Relative retention with reference to enilconazole (retention time = about 10 min): impurity A = about 0.6; impurity B = about 0.7; impurity C = about 0.8; impurity D = about 0.9; impurity E = about 1.03; impurity F = about 1.1.

System suitability: reference solution (a):

- *resolution*: minimum 2.5 between the peaks due to enilconazole and impurity E.

Limits:

- *impurities A, B, C, D, E, F*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent), and not more than 1 such peak has an area greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- *unspecified impurities*: for each impurity, not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.20 per cent);
- *total*: not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent);
- *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (*2.2.32*): maximum 0.5 per cent, determined on 1.000 g by drying *in vacuo* at 40 °C for 4 h.

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

ASSAY

Dissolve 0.230 g in 50 mL of a mixture of 1 volume of *anhydrous acetic acid R* and 7 volumes of *methyl ethyl ketone R*. Titrate with *0.1 M perchloric acid* using 0.2 mL of *naphtholbenzein solution R* as indicator.

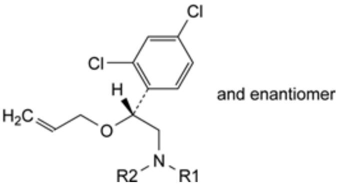
1 mL of *0.1 M perchloric acid* is equivalent to 29.72 mg of $C_{14}H_{14}Cl_2N_2O$.

STORAGE

In an airtight container, protected from light.

IMPURITIES

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

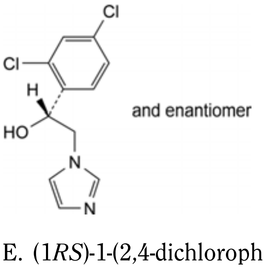


A. R1 = R2 = H: (2*RS*)-2-(2,4-dichlorophenyl)-2-(prop-2-enyloxy)ethanamine,

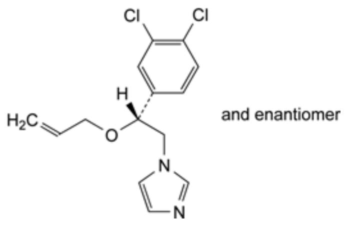
B. R1 = H, R2 = CH_2-CH=CH_2: *N*-[(2*RS*)-2-(2,4-dichlorophenyl)-2-(prop-2-enyloxy)ethyl]prop-2-en-1-amine,

C. R1 = CHO, R2 = H: *N*-[(2*RS*)-2-(2,4-dichlorophenyl)-2-(prop-2-enyloxy)ethyl]formamide,

D. R1 = CHO, R2 = CH_2-CH=CH_2: *N*-[(2*RS*)-2-(2,4-dichlorophenyl)-2-(prop-2-enyloxy)ethyl]-*N*-(prop-2-enyl)formamide,



E. (1*RS*)-1-(2,4-dichlorophenyl)-2-(-1*H*-imidazol-1-yl)ethanol,



F. 1-[(2*RS*)-2-(3,4-dichlorophenyl)-2-(prop-2-enyloxy)ethyl]-1*H*-imidazole.

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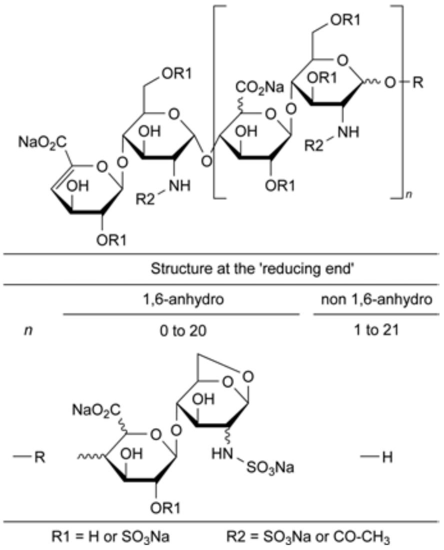
ENOXAPARIN SODIUM

Enoxaparinum natricum



DEFINITION

Enoxaparin sodium is the sodium salt of a low-molecular-mass heparin that is obtained by alkaline depolymerisation of the benzyl ester derivative of heparin from porcine intestinal mucosa. Enoxaparin consists of a complex set of oligosaccharides that have not yet been completely characterised. Based on current knowledge, the majority of the components have a 4-enopyranose uronate structure at the non-reducing end of their chain. 15 per cent to 25 per cent of the components have a 1,6-anhydro structure at the reducing end of their chain.

Enoxaparin sodium complies with the monograph Low-molecular-mass heparins (0828) with the modifications and additional requirements below.

The mass-average relative molecular mass ranges between 3800 and 5000, with a characteristic value of about 4500.

The degree of sulfatation is about 2 per disaccharide unit.

The potency is not less than 90 IU and not more than 125 IU of anti-factor Xa activity per milligram, calculated with reference to the dried substance. The anti-factor IIa activity is not less than 20.0 IU and not more than 35.0 IU per milligram, calculated with reference to the dried substance. The ratio of anti-factor Xa activity to anti-factor IIa activity is between 3.3 and 5.3.

PRODUCTION

Enoxaparin is produced by alkaline depolymerisation of benzyl ester derivatives of heparin from porcine intestinal mucosa under conditions that yield a product complying with the structural requirements stated under Definition.

IDENTIFICATION

Carry out identification test A as described in the monograph *Low-molecular-mass heparins (0828)* using *enoxaparin sodium CRS*.

Carry out identification test C as described in the monograph *Low-molecular-mass heparins (0828)*. The following requirements apply.

The mass-average relative molecular mass ranges between 3800 and 5000. The mass percentage of chains lower than 2000 ranges between 12.0 per cent and 20.0 per cent. The mass percentage of chains between 2000 and 8000 ranges between 68.0 per cent and 82.0 per cent.

TESTS

Appearance of solution. The solution is clear (*2.2.1*) and not more intensely coloured than intensity 6 of the range of reference solutions of the most appropriate colour (*2.2.2, Method II*).

Dissolve 1.0 g in 10 mL of *water R*.

pH (*2.2.3*): 6.2 to 7.7.

Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 10.0 mL with the same solvent.

Specific absorbance (*2.2.25*): 14.0 to 20.0 (dried substance), determined at 231 nm.

Dissolve 50.0 mg in 100 mL of *0.01 M hydrochloric acid*.

Benzyl alcohol. Liquid chromatography (*2.2.29*).

Internal standard solution: 1 g/L solution of *3,4-dimethylphenol R* in *methanol R*.

Test solution. Dissolve about 0.500 g of the substance to be examined in 5.0 mL of *1 M sodium hydroxide*. Allow to stand for 1 h. Add 1.0 mL of *glacial acetic acid R* and 1.0 mL of the internal standard solution and dilute to 10.0 mL with *water R*.

Reference solution. Prepare a 0.25 g/L solution of *benzyl alcohol R* in *water R*. Mix 0.50 mL of this solution with 1.0 mL of the internal standard solution and dilute to 10.0 mL with *water R*.

Precolumn:

- *size*: l = 0.02 m, Ø = 4.6 mm;
- *stationary phase*: *octylsilyl silica gel for chromatography R* (5 μm).

Column:

- *size*: l = 0.15 m, Ø = 4.6 mm;
- *stationary phase*: *octylsilyl silica gel for chromatography R* (5 μm).

Mobile phase: *methanol R, acetonitrile R, water R* (5:15:80 *V/V/V*).

Flow rate: 1 mL/min.

Detection: spectrophotometer at 256 nm.

From the chromatogram obtained with the reference solution, calculate the ratio (R_1) of the height of the peak due to benzyl alcohol to the height of the peak due to the internal standard. From the chromatogram obtained with the test solution, calculate the ratio (R_2) of the height of the peak due to benzyl alcohol to the height of the peak due to the internal standard.

Calculate the percentage content (*m/m*) of benzyl alcohol using the following expression:

$$\frac{0.0125 \times R_2}{m \times R_1}$$

m = mass of the substance to be examined, in grams.

Limit:

- *benzyl alcohol*: maximum 0.1 per cent *m/m*.

Sodium (*2.2.23, Method I*): 11.3 per cent to 13.5 per cent (dried substance).

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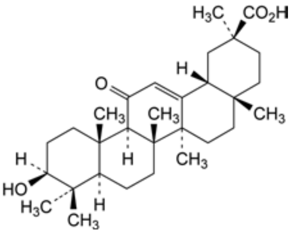
corrected 6.0

ENOXOLONE

Enoxolonum



$C_{30}H_{46}O_4$ M_r 470.7
[471-53-4]

DEFINITION

(20β)-3β-Hydroxy-11-oxo-olean-12-en-29-oic acid.

Content: 98.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white crystalline powder.

Solubility: practically insoluble in water, soluble in ethanol, sparingly soluble in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION

First identification: A.

Second identification: B, C.

A. Examine by infrared absorption spectrophotometry (*2.2.24*).

Comparison: *enoxolone CRS*.

If the spectra obtained in the solid state show differences, dissolve 0.2 g of the substance to be examined and 0.2 g of the reference substance separately in 6 mL of *ethanol R*. Boil under a reflux condenser for 1 h and add 6 mL of *water R*. A precipitate is formed. Cool to about 10 °C and filter with the aid of vacuum. Wash the precipitate with 10 mL of *alcohol R*, dry in an oven at 80 °C and record new spectra.

B. Thin-layer chromatography (*2.2.27*).

Test solution. Dissolve 10 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 *enoxolone CRS* in *methylene chloride R* and dilute to 10 mL with the same solvent.

Plate: *TLC silica gel plate R*.

Mobile phase: *glacial acetic acid R, acetone R, methylene chloride R* (5:10:90 *V/V/V*).

Application: 5 μL.

Development: over 2/3 of the plate.

Drying: in air for 5 min.

Detection: spray with *anisaldehyde solution R* and heat at 100-105 °C for 10 min.

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.

C. Dissolve 50 mg in 10 mL of *methylene chloride R*. To 2 mL of this solution, add 1 mL of *acetic anhydride R* and 0.3 mL of *sulfuric acid R*. A pink colour is produced.