C. Dissolve about 10 mg in 2 mL of *dilute hydrogen peroxide solution R*, add 1 mL of *hydrochloric acid R* and heat. An orange colour develops.

D. Sprinkle about 5 mg on the surface of 1 mL of *sulfomolybdic reagent R2*. A bright-green colour develops.

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

TESTS

Solution S. Dissolve 1.25 g in *carbon dioxide-free water R* and dilute to 25 mL with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and not more intensely coloured than reference solution Y_5 or BY_5 (*2.2.2, Method II*).

pH (*2.2.3*). Dilute 4 mL of solution S to 10 mL with *carbon dioxide-free water R*. The pH of the solution is 4.0 to 6.0.

Specific optical rotation (*2.2.7*). Dissolve in *water R* a quantity of the substance to be examined corresponding to 1.250 g of dried substance and dilute to 25.0 mL with the same solvent. The specific optical rotation is + 16 to + 19, calculated with reference to the dried substance.

Related substances. Examine by thin-layer chromatography (*2.2.27*), using a *TLC silica gel G plate R. Prepare the solutions immediately before use.*

Test solution. Dissolve 50 mg of the substance to be examined in *methanol R* containing 1 per cent *V/V* of *dilute ammonia R2* and dilute to 100 mL with the same solvent.

Reference solution (a). Dissolve 50 mg of *emetine hydrochloride CRS* in *methanol R* containing 1 per cent *V/V* of *dilute ammonia R2* and dilute to 100 mL with the same solvent.

Reference solution (b). Dissolve 10 mg of *isoemetine hydrobromide CRS* in *methanol R* containing 1 per cent *V/V* of *dilute ammonia R2* and dilute to 100 mL with the same solvent. Dilute 5 mL of this solution to 50 mL with *methanol R* containing 1 per cent *V/V* of *dilute ammonia R2*.

Reference solution (c). Dissolve 10 mg of *cephaeline hydrochloride CRS* in *methanol R* containing 1 per cent *V/V* of *dilute ammonia R2* and dilute to 100 mL with the same solvent. Dilute 5 mL of this solution to 50 mL with *methanol R* containing 1 per cent *V/V* of *dilute ammonia R2*.

Reference solution (d). Dilute 1 mL of reference solution (a) to 100 mL with *methanol R* containing 1 per cent *V/V* of *dilute ammonia R2*.

Reference solution (e). To 1 mL of reference solution (a) add 1 mL of reference solution (b) and 1 mL of reference solution (c).

Apply to the plate 10 µL of the test solution and each of reference solutions (a), (b), (c) and (d) and 30 µL of reference solution (e). Develop over a path of 15 cm using a mixture of 0.5 volumes of *diethylamine R*, 2 volumes of *water R*, 5 volumes of *methanol R*, 20 volumes of *ethylene glycol monomethyl ether R* and 100 volumes of *chloroform R*. Allow the plate to dry in air until the solvent has evaporated. Spray in a well ventilated fume-cupboard with *chloroformic solution of iodine R* and heat at 60 °C for 15 min. Examine in ultraviolet light at 365 nm. In the chromatogram obtained with the test solution, any spots corresponding to isoemetine and cephaeline are not more intense than the spots in the chromatograms obtained with reference solutions (b) and (c) respectively (2.0 per cent); any spot, apart from the principal spot and the spots corresponding to isoemetine and cephaeline, is not more intense than the spot in the chromatogram obtained with reference solution (d) (1.0 per cent). The test is not valid unless the chromatogram obtained with reference solution (e) shows three clearly separated spots.

Loss on drying (*2.2.32*). 11.0 per cent to 15.0 per cent, determined on 1.00 g by drying in an oven at 105 °C for 3 h.

Sulfated ash (*2.4.14*). Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.200 g in a mixture of 5.0 mL of *0.01 M hydrochloric acid* and 50 mL of *alcohol R*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the two points of inflexion.

1 mL of *0.1 M sodium hydroxide* is equivalent to 27.68 mg of $C_{29}H_{42}Cl_2N_2O_4$.

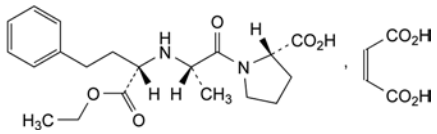
STORAGE

Store protected from light.

07/2010:1420

ENALAPRIL MALEATE

Enalaprili maleas



$C_{24}H_{32}N_2O_9$ M_r 492.5

[76095-16-4]

DEFINITION

(2*S*)-1-[(2*S*)-2-[[(1*S*)-1-(Ethoxycarbonyl)-3-phenylpropyl]-amino]propanoyl]pyrrolidine-2-carboxylic acid (*Z*)-butenedioate.

Content: 98.5 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: sparingly soluble in water, freely soluble in methanol, practically insoluble in methylene chloride. It dissolves in dilute solutions of alkali hydroxides.

mp: about 144 °C.

IDENTIFICATION

Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *enalapril maleate CRS*.

TESTS

Solution S. Dissolve 0.25 g in *carbon dioxide-free water R* and dilute to 25.0 mL with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

pH (*2.2.3*): 2.4 to 2.9 for solution S.

Specific optical rotation (*2.2.7*): – 48 to – 51 (dried substance), determined on solution S.

Related substances. Liquid chromatography (*2.2.29*).

Buffer solution A. Dissolve 2.8 g of *sodium dihydrogen phosphate monohydrate R* in 950 mL of *water R*. Adjust to pH 2.5 with *phosphoric acid R* and dilute to 1000 mL with *water R*.

Buffer solution B. Dissolve 2.8 g of *sodium dihydrogen phosphate monohydrate R* in 950 mL of *water R*. Adjust to pH 6.8 with *strong sodium hydroxide solution R* and dilute to 1000 mL with *water R*.

Dissolution mixture. Mix 50 mL of *acetonitrile R1* and 950 mL of buffer solution A.

Test solution. Dissolve 30 mg of the substance to be examined in the dissolution mixture and dilute to 100.0 mL with the dissolution mixture.

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

Reference solution (b). Dissolve 3 mg of *enalapril for system suitability CRS* (containing impurity A) in the dissolution mixture and dilute to 10.0 mL with the dissolution mixture.

Reference solution (c). Dissolve the contents of a vial of *enalapril impurity mixture CRS* (impurities B, C, D, E and H) in 1.0 mL of the dissolution mixture.

Column:

– *size*: *l* = 0.15 m, Ø = 4.1 mm;

– *stationary phase*: *styrene-divinylbenzene copolymer R* (5 µm);

– *temperature*: 70 °C.

Mobile phase:

– *mobile phase A*: mix 50 mL of *acetonitrile R1* and 950 mL of buffer solution B;

– *mobile phase B*: mix 340 mL of buffer solution B and 660 mL of *acetonitrile R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 20	95 → 40	5 → 60
20 - 25	40	60

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 215 nm.

Injection: 50 µL.

Identification of impurities:

– use the chromatogram supplied with *enalapril impurity mixture CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities B, C, D, E and H;

– use the chromatogram obtained with reference solution (b) to identify the peak due to impurity A.

Relative retention with reference to enalapril (retention time = about 11 min): impurity C = about 0.2; impurity B = about 0.8; impurity A = about 1.1; impurity H = about 1.3; impurity E = about 1.5; impurity D = about 2.1.

System suitability: reference solution (b):

– *peak-to-valley ratio*: minimum 10, 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 enalapril.

Limits:

– *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);

– *impurities B, C, D, E, H*: for each impurity, not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);

– *unspecified impurities*: for each impurity, not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);

– *sum of impurities other than A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);

– *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent); disregard the peak due to maleic acid.

Heavy metals (*2.4.8*): maximum 10 ppm.

2.0 g complies with test C. Prepare the reference solution using 2 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 for 3 h.

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

ASSAY

Dissolve 0.100 g in *carbon dioxide-free water R* and dilute to 30 mL with the same solvent. Titrate with *0.1 M sodium hydroxide* determining the end-point potentiometrically (*2.2.20*). Titrate to the 2nd point of inflexion.

1 mL of *0.1 M sodium hydroxide* is equivalent to 16.42 mg of $C_{24}H_{32}N_2O_9$.

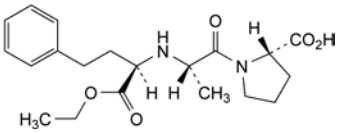
STORAGE

Protected from light.

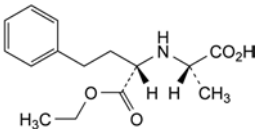
IMPURITIES

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

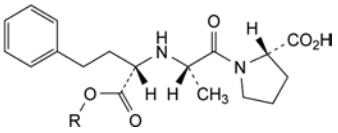
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical 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*): *F, G, I.*



A. (2*S*)-1-[(2*S*)-2-[[(1*R*)-1-(ethoxycarbonyl)-3-phenylpropyl]-amino]propanoyl]pyrrolidine-2-carboxylic acid,



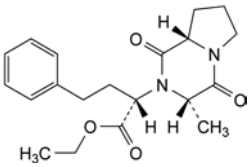
B. (2*S*)-2-[[(1*S*)-1-(ethoxycarbonyl)-3-phenylpropyl]-amino]propanoic acid,



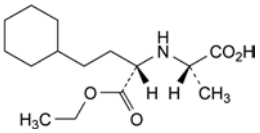
C. R = H: (2*S*)-1-[(2*S*)-2-[[(1*S*)-1-carboxy-3-phenylpropyl]-amino]propanoyl]pyrrolidine-2-carboxylic acid,

E. R = CH_2-CH_2-C_6H_5: (2*S*)-1-[(2*S*)-2-[[(1*S*)-3-phenyl-1-[(2-phenylethoxy)carbonyl]propyl]amino]propanoyl]pyrrolidine-2-carboxylic acid,

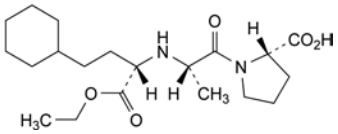
F. R = C_4H_9: (2*S*)-1-[(2*S*)-2-[[(1*S*)-1-(butoxycarbonyl)-3-phenylpropyl]amino]propanoyl]pyrrolidine-2-carboxylic acid,



D. ethyl (2*S*)-2-[(3*S*,8a*S*)-3-methyl-1,4-dioxo-octahydropyrrolo-[1,2-*a*]pyrazin-2-yl]-4-phenylbutanoate,



G. (2*S*)-2-[[(1*S*)-3-cyclohexyl-1-(ethoxycarbonyl)propyl]-amino]propanoic acid,



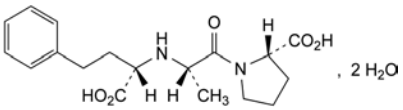
H. (2*S*)-1-[(2*S*)-2-[[(1*S*)-3-cyclohexyl-1-(ethoxycarbonyl)-propyl]amino]propanoyl]pyrrolidine-2-carboxylic acid,

I. 1*H*-imidazole.

01/2008:1749
corrected 7.0

ENALAPRILAT DIHYDRATE

Enalaprilatum dihydricum



$C_{18}H_{24}N_2O_5,2H_2O$ M_r 384.4
[84680-54-6]

DEFINITION

(2*S*)-1-[(2*S*)-2-[[(1*S*)-1-Carboxy-3-phenylpropyl]amino]-propanoyl)pyrrolidine-2-carboxylic acid dihydrate.

Content: 98.5 per cent to 101.5 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white, hygroscopic, crystalline powder.

Solubility: very slightly soluble or slightly soluble in water, sparingly soluble in methanol, practically insoluble in acetonitrile.

It shows pseudopolymorphism (*5.9*).

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation: mulls in *liquid paraffin R*.

Comparison: *enalaprilat dihydrate CRS*.

If the spectra obtained show differences, expose the substance to be examined and the reference substance to a 98 per cent relative humidity for 3 days using a chamber conditioned with a saturated solution of *calcium sulfate R*. Record new spectra.

TESTS

Appearance of solution. The solution is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Dissolve 0.10 g in *water R* and dilute to 100.0 mL with the same solvent.

Specific optical rotation (*2.2.7*): – 53.0 to – 56.0 (anhydrous substance).

Dissolve 0.200 g in *methanol R* and dilute to 20.0 mL with the same solvent.

Related substances. Liquid chromatography (*2.2.29*). *Use freshly prepared solutions.*

Buffer solution. Dissolve 1.36 g of *potassium dihydrogen phosphate R* in 950 mL of *water R*. Adjust to pH 3.0 with *phosphoric acid R* and dilute to 1000 mL with *water R*.

Solvent mixture. Buffer solution, *acetonitrile R1*, *methanol R1* (1:2:2 *V/V/V*).

Dissolution mixture. Solvent mixture, buffer solution (8:92 *V/V*).

Test solution. Dissolve 25.0 mg of the substance to be examined in 2.5 mL of *methanol R1* and dilute to 25.0 mL with the dissolution mixture.

Reference solution (a). Dilute 1.0 mL of the test solution to 100.0 mL with the dissolution mixture. Dilute 5.0 mL of this solution to 10.0 mL with the dissolution mixture.

Reference solution (b). Dissolve 5 mg of *enalaprilat for system suitability CRS* (containing impurity C) in 0.5 mL of *methanol R1* and dilute to 5 mL with the dissolution mixture.

Reference solution (c). Dissolve the contents of a vial of *enalaprilat impurity G CRS* in 1 mL of the test solution.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm;
– *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm);
– *temperature*: 70 °C.

Mobile phase:

– *mobile phase A*: solvent mixture, buffer solution (10:90 *V/V*);
– *mobile phase B*: *acetonitrile R1*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 25	100	0
25 - 50	100 → 90	0 → 10
50 - 80	90	10

Flow rate: 2.0 mL/min.

Detection: spectrophotometer at 210 nm.

Injection: 20 µL.

Identification of impurities: use the chromatogram supplied with *enalaprilat for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peak due to impurity C; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity G.

Relative retention with reference to enalaprilat (retention time = about 21 min): impurity C = about 1.2; impurity G = about 2.9.

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 C and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to enalaprilat.

Limits:

– *impurities C, G*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
– *unspecified impurities*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
– *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent);
– *disregard limit*: 0.1 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 10 ppm.

2.0 g complies with test G. Prepare the reference solution using 2 mL of *lead standard solution (10 ppm Pb) R*.

Water (*2.5.12*): 7.0 per cent to 11.0 per cent, determined on 0.100 g.

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

Bacterial endotoxins (*2.6.14*): less than 0.1 IU/mg.