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

CHARACTERS

Appearance: white or almost white powder or colourless crystals.

Solubility: practically insoluble in water, freely soluble in acetone and in alcohol, soluble in fatty oils.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *Ph. Eur. reference spectrum of testosterone propionate.*

TESTS

Specific optical rotation (*2.2.7*): + 84 to + 90 (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 50.0 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 mg of the substance to be examined and 2 mg of *testosterone acetate 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* = 0.25 m, Ø = 4.6 mm,
– *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase: *water R, methanol R* (20:80 *V/V*).

Flow rate: 1.5 ml/min.

Detection: spectrophotometer at 254 nm.

Injection: 20 µl.

Run time: twice the retention time of testosterone propionate.

Relative retention with reference to testosterone propionate (retention time = about 9 min): impurity C = about 0.5; impurity A = about 0.7; impurity D = about 0.8; impurity B = about 1.4.

System suitability: reference solution (a):

– *resolution*: minimum 4.0 between the peaks due to testosterone propionate and to impurity A.

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 the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 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

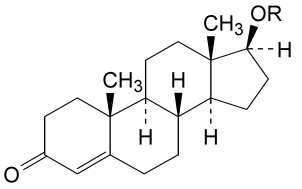
Dissolve 25.0 mg in *ethanol R* and dilute to 250.0 ml with the same solvent. Dilute 10.0 ml of the solution to 100.0 ml with *ethanol R*. Measure the absorbance (*2.2.25*) at the maximum at 240 nm.

Calculate the content of $C_{22}H_{32}O_3$ taking the specific absorbance to be 490.

IMPURITIES

Specified impurities: A, B, C, D.

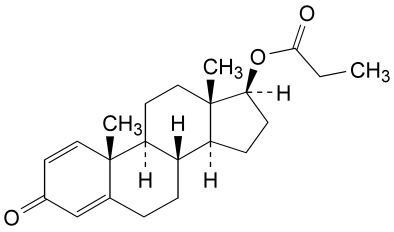
Other detectable impurities: E.



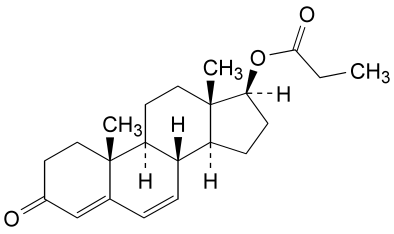
A. R = CO-CH_3: 3-oxoandrost-4-en-17β-yl acetate (testosterone acetate),

B. R = CO-$CH(CH_3)_2$: 3-oxoandrost-4-en-17β-yl 2-methylpropanoate (testosterone isobutyrate),

C. R = H: testosterone,



D. 3-oxoandrosta-1,4-dien-17β-yl propanoate,

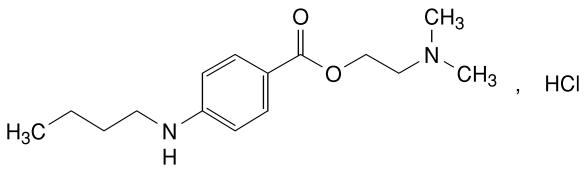


E. 3-oxoandrosta-4,6-dien-17β-yl propanoate.

01/2008:0057
corrected 6.0

TETRACAINE HYDROCHLORIDE

Tetracaini hydrochloridum



$C_{15}H_{25}ClN_2O_2$ M_r 300.8
[136-47-0]

DEFINITION

Tetracaine hydrochloride contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 2-(dimethylamino)ethyl 4-(butylamino)benzoate hydrochloride, calculated with reference to the dried substance.

CHARACTERS

A white or almost white, crystalline powder, slightly hygroscopic, freely soluble in water, soluble in alcohol.

It melts at about 148 °C or it may occur in either of 2 other crystalline forms which melt respectively at about 134 °C and 139 °C. Mixtures of these forms melt within the range 134 °C to 147 °C.

IDENTIFICATION

First identification: A, B, D.

Second identification: B, C, D.

A. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *tetracaine hydrochloride CRS*.

B. To 10 ml of solution S (see Tests) add 1 ml of *ammonium thiocyanate solution R*. A white, crystalline precipitate is formed which, after recrystallisation from *water R* and drying at 80 °C for 2 h, melts (*2.2.14*) at about 131 °C.

C. To about 5 mg add 0.5 ml of *fuming nitric acid R*. Evaporate to dryness on a water-bath, allow to cool and dissolve the residue in 5 ml of *acetone R*. Add 1 ml of *0.1 M alcoholic potassium hydroxide*. A violet colour develops.

D. Solution S gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S. Dissolve 5.0 g in *carbon dioxide-free water R* and dilute to 50 ml with the same solvent.

Appearance of solution. Dilute 2 ml of solution S to 10 ml with *water R*. The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*). Dilute 1 ml of solution S to 10 ml with *carbon dioxide-free water R*. The pH of the solution is 4.5 to 6.5.

Related substances. Examine by thin-layer chromatography (*2.2.27*), using a *TLC silica gel GF_{254} plate R*. Carry out a preliminary development over a path of 12 cm using a mixture of 4 volumes of *glacial acetic acid R*, 16 volumes of *hexane R* and 80 volumes of *dibutyl ether R*. Remove the plate and dry it in a current of warm air for a few minutes. Allow the plate to cool before use.

Test solution. Dissolve 1.0 g of the substance to be examined in *water R* and dilute to 10 ml with the same solvent.

Reference solution. Dissolve 50 mg of *4-aminobenzoic acid R* in *water R* and dilute to 100 ml with the same solvent. Dilute 1 ml of the solution to 10 ml with *water R*.

Apply to the plate 5 µl of each solution. Develop over a path of 10 cm using a mixture of 4 volumes of *glacial acetic acid R*, 16 volumes of *hexane R* and 80 volumes of *dibutyl ether R*. Dry the plate at 100 °C to 105 °C for 10 min 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 (0.05 per cent). The principal spot in the chromatogram obtained with the test solution remains at the starting point.

Heavy metals (*2.4.8*). 12 ml of solution S complies with limit test A for heavy metals (10 ppm). Prepare the standard using *lead standard solution (1 ppm Pb) R*.

Loss on drying (*2.2.32*). Not more than 1.0 per cent, determined on 1.000 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 0.250 g in 50 ml of *alcohol R* and add 5.0 ml of *0.01 M hydrochloric acid*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 30.08 mg of $C_{15}H_{25}ClN_2O_2$.

STORAGE

Store protected from light.

01/2008:0644

TETRACOSACTIDE

Tetracosactidum

H-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-Gly-Lys-Lys-Arg-Arg-Pro-Val-Lys-Val-Tyr-Pro-OH

$C_{136}H_{210}N_{40}O_{31}S$ M_r 2933

[16960-16-0]

DEFINITION

Tetracosactide is a synthetic tetracosapeptide in which the sequence of amino acids is the same as that of the first twenty-four residues of human corticotropin. It is available as an acetate and contains water. It increases the rate at which corticoid hormones are secreted by the adrenal glands. The potency is not less than 800 International Units per milligram, calculated with reference to the anhydrous, acetic acid-free substance.

CHARACTERS

A white or yellow, amorphous powder, sparingly soluble in water.

IDENTIFICATION

A. It increases the amount of corticosterone produced by isolated rat adrenal cells in the conditions of the assay.

B. Examine by electrophoresis (*2.2.31*) and thin-layer chromatography (*2.2.27*) to obtain a two-dimensional separation using two plates with *cellulose for chromatography R1* as the coating substance.

Test solution. Dissolve 1 mg of the substance to be examined in 0.2 ml of a 15.4 g/l solution of *ammonium acetate R* adjusted to pH 8.2 with *dilute ammonia R2*. Add 10 µl of a 2 g/l solution of *trypsin R*, maintain the mixture at 37 °C to 38 °C for 40 min, heat on a water-bath for 3 min and add 5 µl of *glacial acetic acid R*. Evaporate to dryness at 40 °C at a pressure not exceeding 3 kPa, dry the glassy residue at 40 °C for 1 h and dissolve in 0.1 ml of *glacial acetic acid R*. Dry the solution from the frozen state, dissolve the residue in 0.1 ml of *water R* and dry again from the frozen state. Dry the final residue at 45 °C for 1 h at a pressure not exceeding 3 kPa and dissolve in 50 µl of *water R*.

Reference solution. Prepare at the same time and in the same manner as the test solution, using *tetracosactide CRS* instead of the substance to be examined.

Spray the plates with the electrolyte solution which consists of a solution containing 0.2 per cent *V/V* of *glacial acetic acid R* and 0.2 per cent *V/V* of *pyridine R*. Place the filter paper tongues to connect the plates with the appropriate compartment of each trough so that each