Limits:

– *chains with a relative molecular mass less than 2000*: maximum 13 per cent;

– *chains with a relative molecular mass less than 4000*: maximum 39 per cent;

– *chains with a relative molecular mass between 4000 and 8000*: minimum 50 per cent;

– *chains with a relative molecular mass higher than 8000*: maximum 19 per cent;

– *chains with a relative molecular mass higher than 10 000*: maximum 11 per cent.

Nitrogen (*2.5.9*): 2.4 per cent to 3.0 per cent (dried substance).

Nucleic acids: maximum 0.5 per cent (dried substance).

Test solution. Weigh about 50 mg of the dried substance to be examined into a centrifuge tube and dissolve in 200 µl of *water R*.

Reference solution. Dissolve about 50 mg of *ribonucleic acid CRS* in 5 ml of *0.1 M sodium hydroxide* and dilute to 20.0 ml with *water R*. Transfer 200 µl of the solution into a centrifuge tube.

Add 4.0 ml of a 50 g/l solution of *trichloroacetic acid R* to each tube and mix. Place all tubes in boiling water for 30 min. Allow to cool to room temperature. Add again 4.0 ml of a 50 g/l solution of *trichloroacetic acid R* to each tube and mix. If any of the test solutions is not clear, sonicate all the tubes in an ultrasonic bath for 10 min and centrifuge at 1500 *g* for 15 min. Dilute 1.0 ml of the clear supernatant to 4.0 ml with *water R*. Measure the absorbances of the diluted reference and test solutions at 265 nm (*2.2.25*) against a blank solution prepared in the same manner, and calculate the percentage nucleic acid content of the sample.

Total protein (*2.5.33, Method 2*): maximum 0.5 per cent.

Dissolve the substance to be examined in *water R*. Use *bovine albumin R* as the reference substance.

Sodium: 9.0 per cent to 11.0 per cent (dried substance).

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution. Dissolve 0.125 g of the substance to be examined in 100.0 ml of a 1.27 mg/ml solution of *caesium chloride R* in *0.1 M hydrochloric acid*.

Reference solutions. Prepare reference solutions containing 50 ppm, 100 ppm and 150 ppm of Na by diluting *sodium standard solution (1000 ppm Na) R* with a 1.27 mg/ml solution of *caesium chloride R* in *0.1 M hydrochloric acid*.

Source: sodium hollow-cathode lamp.

Wavelength: 330.3 nm.

Atomisation device: air-acetylene flame.

Loss on drying (*2.2.32*): maximum 5.0 per cent, determined on 0.500 g by drying in an oven at 60 °C over *diphosphorus pentoxide R* at a pressure of 670 Pa for 3 h.

Bacterial endotoxins (*2.6.14*): less than 0.02 IU per unit of anti-factor Xa activity, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

The anticoagulant activity of danaparoid sodium is determined *in vitro* by an assay which determines its ability to accelerate the inhibition of factor Xa by antithrombin III (anti-factor Xa assay).

Test solutions. Prepare 2 independent series of dilutions in geometric progression of the substance to be examined in *tris(hydroxymethyl)aminomethane EDTA buffer solution pH 8.4 R* and in the concentration range of 0.1 to 0.32 units of anti-factor Xa activity per millilitre.

Reference solutions. Prepare 2 independent series of dilutions in geometric progression of *danaparoid sodium CRS* in *tris(hydroxymethyl)aminomethane EDTA buffer solution pH 8.4 R* and in the concentration range of 0.08 to 0.35 units of anti-factor Xa activity per millilitre.

Transfer 40 µl of each solution into the wells of a 96-well microtitre plate. Add 40 µl of *antithrombin III solution R4* to each well and shake the microtitre plate but do not allow bubbles to form. Add 40 µl of *bovine factor Xa solution R1* to each well. Exactly 2 min after the addition of the factor Xa solution, add 80 µl of *chromogenic substrate R5*. Measure the absorbance at 405 nm (*2.2.25*) using a suitable reading device, exactly 4 min after the addition of the factor Xa solution. The reaction may be stopped using 75 µl of a 20 per cent *V/V* solution of *glacial acetic acid R*. Determine the blank amidolytic activity in the same manner, using *tris(hydroxymethyl)aminomethane EDTA buffer solution pH 8.4 R* as the blank (minimum 8 blanks per microtitre plate). Calculate the potency of the substance to be examined in units of anti-factor Xa activity per milligram using a suitable statistical method, for example the parallel-line assay.

STORAGE

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

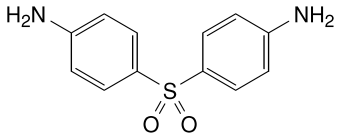
LABELLING

The label states the number of units of anti-factor Xa activity per milligram.

01/2008:0077
corrected 6.0

DAPSONE

Dapsonum



$C_{12}H_{12}N_2O_2S$ M_r 248.3
[80-08-0]

DEFINITION

Dapsone contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of 4,4′-sulphonyldianiline, calculated with reference to the dried substance.

CHARACTERS

A white or slightly yellowish-white, crystalline powder, very slightly soluble in water, freely soluble in acetone, sparingly soluble in alcohol. It dissolves freely in dilute mineral acids.

IDENTIFICATION

A. Melting point (*2.2.14*): 175 °C to 181 °C.

B. Dissolve 50.0 mg in *methanol R* and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of this solution to 100.0 ml with *methanol R*. Examined between 230 nm and 350 nm (*2.2.25*), the solution shows 2 absorption maxima, at 260 nm and 295 nm. The specific absorbances at these maxima are 700 to 760 and 1150 to 1250, respectively.

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).

TESTS

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 *methanol R* and dilute to 10 ml with the same solvent.

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

Reference solution (a). Dissolve 10 mg of *dapsone CRS* in *methanol R* and dilute to 10 ml with the same solvent.

Reference solution (b). Dilute 1 ml of test solution (b) to 10 ml with *methanol R*.

Reference solution (c). Dilute 2 ml of reference solution (b) to 10 ml with *methanol R*.

Apply separately to the plate 1 µl of test solution (b), 1 µl of reference solution (a), 10 µl of test solution (a), 10 µl of reference solution (b) and 10 µl of reference solution (c). Develop in an unsaturated tank over a path of 15 cm using a mixture of 1 volume of *concentrated ammonia R*, 6 volumes of *methanol R*, 20 volumes of *ethyl acetate R* and 20 volumes of *heptane R*. Allow the plate to dry in air. Spray the plate with a 1 g/l solution of *4-dimethylaminocinnamaldehyde R* in a mixture of 1 volume of *hydrochloric acid R* and 99 volumes of *alcohol R*. Examine in daylight. 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) and not more than 2 such spots are more intense than the spot in the chromatogram obtained with reference solution (c) (0.2 per cent).

Loss on drying (*2.2.32*). Not more than 1.5 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 0.100 g in 50 ml of *dilute hydrochloric acid R*. Carry out the determination of primary aromatic amino-nitrogen (*2.5.8*).

1 ml of *0.1 M sodium nitrite* is equivalent to 12.42 mg of $C_{12}H_{12}N_2O_2S$.

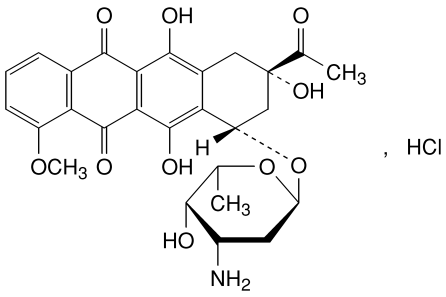
STORAGE

Store protected from light.

01/2008:0662

DAUNORUBICIN HYDROCHLORIDE

Daunorubicini hydrochloridum



$C_{27}H_{30}ClNO_{10}$ M_r 564.0
[23541-50-6]

DEFINITION

(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 hydrochloride.

Substance produced by certain strains of *Streptomyces coeruleorubidus* or of *Streptomyces peucetius* or obtained by any other means.

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

PRODUCTION

It is produced by methods of manufacture designed to eliminate or minimise the presence of histamine.

CHARACTERS

Appearance: crystalline, orange-red powder, hygroscopic.

Solubility: freely soluble in water and in methanol, slightly soluble in alcohol, practically insoluble in acetone.

IDENTIFICATION

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

B. 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*): 4.5 to 6.5.

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*). *Prepare the solutions immediately before use.*

Test solution. Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a). Dissolve 50.0 mg of *daunorubicin hydrochloride CRS* in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (b). Dissolve 10 mg of *doxorubicin hydrochloride CRS* and 10 mg of *epirubicin hydrochloride CRS* in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.