F. 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.

Dilute 1 ml of solution S to 5 ml with *carbon dioxide-free water R*.

Specific optical rotation (*2.2.7*): + 98 to + 104 (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 *betamethasone sodium phosphate CRS* and 25 mg of *dexamethasone sodium phosphate 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* = 0.25 m, Ø = 4.6 mm;

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

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

Flow rate: 1 ml/min.

Detection: spectrophotometer at 254 nm.

Equilibration: with the mobile phase for about 45 min.

Injection: 20 µl.

Run time: twice the retention time of betamethasone sodium phosphate.

Retention time: betamethasone sodium phosphate = about 14 min; dexamethasone sodium phosphate = about 15.5 min.

System suitability: reference solution (a):

– *resolution*: minimum 2.0 between the peaks due to betamethasone sodium phosphate and dexamethasone sodium phosphate; if necessary, increase the concentration of acetonitrile or increase 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 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 241 nm.

Calculate the content of $C_{22}H_{28}FNa_2O_8P$ taking the specific absorbance to be 297.

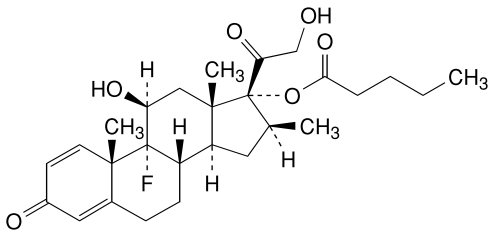
STORAGE

In an airtight container, protected from light.

01/2008:0811
corrected 6.0

BETAMETHASONE VALERATE

Betamethasoni valeras



$C_{27}H_{37}FO_6$ M_r 476.6
[2152-44-5]

DEFINITION

Betamethasone valerate contains not less than 97.0 per cent and not more than the equivalent of 103.0 per cent of 9-fluoro-11β,21-dihydroxy-16β-methyl-3,20-dioxopregna-1,4-dien-17-yl pentanoate, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, practically insoluble in water, freely soluble in acetone and in methylene chloride, soluble in ethanol (96 per cent).

It melts at about 192 °C, with decomposition.

IDENTIFICATION

First identification: C, D.

Second identification: A, B, E, F, G.

A. It complies with the test for specific optical rotation (see Tests).

B. Dissolve 10.0 mg in *anhydrous ethanol R* and dilute to 100.0 ml with the same solvent. 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*) of the solution measured at 419 nm is not more than 0.10.

C. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *betamethasone 17-valerate 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 *chloroform R*, evaporate to dryness on a water-bath and record new spectra using the residues.

D. Examine by thin-layer chromatography (*2.2.27*), using as the coating substance a suitable silica gel with a fluorescent indicator having an optimal intensity at 254 nm.

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 (a). Dissolve 10 mg of *betamethasone 17-valerate 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.

Reference solution (b). Dissolve 5 mg of *betamethasone 21-valerate CRS* in a mixture of 1 volume of *methanol R* and 9 volumes of *methylene chloride R* and dilute to 5 ml with the same mixture of solvents, then dilute to 10 ml with reference solution (a).

Apply to the plate 5 µl of each solution. Prepare the mobile phase by adding a mixture of 1.2 volumes of *water R* and 8 volumes of *methanol R* to a mixture of 15 volumes of *ether R* and 77 volumes of *methylene chloride R*. Develop over a path of 15 cm. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. 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). Spray the plate with *alcoholic solution of sulphuric acid R*. Heat at 120 °C for 10 min or until the spots appear. Allow to cool. Examine in daylight and in ultraviolet light at 365 nm. 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). The test is not valid unless the chromatogram obtained with reference solution (b) shows 2 clearly separated spots.

E. Examine by thin-layer chromatography (*2.2.27*), using as the coating substance a suitable silica gel with a fluorescent indicator having an optimal intensity at 254 nm.

Test solution (a). Dissolve 25 mg of the substance to be examined in *methanol R* with gentle heating and dilute to 5 ml with the same solvent. This solution is also used to prepare test solution (b). Dilute 2 ml of the solution to 10 ml with *methylene chloride R*.

Test solution (b). Transfer 2 ml of the solution obtained during preparation of test solution (a) to a 15 ml glass tube with a ground-glass stopper or a polytetrafluoroethylene cap. Add 10 ml of *saturated methanolic potassium hydrogen carbonate solution R* and immediately pass a current of *nitrogen R* briskly through the solution for 5 min. Stopper the tube. Heat in a water-bath at 45 °C, protected from light, for 3 h. Allow to cool.

Reference solution (a). Dissolve 25 mg of *betamethasone 17-valerate CRS* in *methanol R* with gentle heating and dilute to 5 ml with the same solvent. This solution is also used to prepare reference solution (b). Dilute 2 ml of the solution to 10 ml with *methylene chloride R*.

Reference solution (b). Transfer 2 ml of the solution obtained during preparation of reference solution (a) to a 15 ml glass tube with a ground glass-stopper or a polytetrafluoroethylene cap. Add 10 ml of *saturated methanolic potassium hydrogen carbonate solution R* and immediately pass a current of *nitrogen R* briskly through the solution for 5 min. Stopper the tube. Heat in a water-bath at 45 °C, protected from light, for 3 h. Allow to cool.

Apply to the plate 5 µl of each solution. Prepare the mobile phase by adding a mixture of 1.2 volumes of *water R* and 8 volumes of *methanol R* to a mixture of 15 volumes of *ether R* and 77 volumes of *methylene chloride R*. Develop over a path of 15 cm. Allow the plate to dry in air and examine under ultraviolet light at 254 nm. The principal spot in each of the chromatograms obtained with the test solutions is similar in position and size to the principal spot in the chromatogram obtained with the corresponding reference solution. Spray with *alcoholic solution of sulphuric acid R*. Heat at 120 °C for 10 min or until the spots appear. Allow to cool. Examine in daylight and in ultraviolet light at 365 nm. The principal spot in each of the chromatograms obtained with the test solutions is similar in position, colour in daylight, fluorescence in ultraviolet light at 365 nm and size to the principal spot in the chromatograms obtained with the corresponding reference solution. The principal spot in each of the chromatograms obtained with test solution (b) and reference solution (b) has an R_F value distinctly lower than that of the principal spots in each of the chromatograms obtained with test solution (a) and reference solution (a).

F. Add about 2 mg to 2 ml of *sulphuric acid R* and shake to dissolve. Within 5 min, a deep reddish-brown colour develops. Add the solution to 10 ml of *water R* and mix. The colour is discharged and a clear solution remains.

G. Mix about 5 mg with 45 mg of *heavy magnesium oxide R* and ignite in a crucible until an almost white residue is obtained (usually less than 5 min). Allow to cool, add 1 ml of *water R*, 0.05 ml of *phenolphthalein solution R1* and about 1 ml of *dilute hydrochloric acid R* to render the solution colourless. Filter. Add 1.0 ml of the filtrate to a freshly prepared mixture of 0.1 ml of *alizarin S solution R* and 0.1 ml of *zirconyl nitrate solution R*. Mix, allow to stand for 5 min and compare the colour of the solution with that of a blank prepared in the same manner. The test solution is yellow and the blank solution is red.

TESTS

Specific optical rotation (*2.2.7*). Dissolve 0.250 g in *dioxan R* and dilute to 25.0 ml with the same solvent. The specific optical rotation is + 75 to + 82, calculated with reference to the dried substance.

Related substances. Examine by liquid chromatography (*2.2.29*).

Solution A. To 1000 ml of the mobile phase add 1 ml of *glacial acetic acid R* and mix carefully.

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

Reference solution (a). Dissolve 2 mg of *betamethasone 17-valerate CRS* and 2 mg of *betamethasone 21-valerate CRS* in solution A and dilute to 50.0 ml with solution A.

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

The chromatographic procedure may be carried out using:

– a stainless steel column 0.25 m long and 4.6 mm in internal diameter packed with *octadecylsilyl silica gel for chromatography R* (5 µm);

– as mobile phase at a flow rate of 1 ml/min a mixture prepared as follows: mix 350 ml of *water R* with 600 ml of *acetonitrile R* and allow to equilibrate; adjust the volume to 1000 ml with *water R* and mix again;

– as detector a spectrophotometer set at 254 nm.

Equilibrate the column with the mobile phase for about 45 min.

Adjust the sensitivity so that the height of the principal peak in the chromatogram obtained with reference solution (b) is 70 per cent to 90 per cent of the full scale of the recorder.

Inject 20 µl of reference solution (a). When the chromatograms are recorded in the prescribed conditions, the retention times are: betamethasone 17-valerate, about 7 min; betamethasone 21-valerate, about 9 min. The test is not valid unless the resolution between the peaks due to betamethasone 17-valerate and betamethasone 21-valerate is at least 5.0; if necessary, adjust the concentration of acetonitrile in the mobile phase.

Inject 20 µl of the test solution and 20 µl of reference solution (b). Continue the chromatography for 2.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution: the area of any peak apart from the principal peak is not greater than 0.75 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent) and not more than one such peak has an area greater than half the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent); the sum of the areas of all the peaks, apart from the principal peak, is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3.0 per cent). Disregard any peak with an area less than 0.025 times the area of the principal peak in the chromatogram obtained with reference solution (b).

Loss on drying (*2.2.32*). Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

ASSAY

Dissolve 50.0 mg in *ethanol (96 per cent) R* and dilute to 100.0 ml with the same solvent. Dilute 2.0 ml of the solution to 50.0 ml with *ethanol (96 per cent) R* . Measure the absorbance (*2.2.25*) at the maximum at 240 nm.

Calculate the content of $C_{27}H_{37}FO_6$ taking the specific absorbance to be 325.

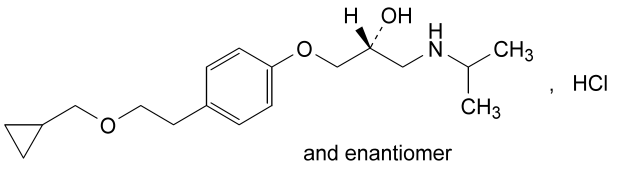
STORAGE

Protected from light.

01/2008:1072
corrected 6.0

BETAXOLOL HYDROCHLORIDE

Betaxololi hydrochloridum



$C_{18}H_{30}ClNO_3$ M_r 343.9
[63659-19-8]

DEFINITION

(2*RS*)-1-[4-[2-(Cyclopropylmethoxy)ethyl]phenoxy]-3-[(1-methylethyl)amino]propan-2-ol hydrochloride.

Content: 98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

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

IDENTIFICATION

First identification: B, D.

Second identification: A, C, D.

A. Melting point (*2.2.14*): 113 °C to 117 °C.

B. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *betaxolol hydrochloride CRS.*

C. Thin-layer chromatography (*2.2.27*).
Test solution. Dissolve 10 mg of the substance to be examined in 1 ml of *methanol R.*
Reference solution (a). Dissolve 20 mg of *betaxolol hydrochloride CRS* in 2 ml of *methanol R.*
Reference solution (b). Dissolve 10 mg of *oxprenolol hydrochloride CRS* in 1 ml of reference solution (a).
Plate: *TLC octadecylsilyl silica gel F_{254} plate R.*
Mobile phase: *perchloric acid R, methanol R, water R* (0.5:50:50 *V/V/V*).
Application: 2 µl.
Development: over a path of 10 cm.
Drying: in air.
System suitability: reference solution (b):
– the chromatogram shows 2 clearly separated spots.
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 a 50 g/l solution of *vanillin R* in a mixture of 5 volumes of *sulphuric acid R,* 10 volumes of *glacial acetic acid R* and 85 volumes of *methanol R.* Heat at 100-105 °C until the colour of the spots reaches maximum intensity (10-15 min). Examine in daylight.
Results B: 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 reference solution (a).

D. It gives reaction (a) of chlorides (*2.3.1*).