Solubility: freely soluble in water and in methanol, sparingly soluble in methylene chloride, slightly soluble in acetonitrile.

IDENTIFICATION

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

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

TESTS

Acidity or alkalinity. Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 10 mL with the same solvent. Add 0.1 mL of *methyl red mixed solution R* and 0.25 mL of *0.01 M sodium hydroxide*. The solution is green. Add 0.5 mL of *0.01 M hydrochloric acid*. The solution is reddish-violet.

Related substances. Liquid chromatography (*2.2.29*).

Buffer solution pH 4.4. Dissolve 3.8 g of *sodium pentanesulfonate monohydrate R* and 4.0 g of *potassium dihydrogen phosphate R* in *water R*, adjust to pH 4.4 with *phosphoric acid R* and dilute to 1000.0 mL with *water R*.

Solvent mixture: mobile phase B, mobile phase A (25:75 *V/V*).

Test solution. Dissolve 50 mg of the substance to be examined in the solvent mixture and dilute to 100.0 mL with the solvent mixture.

Reference solution (a). Dilute 10.0 mL of the test solution to 100.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 100.0 mL with the solvent mixture.

Reference solution (b). Dissolve 5 mg of *epinastine for system suitability CRS* (containing impurities A and B) in 10.0 mL of the solvent mixture.

Column:

- *size*: *l* = 0.10 m, Ø = 3.0 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3 µm);
- *temperature*: 50 °C.

Mobile phase :

- *mobile phase A*: *methanol R2,* buffer solution pH 4.4 (15:85 *V/V*);
- *mobile phase B*: *methanol R2, acetonitrile R1* (15:85 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 4	80	20
4 - 13	80 → 30	20 → 70

Flow rate: 1.4 mL/min.

Detection: spectrophotometer at 220 nm.

Injection: 10 µL.

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

Relative retention with reference to epinastine (retention time = about 4 min): impurity A = about 1.2; impurity B = about 2.0.

System suitability: reference solution (b):

- *peak-to-valley ratio*: minimum 2.0, where H_p = height above the baseline of the peak due to impurity A and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to epinastine.

Limits:

- *impurity B*: not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
- *impurity A*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 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 7 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.7 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).

Heavy metals (*2.4.8*): maximum 20 ppm.

Solvent: water R.

0.250 g complies with test H. Prepare the reference solution using 0.5 mL of *lead standard solution (10 ppm Pb) R*.

Loss on drying (*2.2.32*): maximum 1.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

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

ASSAY

Dissolve 0.200 g in 100 mL of a mixture of 1 volume of *anhydrous acetic acid R* and 2 volumes of *acetic anhydride R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*).

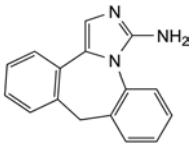
1 mL of *0.1 M perchloric acid* is equivalent to 28.58 mg of $C_{16}H_{16}ClN_3$.

STORAGE

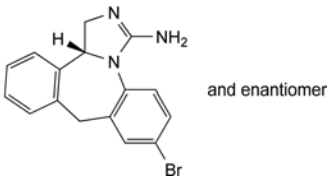
In an airtight container.

IMPURITIES

Specified impurities: A, B.



A. 9*H*-dibenzo[*c,f*]imidazo[1,5-*a*]azepin-3-amine,

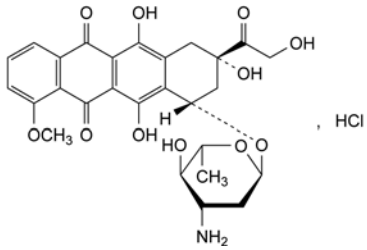


B. (13b*RS*)-7-bromo-9,13b-dihydro-1*H*-dibenzo[*c,f*]imidazo-[1,5-*a*]azepin-3-amine.

01/2008:1590

EPIRUBICIN HYDROCHLORIDE

Epirubicini hydrochloridum



$C_{27}H_{30}ClNO_{11}$ M_r 580.0
[56390-09-1]

DEFINITION

(8*S*,10*S*)-10-[(3-Amino-2,3,6-trideoxy-α-L-*arabino*-hexopyranosyl)oxy]-6,8,11-trihydroxy-8-(hydroxyacetyl)-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione hydrochloride.

Substance obtained by chemical transformation of a substance produced by certain strains of *Streptomyces peucetius*.

Content: 97.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance: orange-red powder.

Solubility: soluble in water and in methanol, slightly soluble in anhydrous ethanol, practically insoluble in acetone.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *epirubicin hydrochloride CRS*.

B. Examine the chromatograms obtained in the assay.

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

C. Dissolve about 10 mg in 0.5 mL of *nitric acid R*, add 0.5 mL of *water R* and heat over a flame for 2 min. Allow to cool and add 0.5 mL of *silver nitrate solution R1*. A white precipitate is formed.

TESTS

pH (*2.2.3*): 4.0 to 5.5.

Dissolve 50 mg in *carbon dioxide-free water R* and dilute to 10 mL with the same solvent.

Related substances. Liquid chromatography (*2.2.29*). *Allow the solutions to stand for 3 h before use.*

Test solution. Dissolve 25.0 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 25.0 mg of *epirubicin hydrochloride CRS* in the mobile phase and dilute to 25.0 mL with the mobile phase.

Reference solution (b). Dissolve 10 mg of *epirubicin hydrochloride CRS* and 10 mg of *doxorubicin hydrochloride CRS* in the mobile phase and dilute to 100 mL with the mobile phase.

Reference solution (c). Dissolve 10 mg of *doxorubicin hydrochloride CRS* in a mixture of 5 mL of *water R* and 5 mL of *phosphoric acid R*. Allow to stand for 30 min. Adjust to pH 2.6 with an 80 g/L solution of *sodium hydroxide R*. Add 15 mL of *acetonitrile R* and 10 mL of *methanol R*. Mix.

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

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm;
– *stationary phase*: *trimethylsilyl silica gel for chromatography R* (6 µm);
– *temperature*: 35 °C.

Mobile phase: mix 17 volumes of *methanol R*, 29 volumes of *acetonitrile R* and 54 volumes of a solution containing 3.7 g/L of *sodium laurilsulfate R* and 2.8 per cent *V/V* of *dilute phosphoric acid R*.

Flow rate: 2.5 mL/min.

Detection: spectrophotometer at 254 nm.

Injection: 10 µL of the test solution and reference solutions (b), (c) and (d).

Run time: 3.5 times the retention time of epirubicin.

Identification of impurities: use the 2nd most abundant peak present in the chromatogram obtained with reference solution (c) to identify impurity A.

Relative retention with reference to epirubicin (retention time = about 9.5 min): impurity A = about 0.3; impurity B = about 0.4; impurity C = about 0.8; impurity E = about 1.1; impurity D = about 1.5; impurity F = about 1.7; impurity G = about 2.1.

System suitability: reference solution (b):

– *resolution*: minimum 2.0 between the peaks due to impurity C and epirubicin.

Limits:

– *correction factor*: for the calculation of content, multiply the peak area of impurity A by 0.7;
– *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (1.0 per cent);
– *impurity C*: not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (1.0 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 (d) (0.5 per cent);
– *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (d) (2.0 per cent);
– *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (d) (0.05 per cent).

Acetone (*2.4.24*): maximum 1.5 per cent.

Water (*2.5.12*): maximum 4.0 per cent, determined on 0.100 g.

Bacterial endotoxins (*2.6.14*): less than 1.1 IU/mg, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for removal of bacterial endotoxins.

ASSAY

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

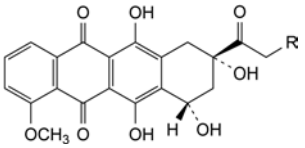
Injection: test solution and reference solution (a).

Calculate the percentage content of $C_{27}H_{30}ClNO_{11}$.

STORAGE

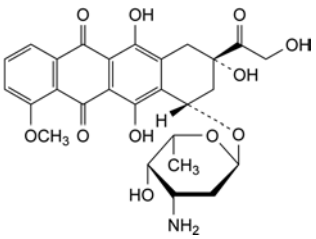
In an airtight container, protected from light, at a temperature of 2 °C to 8 °C. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

IMPURITIES

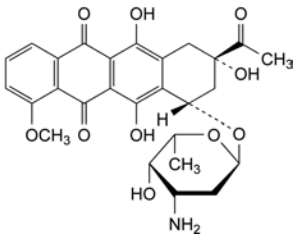


A. R = OH: (8*S*,10*S*)-6,8,10,11-tetrahydroxy-8-(hydroxyacetyl)-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione (doxorubicinone),

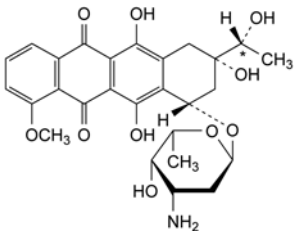
B. R = H: (8*S*,10*S*)-8-acetyl-6,8,10,11-tetrahydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione (daunorubicinone),



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

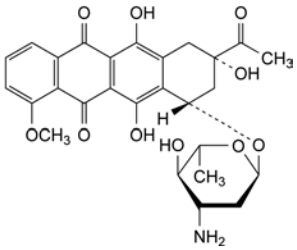


D. (8*S*,10*S*)-8-acetyl-10-[(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-6,8,11-trihydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione (daunorubicin),

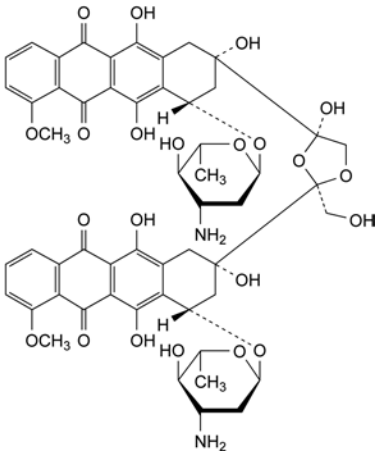


and epimer at C*

E. (8*S*,10*S*)-10-[(3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)oxy]-6,8,11-trihydroxy-8-[(1*RS*)-1-hydroxyethyl]-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione (dihydrodaunorubicin),



F. (8*S*,10*S*)-8-acetyl-10-[(3-amino-2,3,6-trideoxy-α-L-*arabino*-hexopyranosyl)oxy]-6,8,11-trihydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione (*epi*-daunorubicin),

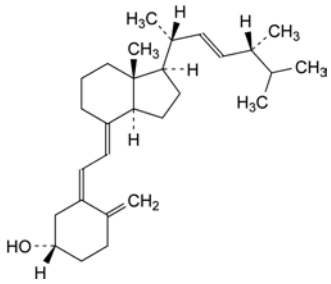


G. 8,8′-[(2*R*,4*R*)-4-hydroxy-2-(hydroxymethyl)-1,3-dioxolan-2,4-diyl]bis[(8*S*,10*S*)-10-[(3-amino-2,3,6-trideoxy-α-L-*arabino*-hexopyranosyl)oxy]-6,8,11-trihydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione] (epirubicin dimer).

01/2008:0082
corrected 6.3

ERGOCALCIFEROL

Ergocalciferolum



$C_{28}H_{44}O$ M_r 396.7
[50-14-6]

DEFINITION

Ergocalciferol contains not less than 97.0 per cent and not more than the equivalent of 103.0 per cent of (5*Z*,7*E*,22*E*)-9,10-secoergosta-5,7,10(19),22-tetraen-3β-ol.

1 mg of ergocalciferol is equivalent to 40 000 IU of antirachitic activity (vitamin D) in rats.

CHARACTERS

A white or slightly yellowish, crystalline powder or white or almost white crystals, practically insoluble in water, freely soluble in alcohol, soluble in fatty oils. It is sensitive to air, heat and light. Solutions in volatile solvents are unstable and are to be used immediately.

A reversible isomerisation to pre-ergocalciferol takes place in solution, depending on temperature and time. The activity is due to both compounds.

IDENTIFICATION

Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *ergocalciferol CRS*. Examine the substances prepared as discs.

TESTS

Specific optical rotation (*2.2.7*). Dissolve 0.200 g rapidly and without heating in *aldehyde-free alcohol R* and dilute to 25.0 mL with the same solvent. The specific optical rotation, determined within 30 min of preparing the solution, is + 103 to + 107.

Reducing substances. Dissolve 0.1 g in *aldehyde-free alcohol R* and dilute to 10.0 mL with the same solvent. Add 0.5 mL of a 5 g/L solution of *tetrazolium blue R* in *aldehyde-free alcohol R* and 0.5 mL of *dilute tetramethylammonium hydroxide solution R*. Allow to stand for exactly 5 min and add 1.0 mL of *glacial acetic acid R*. Prepare a reference solution at the same time and in the same manner using 10.0 mL of a solution containing 0.2 µg/mL of *hydroquinone R* in *aldehyde-free alcohol R*. Measure the absorbance (*2.2.25*) of the two solutions at 525 nm using as the compensation liquid 10.0 mL of *aldehyde-free alcohol R* treated in the same manner. The absorbance of the test solution is not greater than that of the reference solution (20 ppm).

Ergosterol. Examine by thin-layer chromatography (*2.2.27*), using a *TLC silica gel G plate R*.

Test solution. Dissolve 0.25 g of the substance to be examined in *ethylene chloride R* containing 10 g/L of *squalane R* and 0.1 g/L of *butylhydroxytoluene R* and dilute to 5 mL with the same solvent. Prepare immediately before use.