Reference solution (b). Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase.

Column:

– *size*: *l* = 0.15 m, Ø = 4.6 mm;

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

Mobile phase: carefully mix 19 ml of *butyl acetate R1* with 37 ml of *tetrahydrofuran R* and 213 ml of *ethylene glycol monomethyl ether R*, then add with 231 ml of *water R*; mix, allow to equilibrate for 1 h and filter through a 0.45 µm filter.

Flow rate: 1 ml/min.

Detection: spectrophotometer at 254 nm.

Equilibration: with the mobile phase for about 30 min.

Injection: 20 µl.

Run time: 1.5 times the retention time of prednisolone pivalate.

Retention time: prednisolone acetate = about 3.5 min; cortisone acetate = about 4.5 min; prednisolone pivalate = about 13 min.

System suitability: reference solution (a):

– *resolution*: minimum 2.5 between the peaks due to prednisolone acetate and cortisone acetate; if necessary, adjust the concentration of water in the mobile phase.

Limits:

– *any impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent), and not more than one such peak has an area greater than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);

– *total*: not more than 1.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (2.5 per cent);

– *disregard limit*: 0.025 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 1.000 g by drying in an oven at 105 °C.

ASSAY

Dissolve 0.100 g in *ethanol (96 per cent) R* and dilute to 100.0 ml with the same solvent. Dilute 5.0 ml of this solution to 250.0 ml with *ethanol (96 per cent) R*. Measure the absorbance (*2.2.25*) at the absorption maximum at 243 nm.

Calculate the content of $C_{26}H_{36}O_6$ taking the specific absorbance to be 337.

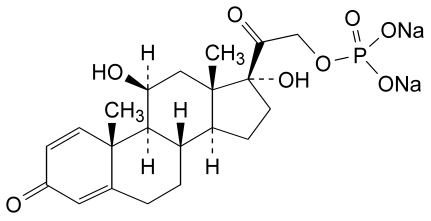
STORAGE

Protected from light.

01/2008:0735

PREDNISOLONE SODIUM PHOSPHATE

Prednisoloni natrii phosphas



$C_{21}H_{27}Na_2O_8P$ M_r 484.4
[125-02-0]

DEFINITION

11β,17-Dihydroxy-3,20-dioxopregna-1,4-dien-21-yl disodium phosphate.

Content: 96.0 per cent to 103.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white, hygroscopic, crystalline powder.

Solubility: freely soluble in water, very slightly soluble in ethanol (96 per cent).

IDENTIFICATION

First identification: B, C.

Second identification: A, C, D, E.

A. Dissolve 10.0 mg in 5 ml of *water R* and dilute to 100.0 ml with *anhydrous ethanol R*. Place 2.0 ml of this solution in a ground-glass-stoppered tube, add 10.0 ml of *phenylhydrazine-sulphuric acid solution R*, mix and heat in a water-bath at 60 °C for 20 min. Cool immediately. The absorbance (*2.2.25*) at the absorption maximum at 415 nm is 0.10 to 0.20.

B. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *prednisolone sodium phosphate CRS*.
If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *ethanol (96 per cent) R*, evaporate to dryness on a water-bath and record new spectra using the residues.

C. Thin-layer chromatography (*2.2.27*).
Test solution. Dissolve 10 mg of the substance to be examined in *methanol R* and dilute to 10 ml with the same solvent.
Reference solution (a). Dissolve 10 mg of *prednisolone sodium phosphate CRS* in *methanol R* and dilute to 10 ml with the same solvent.
Reference solution (b). Dissolve 10 mg of *dexamethasone sodium phosphate CRS* in *methanol R* and dilute to 10 ml with the same solvent. Dilute 5 ml of this solution to 10 ml with reference solution (a).
Plate: *TLC silica gel F_{254} plate R.*
Mobile phase: *glacial acetic acid R, water R, butanol R* (20:20:60 *V/V/V*).
Application: 5 µl.
Development: over a path of 15 cm.
Drying: in air.
Detection A: examine in ultraviolet light at 254 nm.

Results A: the principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

Detection B: spray with *alcoholic solution of sulphuric acid R*, heat at 120 °C for 10 min or until the spots appear, and allow to cool; examine in daylight and in ultraviolet light at 365 nm.

Results B: the principal spot in the chromatogram obtained with the test solution is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatogram obtained with reference solution (a).

System suitability: reference solution (b):

– the chromatogram shows 2 spots which may, however, not be completely separated.

D. To 2 ml of *sulphuric acid R* add about 2 mg and shake to dissolve. Within 5 min, an intense red colour develops. When examined in ultraviolet light at 365 nm, a reddish-brown fluorescence is seen. Add this solution to 10 ml of *water R* and mix. The colour fades and there is a greenish-yellow fluorescence in ultraviolet light at 365 nm.

E. To about 40 mg add 2 ml of *sulphuric acid R* and heat gently until white fumes are evolved. Add *nitric acid R* dropwise, continue the heating until the solution is almost colourless, and cool. Add 2 ml of *water R*, heat until white fumes are again evolved, cool, add 10 ml of *water R* and neutralise to *red litmus paper R* with *dilute ammonia R1*. The solution gives reaction (a) of sodium (*2.3.1*) and reaction (b) of phosphates (*2.3.1*).

TESTS

Solution S. Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 20 ml with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution B_7 (*2.2.2, Method II*).

pH (*2.2.3*): 7.5 to 9.0 for solution S.

Specific optical rotation (*2.2.7*): + 94 to + 100 (anhydrous substance).

Dissolve 0.250 g in *water R* and dilute to 25.0 ml with the same solvent.

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 62.5 mg of the substance to be examined in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (a). Dissolve 25 mg of *prednisolone sodium phosphate CRS* and 25 mg of *prednisolone CRS* in the mobile phase and dilute to 25.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 25.0 ml with the mobile phase.

Reference solution (b). Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase.

Column:

– *size*: *l* = 0.15 m, Ø = 4.6 mm;

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

Mobile phase: into a 250 ml conical flask weigh 1.360 g of *potassium dihydrogen phosphate R* and 0.600 g of *hexylamine R*, mix, allow to stand for 10 min, then dissolve in 185 ml of *water R*; add 65 ml of *acetonitrile R*, mix, and filter through a 0.45 µm filter.

Flow rate: 1 ml/min.

Detection: spectrophotometer at 254 nm.

Equilibration: with the mobile phase for about 30 min.

Injection: 20 µl.

Run time: 3 times the retention time of prednisolone sodium phosphate.

Retention time: prednisolone sodium phosphate = about 6.5 min; prednisolone = about 8.5 min.

System suitability: reference solution (a):

– *resolution*: minimum 4.5 between the peaks due to prednisolone sodium phosphate and prednisolone; if necessary, increase the concentration of *acetonitrile R* or *water R* in the mobile phase.

Limits:

– *any impurity*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2 per cent), and not more than 1 such peak has an area greater than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent);

– *total*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3 per cent);

– *disregard limit*: 0.025 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Inorganic phosphate: maximum 1 per cent.

Dissolve 50 mg in *water R* and dilute to 100 ml with the same solvent. To 10 ml of this solution add 5 ml of *molybdovanadic reagent R*, mix, and allow to stand for 5 min. Any yellow colour in the solution is not more intense than that in a standard prepared at the same time and in the same manner using 10 ml of *phosphate standard solution (5 ppm PO_4) R*.

Water (*2.5.12*): maximum 8.0 per cent, determined on 0.200 g.

ASSAY

Dissolve 0.100 g in *water R* and dilute to 100.0 ml with the same solvent. Dilute 5.0 ml of this solution to 250.0 ml with *water R*. Measure the absorbance (*2.2.25*) at the absorption maximum at 247 nm.

Calculate the content of $C_{21}H_{27}Na_2O_8P$ taking the specific absorbance to be 312.

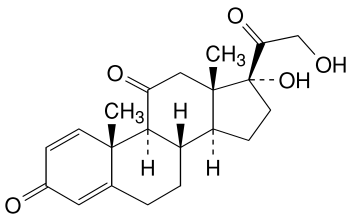
STORAGE

Protected from light.

01/2008:0354
corrected 6.0

PREDNISONE

Prednisonum



$C_{21}H_{26}O_5$ M_r 358.4
[53-03-2]

DEFINITION

17,21-Dihydroxypregna-1,4-diene-3,11,20-trione.