A. Determine the melting point (*2.2.14*) of the substance to be examined. Mix equal parts of the substance to be examined and *cyclophosphamide CRS* and determine the melting point of the mixture. The difference between the melting points (which are about 51 °C) is not greater than 2 °C.

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

C. Examine the chromatograms obtained in the test for related substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Dissolve 0.1 g in 10 mL of *water R* and add 5 mL of *silver nitrate solution R1*; the solution remains clear. Boil, a white precipitate is formed which dissolves in *concentrated ammonia R* and is reprecipitated on the addition of *dilute nitric acid R*.

TESTS

Solution S. Dissolve 0.50 g in *carbon dioxide-free water R* and dilute to 25.0 mL with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

pH (*2.2.3*). The pH of solution S is 4.0 to 6.0, determined immediately after preparation of the solution.

Related substances. Examine by thin-layer chromatography (*2.2.27*), using *silica gel G R* as the coating substance.

Test solution (a). Dissolve 0.10 g of the substance to be examined in *alcohol R* and dilute to 5 mL with the same solvent.

Test solution (b). Dilute 1 mL of test solution (a) to 10 mL with *alcohol R*.

Reference solution (a). Dissolve 10 mg of *cyclophosphamide CRS* in *alcohol R* and dilute to 5 mL with the same solvent.

Reference solution (b). Dilute 0.1 mL of test solution (a) to 10 mL with *alcohol R*.

Apply separately to the plate 10 µL of each solution. Develop over a path of 15 cm using a mixture of 2 volumes of *anhydrous formic acid R*, 4 volumes of *acetone R*, 12 volumes of *water R* and 80 volumes of *methyl ethyl ketone R*. Dry the plate in a current of warm air and heat at 110 °C for 10 min. At the bottom of a chromatographic tank, place an evaporating dish containing a 50 g/L solution of *potassium permanganate R* and add an equal volume of *hydrochloric acid R*. Place the plate whilst still hot in the tank and close the tank. Leave the plate in contact with the chlorine gas for 2 min. Withdraw the plate and place it in a current of cold air until the excess of chlorine is removed and an area of coating below the points of application gives at most a very faint blue colour with a drop of *potassium iodide and starch solution R*. Avoid prolonged exposure to cold air. Spray with *potassium iodide and starch solution R* and allow to stand for 5 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (1.0 per cent). Disregard any spot remaining at the point of application.

Chlorides (*2.4.4*). Dissolve 0.15 g in *water R* and dilute to 15 mL with the same solvent. The freshly prepared solution complies with the limit test for chlorides (330 ppm).

Phosphates (*2.4.11*). Dissolve 0.10 g in *water R* and dilute to 100 mL with the same solvent. The solution complies with the limit test for phosphates (100 ppm).

Heavy metals (*2.4.8*). 1.0 g complies with limit test C for heavy metals (20 ppm). Prepare the standard using 2 mL of *lead standard solution (10 ppm Pb) R*.

Water (*2.5.12*): 6.0 per cent to 7.0 per cent, determined on 0.300 g by the semi-micro determination of water.

ASSAY

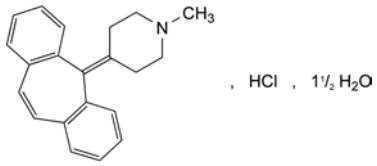
Dissolve 0.100 g in 50 mL of a 1 g/L solution of *sodium hydroxide R* in *ethylene glycol R* and boil under a reflux condenser for 30 min. Allow to cool and rinse the condenser with 25 mL of *water R*. Add 75 mL of *2-propanol R*, 15 mL of *dilute nitric acid R*, 10.0 mL of *0.1 M silver nitrate* and 2.0 mL of *ferric ammonium sulfate solution R2* and titrate with *0.1 M ammonium thiocyanate*.

1 mL of *0.1 M silver nitrate* is equivalent to 13.05 mg of $C_7H_{15}Cl_2N_2O_2P$.

07/2009:0817

CYPROHEPTADINE HYDROCHLORIDE

Cyproheptadini hydrochloridum



$C_{21}H_{22}ClN,1^1/_2H_2O$ M_r 350.9

[41354-29-4]

DEFINITION

4-(5*H*-Dibenzo[*a,d*][7]annulen-5-ylidene)-1-methylpiperidine hydrochloride sesquihydrate.

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

CHARACTERS

Appearance: white or slightly yellow, crystalline powder.

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

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *cyproheptadine hydrochloride CRS*.

B. A saturated solution gives reaction (b) of chlorides (*2.3.1*).

TESTS

Acidity. Dissolve 0.10 g in *water R* and dilute to 25 mL with the same solvent. Add 0.1 mL of *methyl red solution R*. Not more than 0.15 mL of *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 40.0 mg of the substance to be examined in mobile phase A and dilute to 20.0 mL with mobile phase A.

Reference solution (a). Dilute 1.0 mL of the test solution to 100.0 mL with mobile phase A. Dilute 1.0 mL of this solution to 10.0 mL with mobile phase A.

Reference solution (b). Dissolve 2.0 mg of *dibenzocycloheptene CRS* (impurity A), 2.0 mg of *dibenzosuberone CRS* (impurity B) and 2.0 mg of *cyproheptadine impurity C CRS* in mobile phase A, add 1.0 mL of the test solution and dilute to 100.0 mL with mobile phase A.

Reference solution (c). Dilute 1.0 mL of reference solution (b) to 10.0 mL with mobile phase A.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm;
– *stationary phase*: *octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

– *mobile phase A*: dissolve 6.12 g of *potassium dihydrogen phosphate R* in 900 mL of *water R*, adjust to pH 4.5 with *phosphoric acid R* and dilute to 1000 mL with *water R*; mix 60 volumes of this solution and 40 volumes of *acetonitrile for chromatography R*;

– *mobile phase B*: dissolve 6.12 g of *potassium dihydrogen phosphate R* in 900 mL of *water R*, adjust to pH 4.5 with *phosphoric acid R* and dilute to 1000 mL with *water R*; mix 40 volumes of this solution and 60 volumes of *acetonitrile for chromatography R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10.0	100	0
10.0 - 10.1	100 → 0	0 → 100
10.1 - 35	0	100

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 230 nm.

Injection: 10 µL.

Relative retention with reference to cyproheptadine (retention time = about 8 min): impurity C = about 0.7; impurity B = about 2.6; impurity A = about 3.9.

System suitability: reference solution (b):

– *resolution*: minimum 7.0 between the peaks due to impurity C and cyproheptadine.

Limits:

– *impurities A, B, C*: for each impurity, not more than 1.5 times the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.15 per cent);
– *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
– *total*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
– *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (*2.5.12*): 7.0 per cent to 9.0 per cent, determined on 0.200 g.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in a mixture of 5.0 mL of *0.01 M hydrochloric acid* and 50 mL of *ethanol (96 per cent) R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

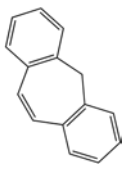
1 mL of *0.1 M sodium hydroxide* is equivalent to 32.39 mg of $C_{21}H_{22}ClN$.

STORAGE

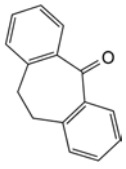
Protected from light.

IMPURITIES

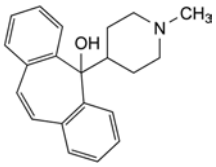
Specified impurities: A, B, C.



A. 5*H*-dibenzo[*a,d*][7]annulene (dibenzocycloheptene),



B. 10,11-dihydro-5*H*-dibenzo[*a,d*][7]annulen-5-one (dibenzosuberone),

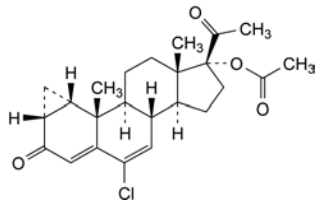


C. 5-(1-methylpiperidin-4-yl)-5*H*-dibenzo[*a,d*][7]annulen-5-ol.

07/2010:1094

CYPROTERONE ACETATE

Cyproteroni acetas



$C_{24}H_{29}ClO_4$ M_r 416.9

[427-51-0]

DEFINITION

6-Chloro-3,20-dioxo-1β,2β-dihydro-3′*H*-cyclopropa-[1,2]pregna-1,4,6-trien-17-yl acetate.

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

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: practically insoluble in water, very soluble in methylene chloride, freely soluble in acetone, soluble in methanol, sparingly soluble in anhydrous ethanol.

mp: about 210 °C.

IDENTIFICATION

First identification: A.

Second identification: B, C, D, E.

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *cyproterone acetate CRS*.

B. Thin-layer chromatography (*2.2.27*).
Test solution. Dissolve 20 mg of the substance to be examined in *methylene chloride R* and dilute to 10 mL with the same solvent.
Reference solution. Dissolve 10 mg of *cyproterone acetate CRS* in *methylene chloride R* and dilute to 5 mL with the same solvent.
Plate: *TLC silica gel F_{254} plate R*.
Mobile phase: *cyclohexane R, ethyl acetate R* (50:50 *V/V*).
Application: 5 µL.
Development: twice over 3/4 of the plate; dry the plate in air between the 2 developments.
Drying: in air.
Detection: examine in ultraviolet light at 254 nm.
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.

C. To about 1 mg add 2 mL of *sulfuric acid R* and heat on a water-bath for 2 min. A red colour develops. Cool. Add this solution cautiously to 4 mL of *water R* and shake. The solution becomes violet.

D. Incinerate about 30 mg with 0.3 g of *anhydrous sodium carbonate R* over a naked flame for about 10 min. Cool and dissolve the residue in 5 mL of *dilute nitric acid R*. Filter. To 1 mL of the filtrate add 1 mL of *water R*. The solution gives reaction (a) of chlorides (*2.3.1*).