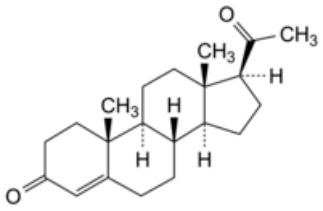
Edition: BP 2025 (Ph. Eur. 11.6 update)

Progesterone

General Notices

(Ph. Eur. monograph 0429)



$C_{21}H_{30}O_2$ 314.5 *57-83-0*

Action and use

Progestogen.

Preparation

Progesterone Injection

Ph Eur

DEFINITION

Pregn-4-ene-3,20-dione.

Content

97.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Practically insoluble in water, freely soluble in anhydrous ethanol, sparingly soluble in acetone and in fatty oils.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Infrared absorption spectrophotometry (2.2.24).

Comparison progesterone CRS.

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

B. Thin-layer chromatography (2.2.27).

Test solution Dissolve 10 mg of the substance to be examined in a mixture of 1 volume of methanol R and 9 volumes of methylene chloride R and dilute to 10 mL with the same mixture of solvents.

Reference solution Dissolve 10 mg of progesterone CRS in a mixture of 1 volume of methanol R and 9 volumes of methylene chloride R and dilute to 10 mL with the same mixture of solvents.

Plate TLC silica gel F_{254} plate R.

Mobile phase ethyl acetate R, methylene chloride R (33:66 *V/V*).

Application 5 µL.

Development Over 3/4 of the plate.

Drying In air.

Detection Examine in ultraviolet light at 254 nm.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Specific optical rotation (2.2.7)

+ 186 to + 194 (dried substance).

Dissolve 0.250 g in anhydrous ethanol R and dilute to 25.0 mL with the same solvent.

Related substances

Liquid chromatography (2.2.29).

Test solution Dissolve 20.0 mg of the substance to be examined in mobile phase B and dilute to 50.0 mL with mobile phase B.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with mobile phase B. Dilute 1.0 mL of this solution to 10.0 mL with mobile phase B.

Reference solution (b) Dissolve 2 mg of progesterone for system suitability CRS (containing impurities B, C, G, I and M) in mobile phase B and dilute to 5.0 mL with mobile phase B.

Reference solution (c) Dissolve 2 mg of progesterone for peak identification CRS (containing impurities D, E, J, K and L) in mobile phase B and dilute to 5.0 mL with mobile phase B.

Reference solution (d) Dissolve 10.0 mg of progesterone for impurity H identification CRS (with an assigned content of impurity H) in mobile phase B and dilute to 25.0 mL with mobile phase B.

Reference solution (e) Dissolve 20.0 mg of progesterone CRS in mobile phase B and dilute to 50.0 mL with mobile phase B.

Column:

— *size*: *l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: *end-capped extra-dense bonded octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

— *mobile phase A*: *acetonitrile R*, *water R* (50:50 *V/V*);

— *mobile phase B*: *water R*, *acetonitrile R* (20:80 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 20	100	0
20 - 27	100 → 0	0 → 100
27 - 45	0	100

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 241 nm and, for impurity H, at 286 nm.

Injection 10 µL of the test solution and reference solutions (a), (b), (c) and (d).

Identification of impurities Use the chromatogram supplied with *progesterone for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities B, C, G, I and M; use the chromatogram supplied with *progesterone for peak identification CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities D + E, J, K and L; use the chromatogram obtained with reference solution (d) to identify the peak due to impurity H.

Relative retention With reference to progesterone (retention time = about 14 min): impurity B = about 0.60; impurity J = about 0.65; impurity H = about 0.82; impurity K = about 0.85; impurity C = about 0.93; impurity M = about 1.1; impurity L = about 1.90; impurity I = about 1.95; impurities D and E = about 2.05; impurity G = about 2.65.

System suitability Reference solution (b):

— *peak-to-valley ratio*: minimum 4.0, where H_p = height above the baseline of the peak due to impurity M and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to progesterone.

Limits:

— *impurity I (sum of the 2 epimers)*: not more than 6 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.6 per cent);

— *impurity C*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

— *impurity B*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);

— *sum of impurities D and E*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent);

— *impurities G, J, K, L, M*: for each impurity, not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent);

— *impurity H at 286 nm*: maximum 0.15 per cent, calculated from the area of the corresponding peak in the chromatogram obtained with reference solution (d) and taking into account the assigned content of impurity H in *progesterone for impurity H identification CRS*;

— *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

— *sum of impurities other than H*: not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);

— *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 0.500 g by drying in an oven at 105 °C for 2 h.

ASSAY

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

Injection Test solution and reference solution (e).

Calculate the percentage content of $C_{21}H_{30}O_2$ taking into account the assigned content of *progesterone CRS*.

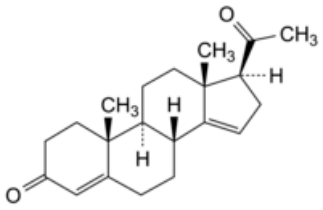
STORAGE

Protected from light.

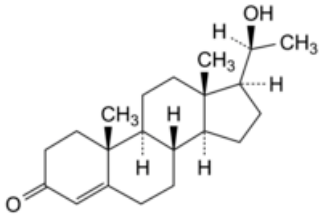
IMPURITIES

Specified impurities *B, C, D, E, G, H, I, J, K, L, M.*

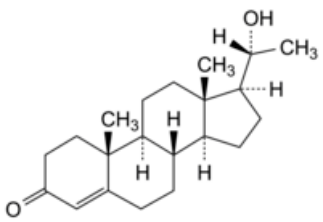
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *A.*



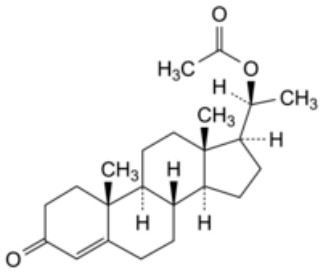
A. pregna-4,14-diene-3,20-dione,



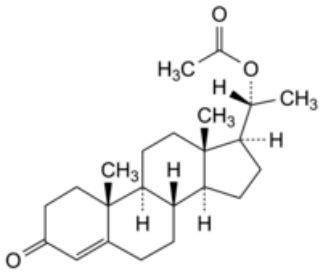
B. (20*S*)-20-hydroxypregn-4-en-3-one,



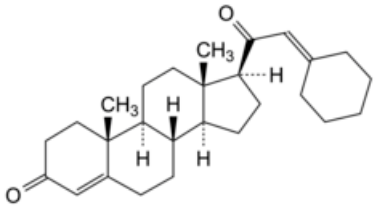
C. (20*R*)-20-hydroxypregn-4-en-3-one,



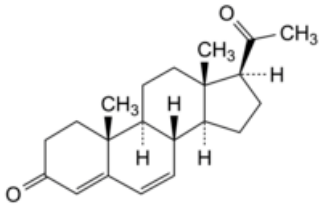
D. (20*S*)-3-oxopregn-4-en-20-yl acetate,



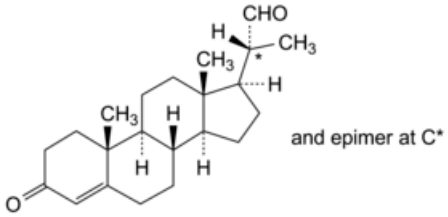
E. (20*R*)-3-oxopregn-4-en-20-yl acetate,



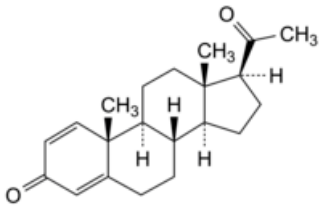
G. 21-(cyclohexylidene)pregn-4-ene-3,20-dione,



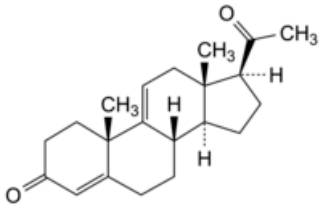
H. pregna-4,6-diene-3,20-dione (Δ6-progesterone),



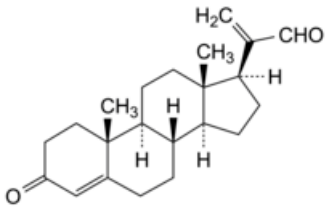
I. (20*RS*)-20-methyl-3-oxopregn-4-en-21-al,



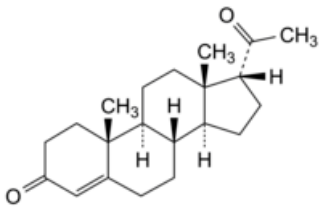
J. pregna-1,4-diene-3,20-dione,



K. pregna-4,9(11)-diene-3,20-dione,



L. 20-methylidene-3-oxopregn-4-en-21-al,



M. (17α)-pregn-4-ene-3,20-dione.

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