

Mobile phase: mixture prepared with *carbon dioxide-free water R* containing 52 g/l of *anhydrous sodium sulphate R*, 1.5 g/l of *sodium octanesulphonate R*, 3 ml/l of *tetrahydrofuran R* stabilised with *butylhydroxytoluene R*, 50 ml/l of 0.2 M *potassium dihydrogen phosphate R* previously adjusted to pH 3.0 with *dilute phosphoric acid R*. Degas.

Flow rate: 1.0 ml/min.

Post-column solution: *carbonate-free sodium hydroxide solution R* diluted 1 to 25 with *carbon dioxide-free water R*, which is added pulseless to the column effluent using a 375 μ l polymeric mixing coil.

Flow rate: 0.3 ml/min.

Detection: pulsed amperometric detector or equivalent with a gold working electrode, a silver-silver chloride reference electrode and a stainless steel auxiliary electrode which is the cell body, held at respectively + 0.05 V detection, + 0.75 V oxidation and - 0.15 V reduction potentials, with pulse durations according to the instrument used. The temperature of the detector is set at 35 °C.

Note: to prevent problems due to salt precipitation, the electrochemical cell can be flushed with *water R* overnight.

Injection: 20 μ l using a refrigerated injector (4-8 °C); inject test solution (a) and reference solutions (b), (c) and (d).

Run time: 1.5 times the retention time of tobramycin.

Relative retention with reference to tobramycin (retention time = about 18 min): impurity C = about 0.35; impurity B = about 0.40, impurity A = about 0.70.

System suitability:

- **resolution:** minimum of 3.0 between the peaks due to impurity A and to tobramycin in the chromatogram obtained with reference solution (d). If necessary, adjust the concentration of sodium octanesulphonate in the mobile phase;
- **signal-to-noise ratio:** minimum of 10 for the principal peak in the chromatogram obtained with reference solution (b).

Limits:

- **any impurity:** not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (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 (c) (0.5 per cent),
- **total:** not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (c) (1.5 per cent),
- **disregard limit:** the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent).

2-Methyl-1-propanol (2.4.24, System B): maximum 1.0 per cent *m/m*.

Water (2.5.12): maximum 8.0 per cent, determined on 0.30 g.

Sulphated ash (2.4.14): maximum 0.3 per cent, determined on 1.0 g.

Bacterial endotoxins (2.6.14): less than 2.0 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

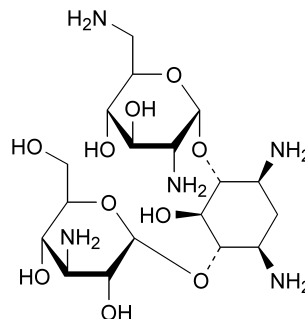
Liquid chromatography (2.2.29) as described in the test for related substances with the following modifications.

Injection: test solution (b) and reference solution (e). Calculate the percentage content of tobramycin.

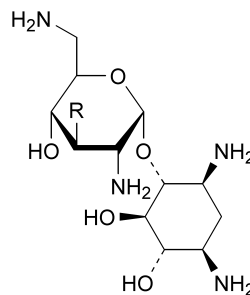
STORAGE

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

IMPURITIES



A. 4-*O*-(3-amino-3-deoxy- α -D-glucopyranosyl)-2-deoxy-6-*O*-(2,6-diamino-2,6-dideoxy- α -D-glucopyranosyl)-L-streptamine (kanamycin B),



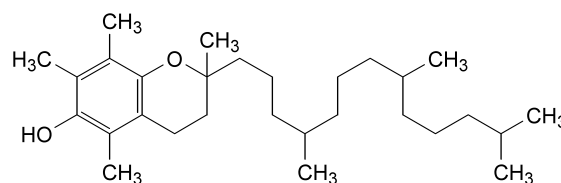
B. R = H: 2-deoxy-4-*O*-(2,6-diamino-2,3,6-trideoxy- α -D-ribohexopyranosyl)-D-streptamine (nebramine),

C. R = OH: 2-deoxy-4-*O*-(2,6-diamino-2,6-dideoxy- α -D-glucopyranosyl)-D-streptamine (neamine).

01/2008:0692

all-*rac*- α -TOCOPHEROL

int-*rac*- α -Tocopherolum



$C_{29}H_{50}O_2$
[59-02-9]

M_r 430.7

DEFINITION

all-*rac*-2,5,7,8-Tetramethyl-2-(4,8,12-trimethyltridecyl)-3,4-dihydro-2*H*-1-benzopyran-6-ol.

Content: 96.0 per cent to 102.0 per cent.

CHARACTERS

Appearance: clear, colourless or yellowish-brown, viscous, oily liquid.

Solubility: practically insoluble in water, freely soluble in acetone, in anhydrous ethanol, in methylene chloride and in fatty oils.

IDENTIFICATION

First identification: A, B.

Second identification: A, C.

A. Optical rotation (2.2.7): -0.01° to $+0.01^\circ$.

Dissolve 2.50 g in *anhydrous ethanol R* and dilute to 25.0 ml with the same solvent.

B. Infrared absorption spectrophotometry (2.2.24).

Comparison: α -tocopherol CRS.

C. Thin-layer chromatography (2.2.27).

Test solution. Dissolve 10 mg of the substance to be examined in 2 ml of *cyclohexane R*.

Reference solution. Dissolve 10 mg of α -tocopherol CRS in 2 ml of *cyclohexane R*.

Plate: TLC silica gel F_{254} plate R.

Mobile phase: ether R, *cyclohexane R* (20:80 V/V).

Application: 10 μ l.

Development: over 2/3 of the plate.

Drying: in a current of air.

Detection: examine in ultraviolet light at 254 nm.

Results: the principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Related substances. Gas chromatography (2.2.28): use the normalisation procedure.

Internal standard solution. Dissolve 1.0 g of *squalane R* in *cyclohexane R* and dilute to 100.0 ml with the same solvent.

Test solution (a). Dissolve 0.100 g of the substance to be examined in 10.0 ml of the internal standard solution.

Test solution (b). Dissolve 0.100 g of the substance to be examined in 10 ml of *cyclohexane R*.

Reference solution (a). Dissolve 0.100 g of α -tocopherol CRS in 10.0 ml of the internal standard solution.

Reference solution (b). Dissolve 10 mg of the substance to be examined and 10 mg of α -tocopheryl acetate R in *cyclohexane R* and dilute to 100.0 ml with the same solvent.

Reference solution (c). Dissolve 10 mg of all-*rac*- α -tocopherol for peak identification CRS (containing impurities A and B) in *cyclohexane R* and dilute to 1 ml with the same solvent.

Reference solution (d). Dilute 1.0 ml of test solution (b) to 100.0 ml with *cyclohexane R*. Dilute 1.0 ml of this solution to 10.0 ml with *cyclohexane R*.

Column:

- **material:** fused silica;
- **size:** $l = 30$ m, $\varnothing = 0.25$ mm;
- **stationary phase:** poly(dimethyl)siloxane R (film thickness 0.25 μ m).

Carrier gas: helium for chromatography R.

Flow rate: 1 ml/min.

Split ratio: 1:100.

Temperature:

- **column:** 280 $^\circ$ C;
- **injection port and detector:** 290 $^\circ$ C.

Detection: flame ionisation.

Injection: 1 μ l of test solution (b) and reference solutions (b), (c) and (d).

Run time: twice the retention time of all-*rac*- α -tocopherol.

Identification of impurities: use the chromatogram supplied with all-*rac*- α -tocopherol for peak identification CRS and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A and B.

Relative retention with reference to all-*rac*- α -tocopherol (retention time = about 13 min): squalane = about 0.5; impurity A = about 0.7; impurity B = about 0.8; impurities C and D = about 1.05 (eluting immediately after the all-*rac*- α -tocopherol peak).

System suitability: reference solution (b):

- **resolution:** minimum 3.5 between the peaks due to all-*rac*- α -tocopherol and α -tocopheryl acetate.

Limits:

- **impurity A:** maximum 0.5 per cent;
- **impurity B:** maximum 1.5 per cent;
- **sum of impurities C and D:** maximum 1.0 per cent;
- **any other impurity:** for each impurity, maximum 0.25 per cent;
- **total:** maximum 2.5 per cent;
- **disregard limit:** the area of the principal peak in the chromatogram obtained with reference solution (d) (0.1 per cent).

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

ASSAY

Gas chromatography (2.2.28) as described in the test for related substances with the following modification.

Injection: test solution (a) and reference solution (a).

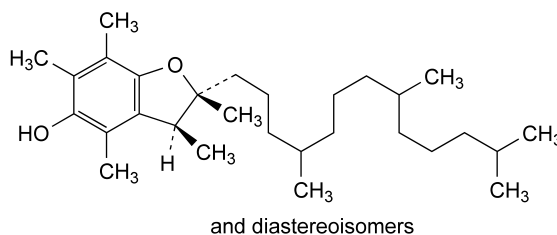
Calculate the percentage content of $C_{29}H_{50}O_2$ from the declared content of α -tocopherol CRS.

STORAGE

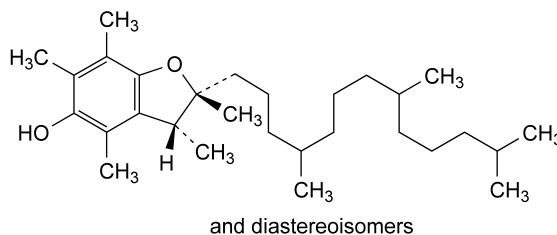
Under an inert gas, protected from light.

IMPURITIES

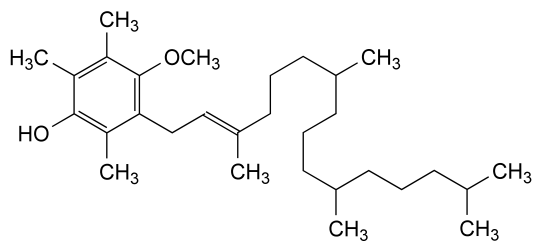
Specified impurities: A, B, C, D.



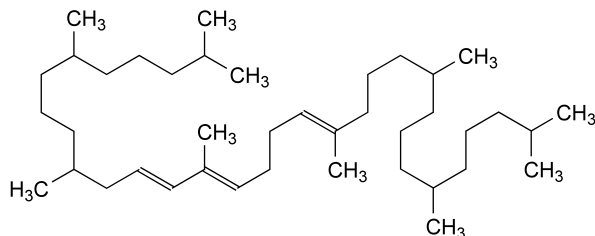
A. all-*rac*-*trans*-2,3,4,6,7-pentamethyl-2-(4,8,12-trimethyltridecyl)-2,3-dihydrobenzofuran-5-ol,



B. all-*rac*-*cis*-2,3,4,6,7-pentamethyl-2-(4,8,12-trimethyltridecyl)-2,3-dihydrobenzofuran-5-ol.



C. 4-methoxy-2,3,6-trimethyl-5-[(all-*RS,E*)-3,7,11,15-tetramethylhexadec-2-enyl]phenol,

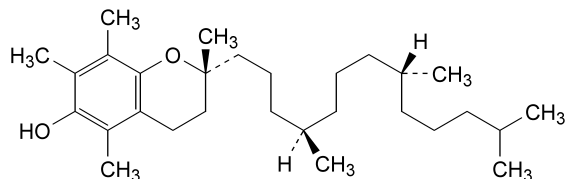


D. (all-*RS,all-E*)-2,6,10,14,19,23,27,31-octamethyldotriacont-12,14,18-triene.

01/2008:1256

RRR- α -TOCOPHEROL

RRR- α -Tocopherolum



$C_{29}H_{50}O_2$
[59-02-9]

M_r 430.7

DEFINITION

(2*R*)-2,5,7,8-Tetramethyl-2-[(4*R,8R*)-4,8,12-trimethyltridecyl]-3,4-dihydro-2*H*-1-benzopyran-6-ol.

Content: 94.5 per cent to 102.0 per cent.

CHARACTERS

Appearance: clear, colourless or yellowish-brown, viscous, oily liquid.

Solubility: practically insoluble in water, freely soluble in acetone, in anhydrous ethanol, in methylene chloride and in fatty oils.

IDENTIFICATION

First identification: A, B.

Second identification: A, C.

A. Optical rotation (2.2.7): + 0.05° to + 0.10°.

Dissolve 2.50 g in *anhydrous ethanol R* and dilute to 25.0 ml with the same solvent.

B. Infrared absorption spectrophotometry (2.2.24).

Comparison: α -tocopherol CRS.

C. Thin-layer chromatography (2.2.27).

Test solution. Dissolve 10 mg of the substance to be examined in 2 ml of *cyclohexane R*.

Reference solution. Dissolve 10 mg of α -tocopherol CRS in 2 ml of *cyclohexane R*.

Plate: TLC silica gel F_{254} plate *R*.

Mobile phase: ether *R*, cyclohexane *R* (20:80 V/V).

Application: 10 μ l.

Development: over 2/3 of the plate.

Drying: in a current of air.

Detection: examine in ultraviolet light at 254 nm.

Results: the principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

TESTS

Related substances. Gas chromatography (2.2.28): use the normalisation procedure.

Internal standard solution. Dissolve 1.0 g of *squalane R* in *cyclohexane R* and dilute to 100.0 ml with the same solvent.

Test solution (a). Dissolve 0.100 g of the substance to be examined in 10.0 ml of the internal standard solution.

Test solution (b). Dissolve 0.100 g of the substance to be examined in 10 ml of *cyclohexane R*.

Reference solution (a). Dissolve 0.100 g of α -tocopherol CRS in 10.0 ml of the internal standard solution.

Reference solution (b). Dissolve 10 mg of α -tocopherol *R* and 10 mg of α -tocopheryl acetate *R* in *cyclohexane R* and dilute to 100.0 ml with the same solvent.

Column:

- *material*: fused silica;
- *size*: $l = 30$ m, $\varnothing = 0.25$ mm;
- *stationary phase*: poly(dimethyl)siloxane *R* (film thickness 0.25 μ m).

Carrier gas: helium for chromatography *R*.

Flow rate: 1 ml/min.

Split ratio: 1:100.

Temperature:

	Time (min)	Temperature (°C)
Column	0 - 15	280
Injection port		290
Detector		290

Detection: flame ionisation.

Injection: 1 μ l of test solution (b) and reference solution (b).

System suitability: reference solution (b):

- *resolution*: minimum 3.5 between the peaks due to α -tocopherol and α -tocopheryl acetate.

Limits:

- *total*: maximum 4.0 per cent;
- *disregard limit*: 0.1 per cent.

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

ASSAY

Gas chromatography (2.2.28) as described in the test for related substances with the following modification.

Injection: test solution (a) and reference solution (a).

Calculate the percentage content of $C_{29}H_{50}O_2$ taking into account the declared content of α -tocopherol CRS.

STORAGE

Under an inert gas, protected from light.