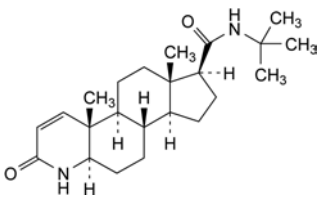
The label states:

- the content, in milligrams of protein per millilitre;
- the potency, in International Units per milligram of protein.

01/2008:1615
corrected 6.0

FINASTERIDE

Finasteridum



$C_{23}H_{36}N_2O_2$ M_r 372.6
[98319-26-7]

DEFINITION

N-(1,1-Dimethylethyl)-3-oxo-4-aza-5α-androst-1-ene-17β-carboxamide.

Content: 98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: practically insoluble in water, freely soluble in ethanol and in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *finasteride CRS*.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

TESTS

Specific optical rotation (*2.2.7*): + 12.0 to + 14.0 (dried substance).

Dissolve 0.250 g in *methanol R* and dilute to 25.0 mL with the same solvent.

Related substances. Liquid chromatography (*2.2.29*).

Test solution (a). Dissolve 25.0 mg of the substance to be examined in a mixture of equal volumes of *acetonitrile R* and *water R* and dilute to 50.0 mL with the same mixture of solvents.

Test solution (b). Dissolve 0.100 g of the substance to be examined in a mixture of equal volumes of *acetonitrile R* and *water R* and dilute to 10.0 mL with the same mixture of solvents.

Reference solution (a). Dissolve 25.0 mg of *finasteride CRS* in a mixture of equal volumes of *acetonitrile R* and *water R* and dilute to 50.0 mL with the same mixture of solvents.

Reference solution (b). Dissolve 50.0 mg of *finasteride for system suitability CRS* in a mixture of equal volumes of *acetonitrile R* and *water R* and dilute to 5.0 mL with the same mixture of solvents.

Reference solution (c). Dilute 2.0 mL of test solution (b) to 100.0 mL in a mixture of equal volumes of *acetonitrile R* and *water R*. Dilute 1.0 mL of this solution to 10.0 mL with a mixture of equal volumes of *acetonitrile R* and *water R*.

Column:

- *size*: *l* = 0.25 m, Ø = 4.0 mm,
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm) with a ratio of specific surface area (m^2g^{-1})/carbon-percentage less than 20,
- *temperature*: 60 °C.

Mobile phase: *acetonitrile R, tetrahydrofuran R, water R* (10:10:80 *V/V/V*).

Flow rate: 1.5 mL/min.

Detection: spectrophotometer at 210 nm.

Injection: 15 µL; inject test solution (b) and reference solutions (b) and (c).

Run time: twice the retention time of finasteride.

Relative retention with reference to finasteride (retention time = about 28 min): impurity A = about 0.94; impurity B = about 1.22; impurity C = about 1.36.

System suitability: reference solution (b):

- *peak-to-valley ratio*: minimum 2.5, where H_p = height above the baseline of the peak due to impurity A, and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to finasteride.

Limits:

- *impurity A*: maximum 0.3 per cent, calculated from the area of the corresponding peak in the chromatogram obtained with reference solution (b) and taking into account the assigned value of impurity A in *finasteride for system suitability CRS*,
- *impurity B*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.3 per cent),
- *impurity C*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.3 per cent),
- *unspecified impurities*: for each impurity, not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.10 per cent),
- *total*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.6 per cent),
- *disregard limit*: 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Loss on drying (*2.2.32*): maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances.

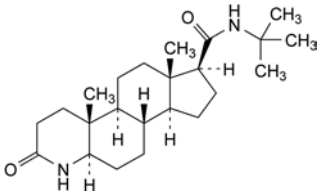
Injection: test solution (a) and reference solution (a).

Calculate the percentage content of $C_{23}H_{36}N_2O_2$.

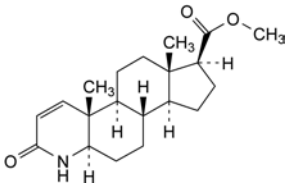
STORAGE

Protected from light.

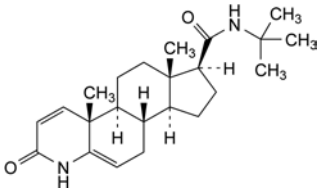
IMPURITIES



A. *N*-(1,1-dimethylethyl)-3-oxo-4-aza-5α-androstane-17β-carboxamide (dihydrofinasteride),



B. methyl 3-oxo-4-aza-5α-androst-1-ene-17β-carboxylate (Δ-1-aza ester),



C. *N*-(1,1-dimethylethyl)-3-oxo-4-azaandrosta-1,5-diene-17β-carboxamide (Δ-1,5-aza amide).

01/2011:1912

FISH OIL, RICH IN OMEGA-3 ACIDS

Piscis oleum omega-3 acidis abundans

DEFINITION

Purified, winterised and deodorised fatty oil obtained from fish of families such as *Engraulidae*, *Carangidae*, *Clupeidae*, *Osmeridae*, *Scombridae* (except the genera *Thunnus* and *Sarda*) and *Ammodytidae* (type I) or from the genera *Thunnus* and *Sarda* within the family *Scombridae* (type II). The omega-3 acids are defined as the following acids: *alpha*-linolenic acid (C18:3 n-3), moroctic acid (C18:4 n-3), eicosatetraenoic acid (C20:4 n-3), timnodonic (eicosapentaenoic) acid (C20:5 n-3; EPA), heneicosapentaenoic acid (C21:5 n-3), clupanodonic acid (C22:5 n-3) and cervonic (docosahexaenoic) acid (C22:6 n-3; DHA).

Content:

	Type I	Type II
EPA, expressed as triglycerides	minimum 13 per cent	4 per cent to 8 per cent
DHA, expressed as triglycerides	minimum 9 per cent	minimum 20 per cent
Total omega-3 acids, expressed as triglycerides	minimum 28 per cent	minimum 28 per cent

Authorised antioxidants in concentrations not exceeding the levels specified by the competent authorities may be added.

CHARACTERS

Appearance: pale yellow liquid.

Solubility: practically insoluble in water, very soluble in acetone and in heptane, slightly soluble in anhydrous ethanol.

IDENTIFICATION

A. Examine the chromatograms obtained in the assay for EPA and DHA.

Results: the peaks due to eicosapentaenoic acid methyl ester and docosahexaenoic acid methyl ester in the chromatogram obtained with test solution (b) are similar in retention time to the corresponding peaks in the chromatogram obtained with reference solution (a).

B. It complies with the limits of the assay for EPA (type I or II).

TESTS

Appearance. The substance to be examined is not more intensely coloured than a reference solution prepared as follows: to 3.0 mL of red primary solution add 25.0 mL of yellow primary solution and dilute to 50.0 mL with a 10 g/L solution of *hydrochloric acid R* (*2.2.2, Method II*).

Absorbance (*2.2.25*): maximum 0.70 (type I) or maximum 0.50 (type II), at 233 nm.

Dilute 0.300 g of the substance to be examined to 50.0 mL with *trimethylpentane R*. Dilute 2.0 mL of this solution to 50.0 mL with *trimethylpentane R*.

Acid value (*2.5.1*): maximum 0.5, determined on 20.0 g.

Anisidine value (*2.5.36*): maximum 30.0 (type I) or maximum 15.0 (type II).

Peroxide value (*2.5.5, Method A*): maximum 10.0 (type I) or maximum 5.0 (type II).

Unsaponifiable matter (*2.5.7*): maximum 1.5 per cent, determined on 5.0 g.

Stearin. 10 mL remains clear after cooling at 0 °C for 3 h.