B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *prochlorperazine maleate CRS*.

C. It complies with the identification of phenothiazines by thin-layer chromatography (*2.3.3*), with the following modifications:

Test solution. Dissolve 20 mg of the substance to be examined in a mixture of equal volumes of *chloroform R* and *methanol R* and dilute to 20 ml with the same mixture of solvents.

Reference solution. Dissolve 20 mg of *prochlorperazine maleate CRS* in a mixture of equal volumes of *chloroform R* and *methanol R* and dilute to 20 ml with the same mixture of solvents.

Apply separately to the plate 4 µl of each solution.

D. Triturate 0.2 g with a mixture of 1 ml of *strong sodium hydroxide solution R* and 3 ml of *water R*. Shake with three quantities, each of 5 ml, of *ether R*. To 0.1 ml of the aqueous layer add a solution of 10 mg of *resorcinol R* in 3 ml of *sulphuric acid R*. Heat in a water-bath for 15 min. No colour develops. To the remainder of the aqueous layer add 2 ml of *bromine solution R*. Heat in a water-bath for 15 min and then heat to boiling. Cool. To 0.1 ml of the solution add a solution of 10 mg of *resorcinol R* in 3 ml of *sulphuric acid R*. Heat in a water-bath for 15 min. A blue colour develops.

TESTS

pH (*2.2.3*). The pH of a freshly prepared saturated solution in *carbon dioxide-free water R* is 3.0 to 4.0.

Related substances. *Carry out the test protected from light.* Examine by thin-layer chromatography (*2.2.27*), using *silica gel* GF_{254} *R* as the coating substance.

Test solution. Dissolve 0.2 g of the substance to be examined in a mixture of 5 volumes of *diethylamine R* and 95 volumes of *methanol R* and dilute to 10 ml with the same mixture of solvents. Prepare immediately before use.

Reference solution. Dilute 1 ml of the test solution to 200 ml with a mixture of 5 volumes of *diethylamine R* and 95 volumes of *methanol R*.

Apply separately to the plate 10 µl of each solution. Develop over a path of 12 cm using a mixture of 10 volumes of *acetone R*, 10 volumes of *diethylamine R* and 80 volumes of *cyclohexane R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent). Disregard any spots remaining at the starting-points.

Loss on drying (*2.2.32*). Not more than 1.0 per cent, determined on 1.00 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*). Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g of the powdered substance to be examined in 50 ml of *anhydrous acetic acid R*, warming on a water-bath. Allow to cool to room temperature. Titrate with *0.1 M perchloric acid* determining the end-point potentiometrically (*2.2.20*).

1 ml of *0.1 M perchloric acid* is equivalent to 30.31 mg of $C_{28}H_{32}ClN_3O_8S$.

STORAGE

Store protected from light.

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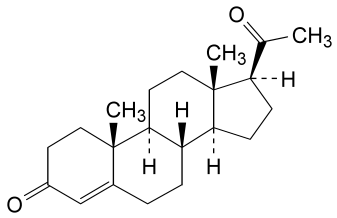
corrected 6.0

PROGESTERONE

Progesteronum



$C_{21}H_{30}O_2$ M_r 314.5
[57-83-0]

DEFINITION

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

Content: 97.0 per cent to 103.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder or colourless crystals.

Solubility: practically insoluble in water, freely soluble in 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 *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 A: examine in ultraviolet light at 254 nm.

Detection B: spray with *alcoholic solution of sulphuric acid R*, heat at 120 °C for 15 min and allow to cool. Examine in daylight and in ultraviolet light at 365 nm.

Results A: 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.

Results B: the principal spot in the chromatogram obtained with the test solution is similar in position, fluorescence in ultraviolet light at 365 nm 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 *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 *methanol R* and dilute to 50.0 ml with the same solvent.

Reference solution (a). Dissolve 2.0 mg of *progesterone CRS* and 2.0 mg of *progesterone impurity C CRS* in *methanol R* and dilute to 50.0 ml with the same solvent.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with *methanol R*.

Column:

– *size*: *l* = 0.15 m, Ø = 4.6 mm,

– *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

– *mobile phase A*: *water R*,

– *mobile phase B*: *acetonitrile R*.

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 20	50	50
20 - 27	50 → 20	50 → 80
27 - 45	20	80
45 - 50	50	50

Flow rate: 0.8 ml/min.

Detection: spectrophotometer at 241 nm.

Injection: 10 µl.

System suitability: reference solution (a):

– *resolution*: minimum 1.5 between the peaks due to impurity C and to progesterone.

Limits:

– *any impurity*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent),

– *total*: not more than 0.8 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.8 per cent),

– *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (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

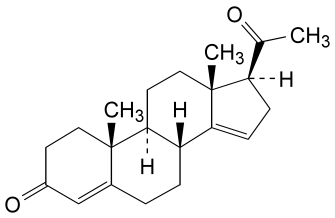
Dissolve 25.0 mg in *alcohol R* and dilute to 250.0 ml with the same solvent. Dilute 5.0 ml of the solution to 50.0 ml with *alcohol R*. Measure the absorbance (*2.2.25*) at the maximum at 241 nm.

Calculate the content of $C_{21}H_{30}O_2$ taking the specific absorbance to be 535.

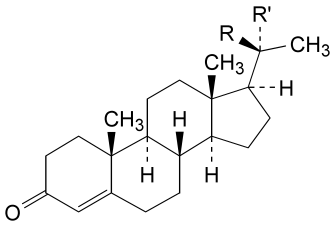
STORAGE

Protected from light.

IMPURITIES



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

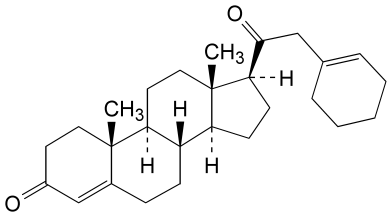


B. R = OH, R′ = H: (20*S*)-20-hydroxypregn-4-en-3-one,

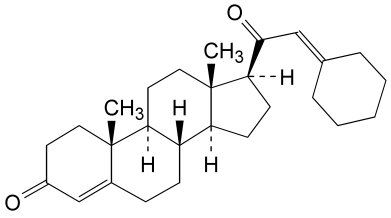
C. R = H, R′ = OH: (20*R*)-20-hydroxypregn-4-en-3-one,

D. R = O-CO-CH_3, R′ = H: (20*S*)-3-oxopregn-4-en-20-yl acetate,

E. R = H, R′ = O-CO-CH_3: (20*R*)-3-oxopregn-4-en-20-yl acetate,



F. 21-(cyclohex-1-enyl)pregn-4-ene-3,20-dione,

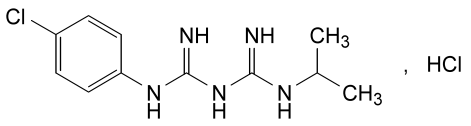


G. 21-(cyclohexylidene)pregn-4-ene-3,20-dione.

01/2008:2002
corrected 6.0

PROGUANIL HYDROCHLORIDE

Proguanili hydrochloridum



$C_{11}H_{17}Cl_2N_5$ M_r 290.2
[637-32-1]

DEFINITION

1-(4-Chlorophenyl)-5-(1-methylethyl)biguanide hydrochloride.

Content: 98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.