ASSAY

Fibrinogen (clottable protein). The estimated content in milligrams of clottable protein is not less than 70 per cent and not more than 130 per cent of the content stated on the label.

Mix 0.2 ml of the reconstituted preparation with 2 ml of a suitable buffer solution (pH 6.6-7.4) containing sufficient *human thrombin R* (approximately 3 IU/ml) and calcium (0.05 mol/l). Maintain at 37 °C for 20 min, separate the precipitate by centrifugation (5000 *g*, 20 min), wash thoroughly with a 9 g/l solution of *sodium chloride R* and determine the protein as nitrogen by sulphuric acid digestion (*2.5.9*). Calculate the protein content by multiplying the result by 6.0. If for a particular preparation this method cannot be applied, use another validated method for determination of fibrinogen.

Factor XIII. Where the label indicates that the human coagulation factor XIII activity is greater than 10 units/ml, the estimated activity is not less than 80 per cent and not more than 120 per cent of the activity stated on the label.

Make at least 3 suitable dilutions of thawed or reconstituted component 1 and of human normal plasma (reference preparation) using as diluent coagulation factor XIII deficient plasma or another suitable diluent. Add to each dilution suitable amounts of the following reagents:

– activator reagent, containing bovine or human thrombin, a suitable buffer, calcium chloride and a suitable inhibitor such as Gly-Pro-Arg-Pro-Ala-NH_2 which inhibits clotting of the sample but does not prevent coagulation factor XIII activation by thrombin,

– detection reagent, containing a suitable factor XIIIa-specific peptide substrate, such as Leu-Gly-Pro-Gly-Glu-Ser-Lys-Val-Ile-Gly-NH_2 and glycine ethyl ester as 2nd substrate in a suitable buffer solution,

– NADH reagent, containing glutamate dehydrogenase, α-ketoglutarate and NADH in a suitable buffer solution.

After mixing, the absorbance changes (ΔA/min) are measured at a wavelength of 340 nm, after the linear phase of the reaction is reached.

1 unit of factor XIII is equal to the activity of 1 ml of human normal plasma.

Calculate the activity of the test preparation by the usual statistical methods (*5.3*, for example). The confidence limits (P = 0.95) are not less than 80 per cent and not more than 125 per cent of the estimated activity.

Component 2 (thrombin preparation)

IDENTIFICATION

It complies with the limits of the assay of thrombin.

TESTS

pH (*2.2.3*): 5.0 to 8.0.

Solubility. Freeze-dried preparations dissolve within 5 min in the volume of solvent for reconstitution stated on the label, forming a colourless, clear or slightly turbid solution.

Water. Determined by a suitable method, such as the semi-microdetermination (*2.5.12*), loss on drying (*2.2.32*) or near infrared spectrophotometry (*2.2.40*), the water content is within the limits approved by the competent authority.

Sterility (*2.6.1*). It complies with the test for sterility.

ASSAY

Thrombin. The estimated activity is not less than 80 per cent and not more than 125 per cent of the activity stated on the label.

If necessary, dilute the reconstituted preparation to be examined to approximately 2-20 IU of thrombin per millilitre using as diluent a suitable buffer pH 7.3-7.5, such as *imidazole buffer solution pH 7.3 R* containing 10 g/l of *human albumin R* or *bovine albumin R*. To a suitable volume of the dilution, add a suitable volume of fibrinogen solution (1 g/l of clottable protein) warmed to 37 °C and start measurement of the clotting time immediately. Repeat the procedure with each of at least 3 dilutions, in the range stated above, of a reference preparation of thrombin, calibrated in International Units. Calculate the activity of the test preparation by the usual statistical methods (*5.3*, for example). The confidence limits (P = 0.95) are not less than 80 per cent and not more than 125 per cent of the estimated activity.

STORAGE

Protected from light and, for freeze-dried components, in an airtight container.

LABELLING

The label states:

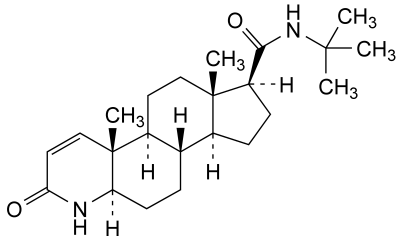
– the amount of fibrinogen (milligrams of clottable protein), thrombin (International Units) per container, and coagulation factor XIII, if this is greater than 10 units/ml,

– where applicable, the name and volume of solvent to be used to reconstitute the components.

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),

– *any other impurity*: not more than half the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 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.

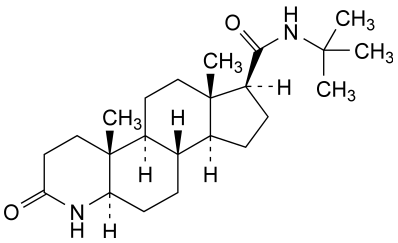
Injection: test solution (a) and reference solution (a).

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

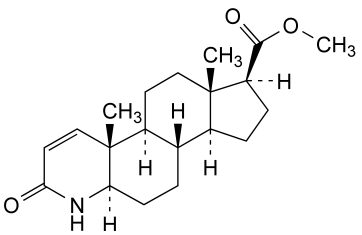
STORAGE

Protected from light.

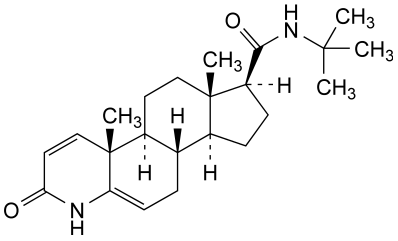
IMPURITIES



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).