Temperature:
- *column*: 280 °C,
- *injection port and detector*: 290 °C.

Detection: flame ionisation.

Injection: 1 µl 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, B and C plus D.

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; impurity C = about 1.05; impurity D = about 1.05.

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).

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).

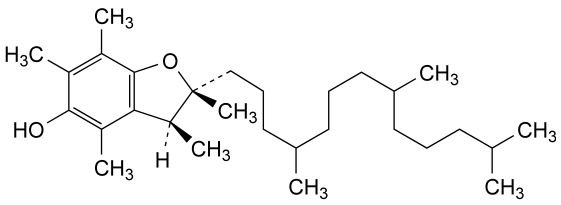
Calculate the percentage content of $C_{29}H_{50}O_2$ taking into account the declared content of *α-tocopherol CRS*.

STORAGE

Under an inert gas, protected from light.

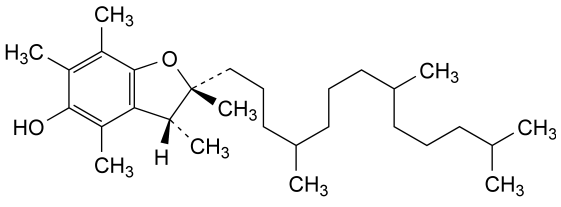
IMPURITIES

Specified impurities: A, B, C, D.



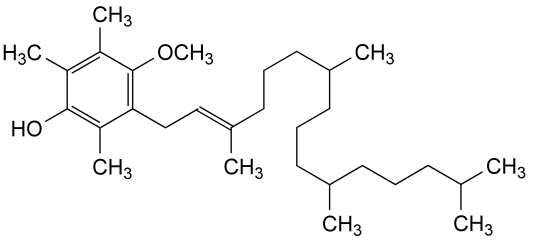
and diastereoisomers

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

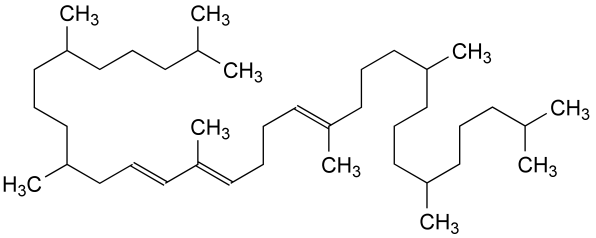


and diastereoisomers

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,

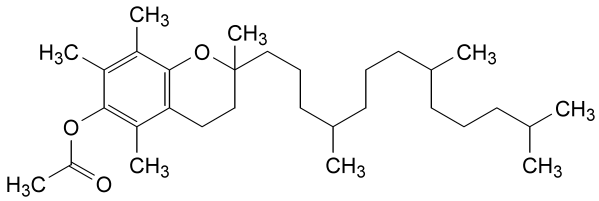


D. (all-*RS*,all-*E*)-2,6,10,14,19,23,27,31-octamethyldotriaconta-12,14,18-triene.

04/2005:0439

all-*rac*-α-TOCOPHERYL ACETATE

int-*rac*-α-Tocopherylis acetas



$C_{31}H_{52}O_3$ M_r 472.7

DEFINITION

All-*rac*-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-3,4-dihydro-2*H*-1-benzopyran-6-yl acetate.

Content: 96.5 per cent to 101.0 per cent.

CHARACTERS

Appearance: clear, colourless or slightly greenish-yellow, viscous, oily liquid.

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

IDENTIFICATION

First identification: A, B.

Second identification: A, C.

A. Optical rotation (*2.2.7*): −0.01° to + 0.01°.
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-tocopheryl acetate CRS*.

C. Thin-layer chromatography (*2.2.27*).
Test solution. Dissolve about 10 mg of the substance to be examined in 2 ml of *cyclohexane R*.
Reference solution. Dissolve about 10 mg of *α-tocopheryl acetate 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 µl.
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-tocopheryl acetate 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-tocopherol R* in *cyclohexane R* and dilute to 100.0 ml with the same solvent.

Reference solution (c). Dissolve 10 mg of *all-rac-α-tocopheryl acetate for peak identification CRS* (containing impurities A, B, E) 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, Ø = 0.25 mm,
- *stationary phase*: *poly(dimethyl)siloxane R* (film thickness 0.25 µm).

Carrier gas: *helium for chromatography R*.

Flow rate: 1 ml/min.

Split ratio: 1:100.

Temperature:

- *column*: 280 °C,
- *injection port and detector*: 290 °C.

Detection: flame ionisation.

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

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

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

Relative retention with reference to all-*rac*-α-tocopheryl acetate (retention time = about 15 min): squalane = about 0.4; impurity A = about 0.7; impurity B = about 0.8; impurity C = about 0.9; impurity D = about 1.05; impurity E = about 1.05.

System suitability:

- *resolution*: minimum 3.5 between the peaks due to impurity C and all-*rac*-α-tocopheryl acetate in the chromatogram obtained with reference solution (b),
- in the chromatogram obtained with reference solution (a), the area of the peak due to impurity C is not greater than 0.2 per cent of the area of the peak due to all-*rac*-α-tocopheryl acetate.

Limits:

- *impurities A, C*: for each impurity, maximum 0.5 per cent,
- *impurity B*: maximum 1.5 per cent,
- *sum of impurities D and E*: 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).

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).

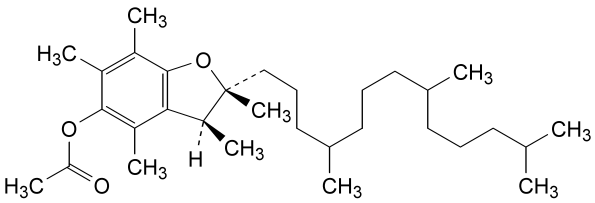
Calculate the percentage content of $C_{31}H_{52}O_3$ taking into account the declared content of *α-tocopheryl acetate CRS*.

STORAGE

Protected from light.

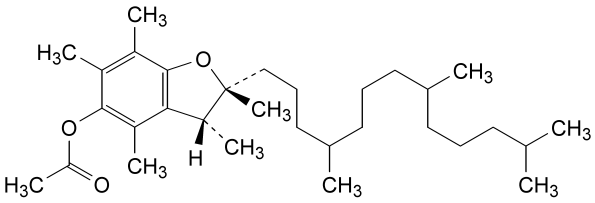
IMPURITIES

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



and diastereoisomers

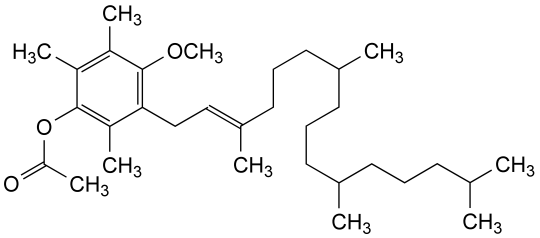
A. all-*rac-trans*-2,3,4,6,7-pentamethyl-2-(4,8,12-trimethyltridecyl)-2,3-dihydrobenzofuran-5-yl acetate,



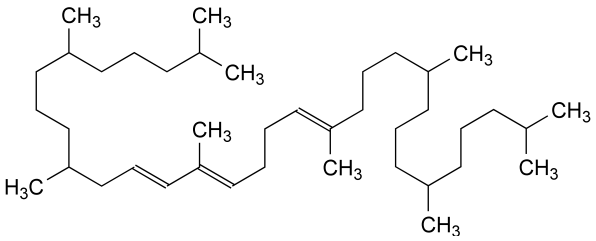
and diastereoisomers

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

C. all-*rac*-α-tocopherol,



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

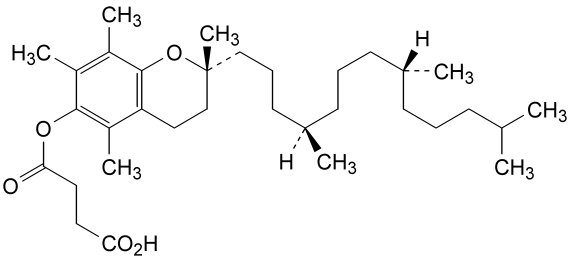


E. (all-*RS*,all-*E*)-2,6,10,14,19,23,27,31-octamethyldotriaconta-12,14,18-triene.

01/2005:1259
corrected

RRR-α-TOCOPHERYL HYDROGEN SUCCINATE

RRR-α-Tocopherylis hydrogenosuccinas



$C_{33}H_{54}O_5$ M_r 530.8

DEFINITION

RRR-α-Tocopheryl hydrogen succinate contains not less than 96.0 per cent and not more than the equivalent of 102.0 per cent of (2*R*)-2,5,7,8-tetramethyl-2-[(4*R*,8*R*)-4,8,12-trimethyltridecyl]-3,4-dihydro-2*H*-1-benzopyran-6-yl hydrogen succinate.

CHARACTERS

A white or almost white, crystalline powder, practically insoluble in water, soluble in acetone and in anhydrous ethanol, very soluble in methylene chloride.

IDENTIFICATION

First identification: B, D.

Second identification: A, C, D.

A. It complies with the test for absorbance (see Tests).

B. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *RRR-α-tocopheryl hydrogen succinate CRS*.

C. Examine by thin-layer chromatography (*2.2.27*), using *silica gel HF_{254} R* as the coating substance.

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

Test solution (b). In a ground-glass-stoppered tube, dissolve 10 mg of the substance to be examined in 2 ml of *2.5 M alcoholic sulphuric acid R*. Heat on a water-bath for 5 min. Cool and add 2 ml of *water R* and 2 ml of *cyclohexane R*. Shake for 1 min. Use the upper layer.

Reference solution (a). Dissolve 10 mg of *RRR-α-tocopheryl hydrogen succinate CRS* in 2 ml of *cyclohexane R*.

Reference solution (b). Prepare as described for test solution (b), using *RRR-α-tocopheryl hydrogen succinate CRS* instead of the substance to be examined.

Apply separately to the plate 10 µl of each solution. Develop over a path of 15 cm using a mixture of 0.2 volumes of *glacial acetic acid R*, 20 volumes of *ether R* and 80 volumes of *cyclohexane R*. Dry the plate in a current of air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with test solution (a) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a). In the chromatograms obtained with test solution (b) and reference solution (b), there are two spots: the spot with the higher R_f value is due to α-tocopherol, the spot with the lower R_f value is due to α-tocopheryl hydrogen succinate and corresponds to the spot obtained with reference solution (a). Depending on the degree of hydrolysis, the lower spot may be weak or even absent. Spray the plate with a mixture of 10 volumes of *hydrochloric acid R*, 40 volumes of a 2.5 g/l solution of *ferric chloride R* in *ethanol (96 per cent) R* and 40 volumes of a 10 g/l solution of *phenanthroline hydrochloride R* in *ethanol (96 per cent) R*. In the chromatograms obtained with test solution (b) and reference solution (b), the spot due to α-tocopherol is orange.

D. After saponification, the resulting *RRR*-α-tocopherol is dextrorotatory (*2.2.7*). The specific optical rotation after oxidation to the quinone form is at least + 24.

Carry out the test avoiding exposure to actinic light. Transfer 1.0 g of the substance to be examined to a round bottomed, ground-glass-stoppered, 250 ml flask and dissolve in 30 ml of *anhydrous ethanol R* and heat under reflux for 3 min. While the solution is boiling, add, through the condenser, 20 ml of *2 M alcoholic potassium hydroxide solution R*. Continue heating under reflux for 20 min and, without cooling, add 4.0 ml of *hydrochloric acid R* dropwise through the condenser. Cool, rinse the condenser with 10 ml of *anhydrous ethanol R*, transfer the contents of the flask to a 500 ml separating funnel, rinse the flask with 4 quantities, each of 25 ml, of *water R* and 4 quantities, each of 25 ml, of *ether R*. Add the rinsings to the separating funnel. Shake vigorously for 2 min, allow the layers to separate and collect each of the 2 layers in individual separating funnels. Shake the aqueous layer with 2 quantities, each of 50 ml, of *ether R* and add these extracts to the main ether extract. Wash the combined ether extracts with 4 quantities, each of 100 ml, of *water R* and discard the washings.

To the ether solution add 40 ml of a 100 g/l solution of *potassium ferricyanide R* in an 8 g/l solution of *sodium hydroxide R* and shake for 3 min. Wash the ether solution with 4 quantities, each of 50 ml, of *water R*, discard the washings and dry the ether layer over *anhydrous sodium sulphate R*. Evaporate the ether on a water-bath under reduced pressure or in an atmosphere of nitrogen until a few millilitres remain, then complete the evaporation removing the last traces of ether without the application of heat. Immediately dissolve the residue in 25.0 ml of *trimethylpentane R* and determine the optical rotation.

Calculate the specific optical rotation of the substance in the test solution using as *c* the number of grams equivalent to α-tocopherol (factor 0.811) in 1000 ml.

TESTS

Absorbance (*2.2.25*). Dissolve 0.150 g in *anhydrous ethanol R* and dilute to 100 ml with the same solvent. Dilute 10.0 ml of the solution to 100.0 ml with *anhydrous ethanol R* (solution a). Dilute 20.0 ml of the initial solution to 50.0 ml with *anhydrous ethanol R* (solution b). Measure the absorbance of solution (a) at the maximum at 284 nm