TESTS

Viscosity (*2.2.9*). Carry out the determination at 20 ± 0.5 °C.

Ethylene oxide units per molecule (nominal value)	Type of macrogol	Viscosity (mPa·s)
4	200	30 to 50
6	300	60 to 80
8	400	80 to 110

Acid value (*2.5.1*): maximum 2.0, determined on 2.0 g.

Hydroxyl value (*2.5.3, Method A*). Use 1.0 g.

Ethylene oxide units per molecule (nominal value)	Type of macrogol	Hydroxyl value
4	200	80 to 120
6	300	140 to 180
8	400	170 to 205

Peroxide value (*2.5.5, Method A*): maximum 6.0, determined on 2.0 g.

Saponification value (*2.5.6*). Use 2.0 g.

Ethylene oxide units per molecule (nominal value)	Type of macrogol	Saponification value
4	200	265 to 285
6	300	170 to 190
8	400	85 to 105

Alkaline impurities. Introduce 5.0 g into a test-tube and carefully add a mixture, neutralised if necessary with *0.01 M hydrochloric acid* or with *0.01 M sodium hydroxide*, of 0.05 mL of a 0.4 g/L solution of *bromophenol blue R* in *ethanol (96 per cent) R*, 0.3 mL of *water R* and 10 mL of *ethanol (96 per cent) R*. Shake and allow to stand. Not more than 1.0 mL of *0.01 M hydrochloric acid* is required to change the colour of the upper layer to yellow.

Free glycerol: maximum 5.0 per cent.

Dissolve 1.20 g in 25.0 mL of *methylene chloride R*. Heat if necessary. After cooling, add 100 mL of *water R*. Shake and add 25.0 mL of *periodic acetic acid solution R*. Shake and allow to stand for 30 min. Add 40 mL of a 75 g/L solution of *potassium iodide R*. Allow to stand for 1 min. Add 1 mL of *starch solution R*. Titrate the iodine with *0.1 M sodium thiosulfate*. Carry out a blank titration.

1 mL of *0.1 M sodium thiosulfate* is equivalent to 2.3 mg of glycerol.

Composition of fatty acids (*2.4.22, Method A*).

Composition of the fatty-acid fraction of the substance:

- *caproic acid*: maximum 2.0 per cent;
- *caprylic acid*: 50.0 per cent to 80.0 per cent;
- *capric acid*: 20.0 per cent to 50.0 per cent;
- *lauric acid*: maximum 3.0 per cent;
- *myristic acid*: maximum 1.0 per cent.

Ethylene oxide and dioxan (*2.4.25*): maximum 1 ppm of ethylene oxide and maximum 10 ppm of dioxan.

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*.

Water (*2.5.12*): maximum 1.0 per cent, determined on 1.0 g. Use a mixture of 30 volumes of *anhydrous methanol R* and 70 volumes of *methylene chloride R* as solvent.

Total ash (*2.4.16*): maximum 0.1 per cent.

LABELLING

The label states the type of macrogol used (mean relative molecular mass) or the number of ethylene oxide units per molecule (nominal value).

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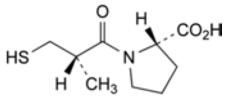
CAPTOPRIL

Captoprilum



$C_9H_{15}NO_3S$ M_r 217.3
[62571-86-2]

DEFINITION

(2*S*)-1-[(2*S*)-2-Methyl-3-sulfanylpropanoyl]pyrrolidine-2-carboxylic acid.

Content: 98.0 per cent to 101.5 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

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

IDENTIFICATION

A. Specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *captopril CRS*.

TESTS

Solution S. Dissolve 0.5 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 colourless (*2.2.2, Method II*).

pH (*2.2.3*): 2.0 to 2.6 for solution S.

Specific optical rotation (*2.2.7*): – 127 to – 132 (dried substance).

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

Impurity F. Gas chromatography (*2.2.28*).

Reagent solution. Add 2.8 mL of *acetyl chloride R* dropwise to 17.2 mL of *anhydrous methanol R* at 0 °C and mix. Allow to stand for 20 min at room temperature before use.

Test solution. Introduce 20.0 mg of the substance to be examined into a vial and add 1.0 mL of the reagent solution. Mix and heat at 60 °C for 30 min. Evaporate to dryness under a stream of *nitrogen R*. Dissolve the residue in 0.5 mL of *ethyl acetate R*, add 0.5 mL of *pentafluoropropionic anhydride R*, mix and heat at 60 °C for 30 min. Evaporate to dryness under a stream of *nitrogen R*. Dissolve the residue in 1.0 mL of *butyl acetate R*.

Reference solution (a). Dissolve the contents of a vial of *captopril for system suitability CRS* (containing impurity F) in 1.0 mL of the reagent solution. Prepare as described for the test solution.

Reference solution (b). Mix 0.25 mL of reference solution (a) and 0.75 mL of *butyl acetate R*.

Column:

- *material*: fused silica;
- *size*: l = 25 m, Ø = 0.32 mm;
- *stationary phase*: *poly(dimethyl)(diphenyl)siloxane R* (film thickness 1 µm).

Carrier gas: *helium for chromatography R*.

Flow rate: 1.2 mL/min.

Split ratio: 1:20.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 10	200
	10 - 14	200 → 240
	14 - 34	240
Injection port		270
Detector		300

Detection: flame ionisation.

Injection: 1 µL.

Relative retention with reference to captopril (retention time = about 6 min): impurity F = about 0.96.

System suitability:

- *resolution*: minimum 1.5 between the peaks due to impurity F and captopril in the chromatogram obtained with reference solution (a);
- *signal-to-noise ratio*: minimum 10 for the peak due to impurity F in the chromatogram obtained with reference solution (b).

Calculate the percentage content of impurity F using the following expression:

$$\frac{A}{A+B} \times 100$$

A = area of the peak due to impurity F in the chromatogram obtained with the test solution;

B = area of the peak due to captopril in the chromatogram obtained with the test solution.

Limit:

- *impurity F*: maximum 0.2 per cent.

Related substances. Liquid chromatography (*2.2.29*).

Solvent mixture: *phosphoric acid R, acetonitrile R1, water R* (0.8:100:900 *V/V/V*).

Test solution. Dissolve 0.125 g of the substance to be examined in the solvent mixture and dilute to 25.0 mL with the solvent mixture.

Reference solution (a). Dissolve 5.0 mg of *captopril impurity B CRS*, 5.0 mg of *captopril impurity C CRS* and 5.0 mg of *captopril impurity D CRS* in the solvent mixture. Add 1.0 mL of the test solution and dilute to 50.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 20.0 mL with the solvent mixture. Prepare immediately before use.

Reference solution (b). Dissolve 5 mg of the substance to be examined and 5 mg of *captopril impurity E CRS* in *acetonitrile R* and dilute to 25.0 mL with the same solvent. Dilute 4 mL of the solution to 50.0 mL with the solvent mixture.

Reference solution (c). In order to prepare impurity A *in situ,* introduce 1.0 mL of the test solution into a volumetric flask and add 230 µL of *0.05 M iodine.* If the solution is not colourless, add *0.1 M sodium thiosulfate* dropwise until it becomes colourless, and dilute to 50.0 mL with the solvent mixture. Dilute 5.0 mL of this solution to 20.0 mL with the solvent mixture.

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

Column:

- *size*: l = 0.3 m, Ø = 3.9 mm;
- *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (10 µm);
- *temperature*: 50 °C.

Mobile phase:

- *mobile phase A*: *phosphoric acid R, water R* (0.8:1000 *V/V*);
- *mobile phase B*: *phosphoric acid R, acetonitrile R1, water R* (0.8:500:500 *V/V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 5	90	10
5 - 20	90 → 50	10 → 50
20 - 45	50	50

Flow rate: 1.5 mL/min.

Detection: spectrophotometer at 210 nm.

Injection: 25 µL.

Identification of impurities: use the chromatogram obtained with reference solution (a) to identify the peaks due to impurities B, C and D; use the chromatogram obtained with reference solution (b) to identify the peak due to impurity E; use the chromatogram obtained with reference solution (c) to identify the peak due to impurity A.

Relative retention with reference to captopril (retention time = about 15 min): impurity C = about 0.6; impurity D = about 0.8; impurity E = about 0.9; impurity B = about 1.3; impurity A = about 1.7.

System suitability:

- *resolution*: minimum 2.0 between the peaks due to impurity E and captopril in the chromatogram obtained with reference solution (b).

Limits:

- *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (d) (1.0 per cent);
- *impurities B, C, D*: for each impurity, not more than 1.5 times the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.15 per cent);
- *impurity E*: not more than 1.5 times the area of the peak due to captopril in the chromatogram obtained with reference solution (a) (0.15 per cent);
- *unspecified impurities*: for each impurity, not more than the area of the peak due to captopril in the chromatogram obtained with reference solution (a) (0.10 per cent);
- *total*: not more than 1.2 times the area of the principal peak in the chromatogram obtained with reference solution (d) (1.2 per cent);
- *disregard limit*: 0.5 times the area of the peak due to captopril in the chromatogram obtained with reference solution (a) (0.05 per cent).

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

Solvent: *water R.*

0.50 g complies with test H. Prepare the reference solution using 1 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 under high vacuum at 60 °C for 3 h.

Sulfated ash (*2.4.14*): maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g in 30 mL of *water R.* Titrate with *0.05 M iodine,* determining the end-point potentiometrically (*2.2.20*). Use a combined platinum electrode.

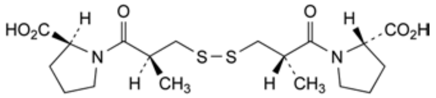
1 mL of *0.05 M iodine* is equivalent to 21.73 mg of $C_9H_{15}NO_3S$.

IMPURITIES

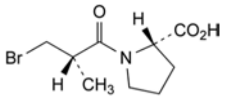
Specified impurities: A, B, C, D, E, F.

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*

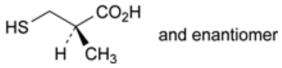
(2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *G, H, I, J, L, M, N, O.*



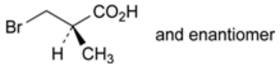
A. 1,1′-[disulfanediylbis[(2*S*)-2-methyl-1-oxopropane-3,1-diyl]]bis[(2*S*)-pyrrolidine-2-carboxylic] acid (captopril disulfide),



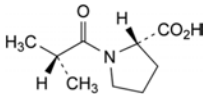
B. (2*S*)-1-[(2*S*)-3-bromo-2-methylpropanoyl]pyrrolidine-2-carboxylic acid,



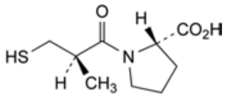
C. (2*RS*)-2-methyl-3-sulfanylpropanoic acid,



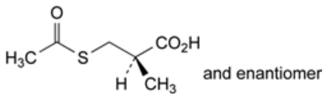
D. (2*RS*)-3-bromo-2-methylpropanoic acid,



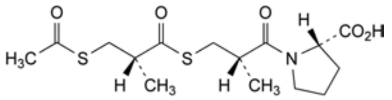
E. (2*S*)-1-(2-methylpropanoyl)pyrrolidine-2-carboxylic acid,



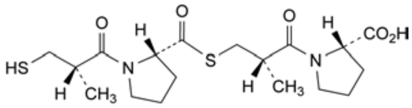
F. (2*S*)-1-[(2*R*)-2-methyl-3-sulfanylpropanoyl]pyrrolidine-2-carboxylic acid (*epi*-captopril),



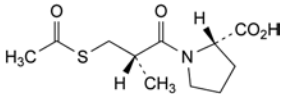
G. (2*RS*)-3-(acetylsulfanyl)-2-methylpropanoic acid,



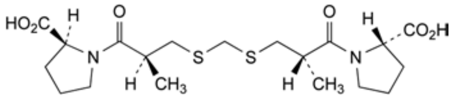
H. (2*S*)-1-[(2*S*)-3-[[(2*R*)-3-(acetylsulfanyl)-2-methylpropanoyl]-sulfanyl]-2-methylpropanoyl]pyrrolidine-2-carboxylic acid,



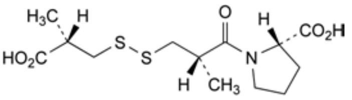
I. (2*S*)-1-[(2*S*)-3-[[[(2*S*)-1-[(2*S*)-2-methyl-3-sulfanylpropanoyl]-pyrrolidin-2-yl]carbonyl]sulfanyl]-2-methylpropanoyl]-pyrrolidine-2-carboxylic acid,



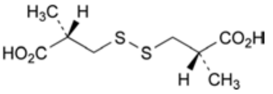
J. (2*S*)-1-[(2*S*)-3-(acetylsulfanyl)-2-methylpropanoyl]pyrrolidine-2-carboxylic acid (acetylcaptopril),



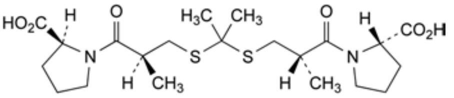
L. 1,1′-[methylenebis[sulfanediyl[(2*S*)-2-methyl-1-oxopropane-3,1-diyl]]]bis[(2*S*)-pyrrolidine-2-carboxylic] acid,



M. (2*S*)-1-[(2*S*)-3-[[(2*S*)-2-carboxypropyl]disulfanyl]-2-methylpropanoyl]pyrrolidine-2-carboxylic acid,



N. 3,3′-disulfanediylbis[(2*S*)-2-methylpropanoic] acid.



O. 1,1′-[propane-2,2-diylbis[sulfanediyl[(2*S*)-2-methyl-1-oxopropane-3,1-diyl]]]bis[(2*S*)-pyrrolidine-2-carboxylic] acid,

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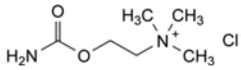
corrected 6.0

CARBACHOL

Carbacholum



$C_6H_{15}ClN_2O_2$ M_r 182.7
[51-83-2]

DEFINITION

2-(Carbamoyloxy)-*N,N,N*-trimethylethanaminium chloride.

Content: 99.0 per cent to 101.5 per cent (dried substance).

CHARACTERS

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

Solubility: very soluble in water, sparingly soluble in alcohol, practically insoluble in acetone.

IDENTIFICATION

First identification: A, C.

Second identification: B, C.

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

B. Examine the chromatograms obtained in the test for related substances.
Results: 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).

C. 0.5 mL of solution S (see Tests) gives reaction (a) of chlorides (*2.3.1*).

TESTS

Solution S. Dissolve 2.5 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 colourless (*2.2.2, Method II*).

Acidity or alkalinity. To 2.0 mL of solution S, add 0.05 mL of *methyl red mixed solution R*. Not more than 0.2 mL of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the indicator.

Related substances. Thin-layer chromatography (*2.2.27*).

Prepare the solutions immediately before use.

Test solution (a). Dissolve 0.20 g of the substance to be examined in *methanol R* and dilute to 5.0 mL with the same solvent.