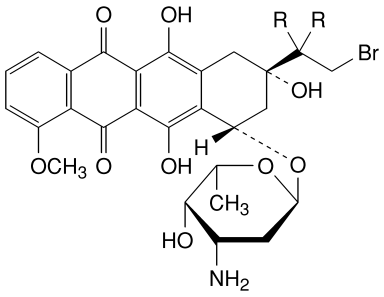
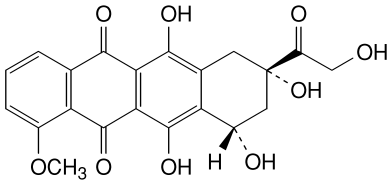
IMPURITIES

A. daunorubicin,



B. R = OCH_3: (8*S*,10*S*)-10[(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-8-(2-bromo-1,1-dimethoxyethyl)-6,8,11-trihydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione,

C. R + R = O: (8*S*,10*S*)-10[(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-8-(bromoacetyl)-6,8,11-trihydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione,

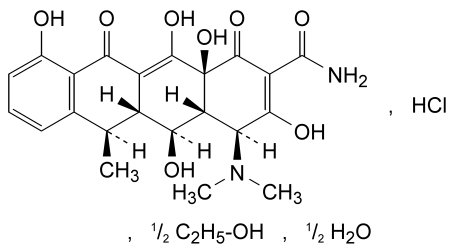


D. (8*S*,10*S*)-6,8,10,11-tetrahydroxy-8-(hydroxyacetyl)-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione (doxorubicin aglycone, doxorubicinone).

01/2008:0272
corrected 6.0

DOXYCYCLINE HYCLATE

Doxycyclini hyclas



$(C_{22}H_{25}ClN_2O_8),{}^1/_2C_2H_6O,{}^1/_2H_2O$ M_r 512.9
[24390-14-5]

DEFINITION

Hydrochloride hemiethanol hemihydrate of (4*S*,4a*R*,5*S*,5a*R*,6*R*,12a*S*)-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide.

Substance obtained from oxytetracycline or metacycline or by any other means.

Semi-synthetic product derived from a fermentation product.

Content: 95.0 per cent to 102.0 per cent of $C_{22}H_{25}ClN_2O_8$ (anhydrous substance).

CHARACTERS

Appearance: yellow, crystalline powder, hygroscopic.

Solubility: freely soluble in water and in methanol, sparingly soluble in ethanol (96 per cent). It dissolves in solutions of alkali hydroxides and carbonates.

IDENTIFICATION

A. Examine the chromatograms obtained in the assay.

Results: the principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

B. To about 2 mg add 5 ml of *sulphuric acid R*. A yellow colour develops.

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

TESTS

pH (*2.2.3*): 2.0 to 3.0.

Dissolve 0.1 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Specific optical rotation (*2.2.7*): – 105 to – 120 (anhydrous substance).

Dissolve 0.250 g in a mixture of 1 volume of *1 M hydrochloric acid* and 99 volumes of *methanol R* and dilute to 25.0 ml with the same mixture of solvents. Carry out the measurement within 5 min of preparing the solution.

Specific absorbance (*2.2.25*): 300 to 335, determined at the absorption maximum at 349 nm (anhydrous substance).

Dissolve 25.0 mg in a mixture of 1 volume of *1 M hydrochloric acid* and 99 volumes of *methanol R* and dilute to 25.0 ml with the same mixture of solvents. Dilute 1.0 ml of the solution to 100.0 ml with a mixture of 1 volume of *1 M hydrochloric acid* and 99 volumes of *methanol R*. Carry out the measurement within 1 h of preparing the solution.

Light-absorbing impurities. The absorbance (*2.2.25*), determined at 490 nm is not greater than 0.07 (anhydrous and ethanol-free substance).

Dissolve 0.10 g in a mixture of 1 volume of *1 M hydrochloric acid* and 99 volumes of *methanol R* and dilute to 10.0 ml with the same mixture of solvents. Carry out the measurement within 1 h of preparing the solution.

Related substances. Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution. Dissolve 20.0 mg of the substance to be examined in *0.01 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Reference solution (a). Dissolve 20.0 mg of *doxycycline hyclate CRS* in *0.01 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Reference solution (b). Dissolve 20.0 mg of *6-epidoxycycline hydrochloride CRS* in *0.01 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Reference solution (c). Dissolve 20.0 mg of *metacycline hydrochloride CRS* in *0.01 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Reference solution (d). Mix 4.0 ml of reference solution (a), 1.5 ml of reference solution (b) and 1.0 ml of reference solution (c) and dilute to 25.0 ml with *0.01 M hydrochloric acid*.

Reference solution (e). Mix 2.0 ml of reference solution (b) and 2.0 ml of reference solution (c) and dilute to 100.0 ml with *0.01 M hydrochloric acid*.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm,

– *stationary phase*: *styrene-divinylbenzene copolymer R* (8 µm),

– *temperature*: 60 °C.

Mobile phase: weigh 60.0 g of *2-methyl-2-propanol R* and transfer to a 1000 ml volumetric flask with the aid of 200 ml of *water R*; add 400 ml of *buffer solution pH 8.0 R*, 50 ml of a 10 g/l solution of *tetrabutylammonium hydrogen sulphate R* adjusted to pH 8.0 with *dilute sodium hydroxide solution R* and 10 ml of a 40 g/l solution of *sodium edetate R* adjusted to pH 8.0 with *dilute sodium hydroxide solution R*; dilute to 1000.0 ml with *water R*.

Flow rate: 1.0 ml/min.

Detection: spectrophotometer at 254 nm.

Injection: 20 µl of the test solution and reference solutions (d) and (e).

Relative retention with reference to doxycycline: impurity E = about 0.2; impurity D = about 0.3; impurity C = about 0.5; impurity F = about 1.2.

System suitability: reference solution (d):

– *resolution*: minimum 1.25 between the peaks due to impurity B (1st peak) and impurity A (2nd peak) and minimum 2.0 between the peaks due to impurity A and doxycycline (3rd peak); if necessary, adjust the 2-methyl-2-propanol content in the mobile phase;

– *symmetry factor*: maximum 1.25 for the peak due to doxycycline.

Limits:

– *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (e) (2.0 per cent),

– *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (e) (2.0 per cent),

– *impurities C, D, E, F*: for each impurity, not more than 0.25 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (e) (0.5 per cent),

– *any other impurity*: for each impurity, not more than 0.25 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (e) (0.5 per cent),

– *disregard limit*: 0.05 times the area of the peak due to impurity A in the chromatogram obtained with reference solution (e) (0.1 per cent).

Ethanol. Gas chromatography (*2.2.28*).

Internal standard solution. Dilute 0.50 ml of *propanol R* to 1000.0 ml with *water R*.

Test solution (a). Dissolve 0.10 g of the substance to be examined in *water R* and dilute to 10.0 ml with the same solvent.

Test solution (b). Dissolve 0.10 g of the substance to be examined in the internal standard solution and dilute to 10.0 ml with the same solution.

Reference solution. Dilute 0.50 ml of *ethanol R* to 100.0 ml with the internal standard solution. Dilute 1.0 ml of this solution to 10.0 ml with the internal standard solution.

Column:

– *size*: *l* = 1.5 m, Ø = 4.0 mm,

– *stationary phase*: *ethylvinylbenzene-divinylbenzene copolymer R* (150-180 µm).

Carrier gas: *nitrogen for chromatography R*.

Temperature:

– *column*: 135 °C,

– *injection port and detector*: 150 °C.

Detection: flame ionisation.

Calculate the content of ethanol taking the density (*2.2.5*) at 20 °C to be 0.790 g/ml.

Limit:

– *ethanol*: 4.3 per cent to 6.0 per cent.

Heavy metals (*2.4.8*): maximum 50 ppm.

0.5 g complies with limit test C. Prepare the reference solution using 2.5 ml of *lead standard solution (10 ppm Pb) R*.

Water (*2.5.12*): 1.4 per cent to 2.8 per cent, determined on 1.20 g.

Sulphated ash (*2.4.14*): maximum 0.4 per cent, determined on 1.0 g.

Bacterial endotoxins (*2.6.14*): less than 1.14 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection: test solution and reference solution (a).

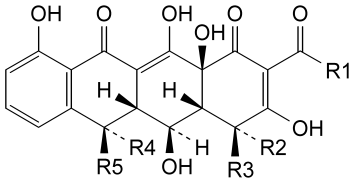
Calculate the percentage content of $C_{22}H_{25}ClN_2O_8$ (M_r = 480.9).

STORAGE

In an airtight container, protected from light. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

IMPURITIES

Specified impurities: A, B, C, D, E, F.



A. R1 = NH_2, R2 = R5 = H, R3 = $N(CH_3)_2$, R4 = CH_3: (4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (6-epidoxycycline),

B. R1 = NH_2, R2 = H, R3 = $N(CH_3)_2$, R4 + R5 = CH_2: (4*S*,4a*R*,5*S*,5a*R*,12a*S*)-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methylene-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (metacycline),

C. R1 = NH_2, R2 = $N(CH_3)_2$, R3 = R4 = H, R5 = CH_3: (4*R*,4a*R*,5*S*,5a*R*,6*R*,12a*S*)-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (4-epidoxycycline),

D. R1 = NH_2, R2 = $N(CH_3)_2$, R3 = R5 = H, R4 = CH_3: (4*R*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide (4-epi-6-epidoxycycline),

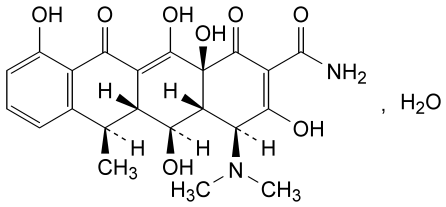
E. R1 = NH_2, R2 = H, R3 = $N(CH_3)_2$, R4 = OH, R5 = CH_3: oxytetracycline,

F. R1 = CH_3, R2 = R4 = H, R3 = $N(CH_3)_2$, R5 = CH_3: (4*S*,4a*R*,5*S*,5a*R*,6*R*,12a*S*)-2-acetyl-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-4a,5a,6,12a-tetrahydrotetracene-1,11(4*H*,5*H*)-dione (2-acetyl-2-decarbamoyldoxycycline).

01/2008:0820
corrected 6.0

DOXYCYCLINE MONOHYDRATE

Doxycyclinum monohydricum



$C_{22}H_{24}N_2O_8,H_2O$ M_r 462.5
[17086-28-1]

DEFINITION

(4*S*,4a*R*,5*S*,5a*R*,6*R*,12a*S*)-4-(Dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide monohydrate.

Substance obtained from oxytetracycline or metacycline or by any other means.

Semi-synthetic product derived from a fermentation product.

Content: 95.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance: yellow, crystalline powder.

Solubility: very slightly soluble in water and in alcohol. It dissolves in dilute solutions of mineral acids and in solutions of alkali hydroxides and carbonates.

IDENTIFICATION

A. Examine the chromatograms obtained in the assay.

Results: the principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

B. To about 2 mg add 5 ml of *sulphuric acid R*. A yellow colour develops.

C. Dissolve 25 mg in a mixture of 0.2 ml of *dilute nitric acid R* and 1.8 ml of *water R*. The solution does not give reaction (a) of chlorides (*2.3.1*).

TESTS

pH (*2.2.3*): 5.0 to 6.5.

Suspend 0.1 g in *carbon dioxide-free water R* and dilute to 10 ml with the same solvent.

Specific optical rotation (*2.2.7*): – 113 to – 130 (anhydrous substance).

Dissolve 0.250 g in a mixture of 0.5 volumes of *hydrochloric acid R* and 99.5 volumes of *methanol R* and dilute to 25.0 ml with the same mixture of solvents. Carry out the measurement within 5 min of preparing the solution.

Specific absorbance (*2.2.25*): 325 to 363 determined at the maximum at 349 nm (anhydrous substance).

Dissolve 25.0 mg in a mixture of 0.5 volumes of *hydrochloric acid R* and 99.5 volumes of *methanol R* and dilute to 50.0 ml with the same mixture of solvents. Dilute 2.0 ml of the solution to 100.0 ml with a mixture of 0.5 volumes of *1 M hydrochloric acid* and 99.5 volumes of *methanol R*. Carry out the measurement within 1 h of preparing the solution.

Light-absorbing impurities. The absorbance (*2.2.25*) determined at 490 nm has a maximum of 0.07 (anhydrous substance).

Dissolve 0.10 g in a mixture of 0.5 volumes of *hydrochloric acid R* and 99.5 volumes of *methanol R* and dilute to 10.0 ml with the same mixture of solvents. Carry out the measurement within 1 h of preparing the solution.

Related substances. Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Test solution. Dissolve 20.0 mg of the substance to be examined in *0.01 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Reference solution (a). Dissolve 20.0 mg of *doxycycline hyclate CRS* in *0.01 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Reference solution (b). Dissolve 20.0 mg of *6-epidoxycycline hydrochloride CRS* in *0.01 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Reference solution (c). Dissolve 20.0 mg of *metacycline hydrochloride CRS* in *0.01 M hydrochloric acid* and dilute to 25.0 ml with the same acid.

Reference solution (d). Mix 4.0 ml of reference solution (a), 1.5 ml of reference solution (b) and 1.0 ml of reference solution (c) and dilute to 25.0 ml with *0.01 M hydrochloric acid*.

Reference solution (e). Mix 2.0 ml of reference solution (b) and 2.0 ml of reference solution (c) and dilute to 100.0 ml with *0.01 M hydrochloric acid*.