

Relative density (2.2.5): 1.180 to 1.186.

Refractive index (2.2.6): 1.535 to 1.538.

ASSAY

Dissolve 0.500 g in 25 mL of *ethanol (96 per cent) R*. Add 0.05 mL of *phenol red solution R* and neutralise with 0.1 M *sodium hydroxide*. To the neutralised solution add 50.0 mL of 0.1 M *sodium hydroxide* and heat under a reflux condenser on a water-bath for 30 min. Cool and titrate with 0.1 M *hydrochloric acid*. Calculate the volume of 0.1 M *sodium hydroxide* used in the saponification. Carry out a blank titration.

1 mL of 0.1 M *sodium hydroxide* is equivalent to 15.21 mg of $C_{20}H_{30}O_2$.

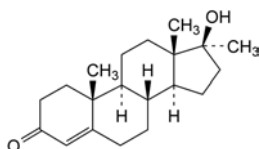
STORAGE

Protected from light.

07/2008:0410
corrected 6.3

METHYLTESTOSTERONE

Methyltestosteronum



$C_{20}H_{30}O_2$
[58-18-4]

M_r 302.5

DEFINITION

17 β -Hydroxy-17-methylandrosta-4-en-3-one.

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

CHARACTERS

Appearance: white or slightly yellowish-white, crystalline powder.

Solubility: practically insoluble in water, freely soluble in ethanol (96 per cent).

IDENTIFICATION

First identification: B.

Second identification: A, C.

A. Melting point (2.2.14): 162 °C to 168 °C.

B. Infrared absorption spectrophotometry (2.2.24).

Comparison: methyltestosterone CRS.

C. Thin-layer chromatography (2.2.27).

Test solution. Dissolve 0.2 g of the substance to be examined in a mixture of 1 volume of *methanol R* and 9 volumes of *chloroform R* and dilute to 10 mL with the same mixture of solvents.

Reference solution. Dissolve 20 mg of *methyltestosterone CRS* in 1 mL of a mixture of 1 volume of *methanol R* and 9 volumes of *chloroform R*.

Plate: TLC silica gel F_{254} plate R.

Mobile phase: anhydrous acetic acid R, light petroleum R, butyl acetate R (1:30:70 V/V/V).

Application: 5 μ L.

Development: over 2/3 of the plate.

Drying: in air.

Detection: examine in ultraviolet light at 254 nm and spray with a saturated solution of *potassium dichromate R* in a mixture of 30 volumes of *water R* and 70 volumes of *sulfuric acid R*. Examine immediately in daylight.

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): + 79 to + 85 (dried substance).

Dissolve 0.250 g in *ethanol (96 per cent) R* and dilute to 25.0 mL with the same solvent.

Related substances. Liquid chromatography (2.2.29).

Test solution. Dissolve 50 mg of the substance to be examined in *methanol R* and dilute to 100.0 mL with the same solvent.

Reference solution (a). Dilute 0.5 mL of the test solution to 100.0 mL with *methanol R*.

Reference solution (b). Dissolve 5 mg of *methyltestosterone for system suitability CRS* (containing impurity A) in *methanol R* and dilute to 10 mL with the same solvent.

Column:

– size: $l = 0.25$ m, $\varnothing = 4.6$ mm;

– stationary phase: end-capped octadecylsilyl silica gel for chromatography R (5 μ m).

Mobile phase:

– mobile phase A: water R;

– mobile phase B: methanol R;

Time (min)	Mobile phase A (per cent V/V)	Mobile phase B (per cent V/V)
0 - 15	30	70
15 - 45	30 \rightarrow 0	70 \rightarrow 100
45 - 50	0	100

Flow rate: 1.5 mL/min.

Detection: spectrophotometer at 254 nm.

Injection: 20 μ L.

Identification of impurities: use the chromatogram supplied with *methyltestosterone for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peak due to impurity A.

Relative retention with reference to methyltestosterone (retention time = about 8 min): impurity A = about 1.5.

System suitability: reference solution (b):

– resolution: minimum 5 between the peaks due to methyltestosterone and impurity A.

Limits:

– impurity A: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);

– unspecified impurities: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

– total: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);

– disregard limit: 0.1 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 2.0 per cent, determined on 0.500 g by drying in an oven at 105 °C for 2 h.

ASSAY

Dissolve 50.0 mg in *ethanol (96 per cent) R* and dilute to 50.0 mL with the same solvent. Dilute 10.0 mL of the solution to 100.0 mL with *ethanol (96 per cent) R*. Dilute 10.0 mL of this solution to 100.0 mL with *ethanol (96 per cent) R*. Measure the absorbance (2.2.25) at the absorption maximum at 241 nm.

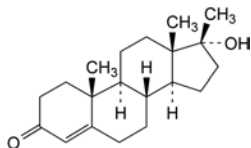
Calculate the content of $C_{20}H_{30}O_2$, taking the specific absorbance to be 540.

STORAGE

Protected from light.

IMPURITIES

Specified impurities: A.

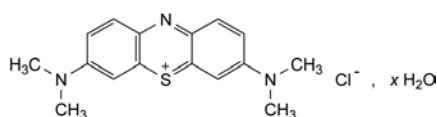


A. 17 α -hydroxy-17-methylandrosta-4-en-3-one.

01/2008:1132
corrected 7.0

METHYLTHIONINIUM CHLORIDE

Methylthioninii chloridum



$C_{16}H_{18}ClN_3 \cdot xH_2O$

M_r 319.9 (anhydrous substance)

DEFINITION

3,7-Bis(dimethylamino)phenothiazin-5-ylum chloride (methylene blue).

Content: 95.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance: dark blue, crystalline powder with a copper-coloured sheen, or green crystals with a bronze-coloured sheen.

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

IDENTIFICATION

A. Ultraviolet and visible absorption spectrophotometry (2.2.25).

Test solution. Dissolve 10 mg in dilute hydrochloric acid R and dilute to 100 mL with the same acid. Dilute 5 mL of the solution to 100 mL with dilute hydrochloric acid R.

Spectral range: 240-800 nm.

Absorption maxima: at 255-260 nm, 285-290 nm, 675-685 nm and 740-750 nm.

B. 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. Dilute 1 mL of the solution to 10 mL with methanol R.

Reference solution. Dissolve 10 mg of methylthioninium chloride CRS in methanol R and dilute to 10 mL with the same solvent. Dilute 1 mL of the solution to 10 mL with methanol R.

Plate: TLC silica gel plate R.

Mobile phase: anhydrous formic acid R, propanol R (20:80 V/V).

Application: 2 μ L.

Development: over a path of 8 cm.

Drying: in air, protected from light.

Detection: examine in daylight.

Results: 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 the reference solution. A secondary spot may appear above the principal spot in both chromatograms.

C. Dissolve about 1 mg in 10 mL of water R. Add 1 mL of glacial acetic acid R and 0.1 g of zinc powder R. Heat to boiling. The solution becomes colourless. Filter and shake the filtrate. It becomes blue on contact with air.

D. Ignite 50 mg with 0.5 g of anhydrous sodium carbonate R. Cool and dissolve the residue in 10 mL of dilute nitric acid R. Filter. The filtrate, without further addition of dilute nitric acid R, gives reaction (a) of chlorides (2.3.1).

TESTS

Methanol-insoluble substances: maximum 10.0 mg (1.0 per cent).

To 1.0 g add 20 mL of methanol R and boil under a reflux condenser for 5 min. Filter through a tared sintered-glass filter (40) (2.1.2) and wash the filter with methanol R until a colourless filtrate is obtained. Dry the filter at 100 °C and weigh.

Related substances. Liquid chromatography (2.2.29).

Test solution. Dissolve 15.0 mg of the substance to be examined in the mobile phase and dilute to 100.0 mL with the mobile phase.

Reference solution (a). Dissolve 7.5 mg of methylthioninium impurity A CRS in the mobile phase and dilute to 50.0 mL with the mobile phase. To 1.0 mL of this solution add 1.0 mL of the test solution and dilute to 10.0 mL with the mobile phase.

Reference solution (b). Dilute 1.0 mL of the test solution to 100.0 mL with the mobile phase.

Column:

- size: $l = 0.25$ m, $\varnothing = 4$ mm,
- stationary phase: octadecylsilyl silica gel for chromatography R (7 μ m).

Mobile phase: mix 27 volumes of acetonitrile R and 73 volumes of a mixture of 3.4 mL of phosphoric acid R and 1000 mL of water R.

Flow rate: 1 mL/min.

Detection: spectrophotometer at 246 nm.

Injection: 20 μ L.

Run time: twice the retention time of methylthioninium.

Relative retention with reference to methylthioninium (retention time = about 11 min): impurity A = about 0.7.

System suitability: reference solution (a):

- resolution: minimum 1.5 between the peaks due to impurity A and methylthioninium. If necessary, adjust the concentration of acetonitrile in the mobile phase.

Limits:

- impurity A: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (5.0 per cent),
- any other impurity: for each 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),
- sum of impurities other than A: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent),
- disregard limit: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Metals. Atomic emission spectrometry (2.2.22) in argon plasma, using as detector a conventional optical system or a mass spectrometer; in the case of a mass spectrometer, use indium as internal standard.

Test solution. In a 10 mL volumetric flask, dissolve with stirring 100 mg of the substance to be examined in 9 mL of water R, add 100.0 μ L of a 10 μ g/mL solution of indium prepared from indium elementary standard solution for atomic spectrometry (1.000 g/L) R in nitric acid R which has been diluted fifty-fold with water R. Dilute to 10.0 mL with water R.