Apply separately to the plate as 10 mm bands 5 μL of each solution. Develop immediately over a path of 15 cm using a mixture of 3 volumes of *water R*, 36 volumes of *methanol R* and 130 volumes of *methylene chloride R*. Dry the plate in a current of warm air, spray with a mixture of 5 volumes of *sulfuric acid R* and 95 volumes of *alcohol R* and heat at 140 °C for 15 min. Examine in daylight. In the chromatogram obtained with test solution (a), any band, apart from the principal band, is not more intense than the band in the chromatogram obtained with reference solution (b) (2.5 per cent) and at most two such bands are more intense than the band in the chromatogram obtained with reference solution (c) (1.0 per cent).

Loss on drying (*2.2.32*). Not more than 5.0 per cent, determined on 0.500 g by drying *in vacuo* at 105 °C.

Sulfated ash (*2.4.14*). Not more than 0.1 per cent, determined on the residue obtained in the test for loss on drying.

ASSAY

Dissolve 50.0 mg in *alcohol R* and dilute to 50.0 mL with the same solvent. Dilute 5.0 mL of this solution to 100.0 mL with *alcohol R*. Prepare a reference solution in the same manner, using 50.0 mg of *deslanoside CRS* (undried). To 5.0 mL of each solution add 3.0 mL of *alkaline sodium picrate solution R* and allow to stand protected from bright light in a water-bath at 20 ± 1 °C for 40 min. Measure the absorbance (*2.2.25*) of each solution at the maximum at 484 nm, using as the compensation liquid a mixture of 3.0 mL of *alkaline sodium picrate solution R* and 5.0 mL of *alcohol R* prepared at the same time.

Calculate the content of $C_{47}H_{74}O_{19}$ from the absorbances measured and the concentrations of the solutions.

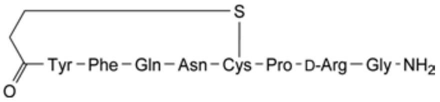
STORAGE

Store in an airtight, glass container, protected from light, at a temperature below 10 °C.

07/2009:0712

DESMOPRESSIN

Desmopressinum



$C_{46}H_{64}N_{14}O_{12}S_2$ M_r 1069
[16679-58-6]

DEFINITION

(3-Sulfanylpropanoyl)-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparaginyl-L-cysteinyl-L-prolyl-D-arginylglycinamide cyclic (1→6)-disulfide.

Synthetic cyclic nonapeptide, available as an acetate.

Content: 95.0 per cent to 105.0 per cent (anhydrous and acetic acid-free substance).

CHARACTERS

Appearance: white or almost white, fluffy powder.

Solubility: soluble in water, in ethanol (96 per cent) and in glacial acetic acid.

IDENTIFICATION

A. Examine the chromatograms obtained in the assay.

Results: the retention time and size of the principal peak in the chromatogram obtained with the test solution are approximately the same as those of the principal peak in the chromatogram obtained with the reference solution.

B. Amino acid analysis (*2.2.56*). For hydrolysis use Method 1 and for analysis use Method 1.

Express the content of each amino acid in moles. Calculate the relative proportions of the amino acids, taking 1/6 of the sum of the number of moles of aspartic acid, glutamic acid, proline, glycine, arginine and phenylalanine as equal to 1. The values fall within the following limits: aspartic acid: 0.90 to 1.10; glutamic acid: 0.90 to 1.10; proline: 0.90 to 1.10; glycine: 0.90 to 1.10; arginine: 0.90 to 1.10; phenylalanine: 0.90 to 1.10; tyrosine: 0.70 to 1.05; half-cystine: 0.30 to 1.05. Lysine, isoleucine and leucine are absent; not more than traces of other amino acids are present.

TESTS

Specific optical rotation (*2.2.7*): – 72 to – 82 (anhydrous and acetic acid-free substance).

Dissolve 10.0 mg in a 1 per cent *V/V* solution of *glacial acetic acid R* and dilute to 5.0 mL with the same acid.

Related substances. Liquid chromatography (*2.2.29*): use the normalisation procedure.

Test solution. Dissolve 1.0 mg of the substance to be examined in 2.0 mL of *water R*.

Resolution solution. Dissolve the contents of a vial of *oxytocin/desmopressin validation mixture CRS* in 500 μL of *water R*.

Column:

- *size*: *l* = 0.12 m, Ø = 4.0 mm;
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 μm).

Mobile phase:

- *mobile phase A*: *0.067 M phosphate buffer solution pH 7.0 R*; filter and degas;
- *mobile phase B*: *acetonitrile for chromatography R*, mobile phase A (50:50 *V/V*); filter and degas.

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 4	76	24
4 - 18	76 → 58	24 → 42
18 - 35	58 → 48	42 → 52
35 - 40	48 → 76	52 → 24
40 - 50	76	24

Flow rate: 1.5 mL/min.

Detection: spectrophotometer at 220 nm.

Injection: 50 μL.

Retention time: desmopressin = about 16 min; oxytocin = about 17 min.

System suitability: resolution solution:

- *resolution*: minimum 1.5 between the peaks due to desmopressin and oxytoxin.

Limits:

- *unspecified impurities*: for each impurity, maximum 0.5 per cent;
- *total*: maximum 1.5 per cent;
- *disregard limit*: 0.05 per cent.

Acetic acid (*2.5.34*): 3.0 per cent to 8.0 per cent.

Test solution. Dissolve 20.0 mg of the substance to be examined in a mixture of 5 volumes of mobile phase B and 95 volumes of mobile phase A and dilute to 10.0 mL with the same mixture of mobile phases.

Water (*2.5.32*): maximum 6.0 per cent, determined on 20.0 mg.

Bacterial endotoxins (*2.6.14*): less than 500 IU/mg, if intended for use in the manufacture of parenteral preparations 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 modifications.

Reference solution. Dissolve the contents of a vial of *desmopressin CRS* in *water R* to obtain a concentration of 0.5 mg/mL.

Mobile phase: mobile phase B, mobile phase A (40:60 *V/V*).

Flow rate: 2.0 mL/min.

Retention time: desmopressin = about 5 min.

Calculate the content of desmopressin ($C_{46}H_{64}N_{14}O_{12}S_2$) from the declared content of $C_{46}H_{64}N_{14}O_{12}S_2$ in *desmopressin CRS.*

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.

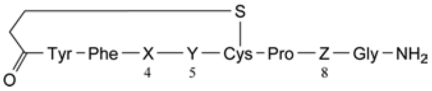
LABELLING

The label states:

- the mass of peptide per container;
- where applicable, that the substance is suitable for use in the manufacture of parenteral preparations.

IMPURITIES

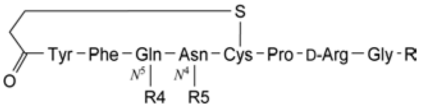
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034).* It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*)*: A, B, C, D, E, F, G.*



A. X = Gln, Y = Asp, Z = D-Arg: [5-L-aspartic acid]desmopressin,

B. X = Glu, Y = Asn, Z = D-Arg: [4-L-glutamic acid]desmopressin,

D. X = Gln, Y = Asn, Z = L-Arg: [8-L-arginine]desmopressin,



C. R = OH, R4 = R5 = H: [9-glycine]desmopressin,

E. R = NH_2, R4 = CH_2-NH-CO-CH_3, R5 = H: $N^{5.4}$-[(acetylamino)methyl]desmopressin,

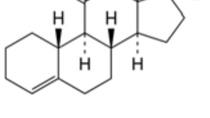
F. R = NH_2, R4 = H, R5 = CH_2-NH-CO-CH_3: $N^{4.5}$-[(acetylamino)methyl]desmopressin,

G. R = $N(CH_3)_2$, R4 = R5 = H: $N^{1.9}$,$N^{1.9}$-dimethyldesmopressin.

01/2008:1717

DESOGESTREL

Desogestrelum



$C_{22}H_{30}O$ M_r 310.5
[54024-22-5]

DEFINITION

13-Ethyl-11-methylidene-18,19-dinor-17α-pregn-4-en-20-yn-17-ol.

Content: 98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

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

IDENTIFICATION

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

B. Specific optical rotation (see Tests).

TESTS

Specific optical rotation (*2.2.7*): + 53 to + 57 (dried substance).

Dissolve 0.250 g in *anhydrous ethanol R* and dilute to 25.0 mL with the same solvent.

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 20.0 mg of the substance to be examined in 25 mL of *acetonitrile R1* and dilute to 50.0 mL with *water R.*

Reference solution (a). Dissolve 4 mg of *desogestrel for system suitability CRS* (containing impurities A, B, C and D) in 5 mL of *acetonitrile R1* and dilute to 10.0 mL with *water R.*

Reference solution (b). Dilute 1.0 mL of the test solution to 100.0 mL with a mixture of equal volumes of *acetonitrile R1* and *water R.*

Reference solution (c). Dilute 1.0 mL of reference solution (b) to 10.0 mL with a mixture of equal volumes of *acetonitrile R1* and *water R.*

Reference solution (d). Dissolve 20.0 mg of *desogestrel CRS* in 25 mL of *acetonitrile R1* and dilute to 50.0 mL with *water R.*

Column:

- *size*: *l* = 0.25 m, Ø = 4.6 mm,
- *stationary phase*: sterically protected *octadecylsilyl silica gel for chromatography R* (5 μm),
- *temperature*: 50 °C.

Mobile phase: *water R, acetonitrile R1* (27:73 *V/V*).

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 205 nm.

Injection: 15 μL of the test solution and reference solutions (a), (b) and (c).

Run time: 2.5 times the retention time of desogestrel.

Identification of impurities: use the chromatogram supplied with *desogestrel for system suitability CRS* and the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B, C and D.

Relative retention with reference to desogestrel (retention time = about 22 min): impurity E = about 0.2; impurity D = about 0.25; impurity B = about 0.7; impurity A = about 0.95; impurity C = about 1.05.

System suitability: reference solution (a):

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

Limits:

- *correction factors*: for the calculation of content, multiply the peak area of the following impurities by the corresponding correction factor: impurity A = 1.8, impurity D = 1.5;
- *impurities A, B, C*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent);
- *impurity D*: not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent);