Drying: in a current of air.

Detection: examine in ultraviolet light at 254 nm.

Results: 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 may be 2 spots depending on the degree of hydrolysis: the spot with the higher R_F value is due to α-tocopheryl acetate and corresponds to the spot in the chromatogram obtained with reference solution (a); the spot with the lower R_F value is due to α-tocopherol.

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 *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, 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:

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

Detection: flame ionisation.

Injection: 1 μl of test solution (b) and reference solutions (a) and (b); inject directly onto the column or via a sufficiently inert, glass-lined injection port using an automatic injection device or other reproducible injection method.

System suitability:

- *resolution*: minimum 3.5 between the peaks due to α-tocopherol and 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 α-tocopherol is not greater than 0.2 per cent of the area of the peak due to 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).

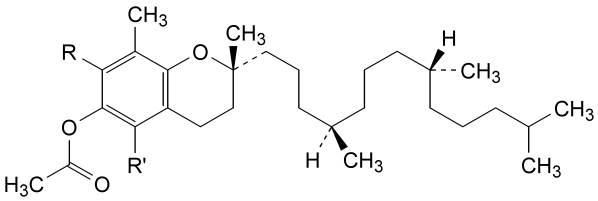
Calculate the percentage content of $C_{31}H_{52}O_3$ taking into account the declared content of *α-tocopheryl acetate CRS*.

STORAGE

Protected from light.

IMPURITIES

A. *RRR*-α-tocopherol,



B. R = H, R′ = CH_3: (2*R*)-2,5,8-trimethyl-2-[(4*R*,8*R*)–4,8,12-trimethyltridecyl]-3,4-dihydro-2*H*-1-benzopyran-6-yl acetate (*RRR*-β-tocopheryl acetate),

C. R = CH_3, R′ = H: (2*R*)-2,7,8-trimethyl-2-[(4*R*,8*R*)–4,8,12-trimethyltridecyl]-3,4-dihydro-2*H*-1-benzopyran-6-yl acetate (*RRR*-γ-tocopheryl acetate),

D. R = R′ = H: (2*R*)-2,8-dimethyl-2-[(4*R*,8*R*)-4,8,12-trimethyltridecyl]-3,4-dihydro-2*H*-1-benzopyran-6-yl acetate (*RRR*-δ-tocopheryl acetate).

01/2008:0691

α-TOCOPHERYL ACETATE CONCENTRATE (POWDER FORM)

α-Tocopherylis acetatis pulvis

DEFINITION

Preparation obtained either by finely dispersing *all-rac-α-Tocopheryl acetate (0439)* in a suitable carrier of suitable quality (for example gelatin, acacia, carbohydrates, lactoproteins or a mixture thereof) or by adsorbing *all-rac-α-Tocopheryl acetate (0439)* on silicic acid of suitable quality.

Content: 90.0 per cent to 115.0 per cent of the α-tocopheryl acetate content stated on the label, which is not less than 25 g per 100 g of concentrate.

CHARACTERS

Appearance: almost white, yellowish or light brown, small particles.

Solubility: practically insoluble or swells or forms a dispersion in water, depending on the formulation.

IDENTIFICATION

Thin-layer chromatography (*2.2.27*).

Test solution. To a quantity of the preparation to be examined corresponding to 50 mg of α-tocopheryl acetate add 5 ml of *0.01 M hydrochloric acid* and treat with ultrasound at 60 °C. Add 5 ml of *anhydrous ethanol R* and 10 ml of *cyclohexane R*, shake for 1 min and centrifuge for 5 min. Use the upper layer.

Reference solution. Dissolve 50 mg of *α-tocopheryl acetate CRS* in *cyclohexane R* and dilute to 10 ml with the same solvent.

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

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

Application: 10 µl.

Development: over a path of 15 cm.

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

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*).

Internal standard solution. Dissolve 0.20 g of *dotriacontane R* in *hexane R* and dilute to 100.0 ml with the same solvent.

Test solution. Weigh accurately a quantity of the preparation to be examined corresponding to about 0.100 g of α-tocopheryl acetate in a 250 ml conical flask. Add 20 ml of *1 M hydrochloric acid* and treat with ultrasound at 70 °C for 20 min. Add 50 ml of *anhydrous ethanol R* and 50.0 ml of the internal standard solution and thoroughly mix the 2 layers for 30 min. Allow to separate, and use the upper layer.

Reference solution. Dissolve 0.100 g of *α-tocopheryl acetate CRS* in the internal standard solution and dilute to 50.0 ml with the same solution.

Column:

– *material*: silanised glass;
– *size*: *l* = 2.0-3.0 m, Ø = 2.2-4.0 mm;
– *stationary phase*: *diatomaceous earth for gas chromatography R* (125-150 µm or 150-180 µm), silanised with dimethyldichlorosilane and impregnated with 1-5 per cent *m/m* of *poly(dimethyl)siloxane R*;
– *plug*: silanised glass wool placed at each end of the column.

Carrier gas: *nitrogen for chromatography R.*

Flow rate: 25-90 ml/min.

Temperature:

– *column*: constant between 245 °C and 280 °C;
– *injection port and detector*: constant between 270 °C and 320 °C.

Detection: flame ionisation.

Injection: 1 µl; inject directly onto the column or via an injection port (preferably glass-lined) using an automatic injection device or other reproducible injection method.

System suitability: reference solution:

– *resolution*: minimum 1.4 between the peaks due to dotriacontane and α-tocopheryl acetate. Set the temperature of the column and the flow rate of the carrier gas in such a manner that the required resolution is achieved. Repeat the injection until the response factor (RF) determined as described below is constant to within ± 2 per cent.

Interference test. Weigh accurately a quantity of the substance to be examined corresponding to about 0.100 g of α-tocopheryl acetate in a 250 ml conical flask. Add 20 ml of *1 M hydrochloric acid* and treat with ultrasound at 70 °C for 20 min. Add 50 ml of *anhydrous ethanol R* and 50 ml of *hexane R* and thoroughly mix the 2 layers for 30 min. Allow to separate. Inject 1 µl of the upper layer and record the chromatogram, choosing an attenuation such that the height of the peak due to α-tocopheryl acetate is greater than 50 per cent of the maximum recorder response; during the recording, change the attenuation so that any peak appearing with the same retention time as that of the peak due to dotriacontane is recorded with a sensitivity at least 8 times greater than for the peak due to α-tocopheryl acetate. If a peak with a height of at least 5 mm for a recorder paper width of 250 mm is detected with the same retention time as that of dotriacontane, use the corrected peak area $S'_{D(corr)}$ for the final calculation.

$$S'_{D(corr)} = S'_D - \frac{S_I \times S'_T}{f \times S_{TI}}$$

S'_D = area of the peak due to dotriacontane in the chromatogram obtained with the test solution;

S_I = area of the peak with the same retention time as that of the peak due to dotriacontane in the chromatogram obtained in the interference test;

S'_T = area of the peak due to α-tocopheryl acetate in the chromatogram obtained with the test solution;

S_{TI} = area of the peak due to α-tocopheryl acetate in the chromatogram obtained in the interference test;

f = factor by which the attenuation was changed.

Record the chromatograms choosing an attenuation such that the peak due to α-tocopheryl acetate is greater than 50 per cent of the maximum recorder response.

Measure the areas of the peaks due to *α-tocopheryl acetate CRS* (S_T) and to dotriacontane (S_D) in the chromatogram obtained with the reference solution and the areas of the peaks due to α-tocopheryl acetate (S'_T) and to dotriacontane (S'_D) in the chromatogram obtained with the test solution.

Determine the response factor (RF) for α-tocopheryl acetate from the areas of the peak due to α-tocopheryl acetate and the peak due to dotriacontane in the chromatogram obtained with the reference solution using the following expression:

$$\frac{S_D \times m_T}{S_T \times m_D}$$

Calculate the percentage content of α-tocopheryl acetate using the following expression:

$$\frac{100 \times S'_T \times m_D \times RF}{S'_{D(corr.)} \times m}$$

S_D = area of the peak due to dotriacontane in the chromatogram obtained with the reference solution;

$S'_{D(corr.)}$ = corrected area of the peak due to dotriacontane in the chromatogram obtained with the test solution;

S_T = area of the peak due to *α-tocopheryl acetate CRS* in the chromatogram obtained with the reference solution;

S'_T = area of the peak due to α-tocopheryl acetate in the chromatogram obtained with the test solution;

m_D = mass of dotriacontane in the test solution and in the reference solution, in milligrams;

m_T = mass of *α-tocopheryl acetate CRS* in the reference solution, in milligrams;

m = mass of the substance to be examined in the test solution, in milligrams.

STORAGE

In an airtight, well-filled container, protected from light.

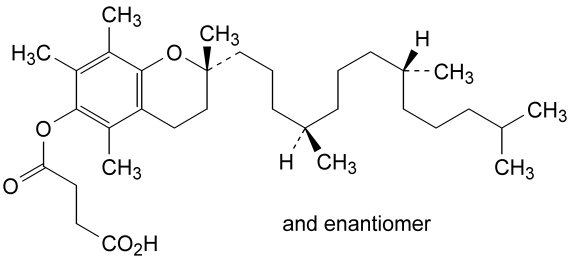
LABELLING

The label states the content of α-tocopheryl acetate, expressed in grams per 100 g of concentrate.

01/2008:1258

DL-α-TOCOPHERYL HYDROGEN SUCCINATE

DL-α-Tocopherylis hydrogenosuccinas



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

DEFINITION

(2*RS*)-2,5,7,8-Tetramethyl-2-[(4*RS*,8*RS*)-4,8,12-trimethyltridecyl]-3,4-dihydro-2*H*-1-benzopyran-6-yl hydrogen succinate.

Content: 96.0 per cent to 102.0 per cent.

CHARACTERS

Appearance: white or almost white, crystalline powder.

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

IDENTIFICATION

First identification: B, D.

Second identification: A, C, D.

A. Absorbance (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *RRR-α-tocopheryl hydrogen succinate CRS.*

C. Thin-layer chromatography (*2.2.27*).

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.

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

Mobile phase: *glacial acetic acid R, ether R, cyclohexane R* (0.2:20:80 *V/V/V*).

Application: 10 µl.

Development: over a path of 15 cm.

Drying: in a current of air.

Detection A: examine in ultraviolet light at 254 nm.

Results A: 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 2 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 DL-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.

Detection B: spray 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.*

Results B: in the chromatograms obtained with test solution (b) and reference solution (b), the spot due to α-tocopherol is orange.

D. Optical rotation (see Tests).

TESTS

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.

Absorbance (*2.2.25*).

Solution A. Dissolve 0.150 g in *anhydrous ethanol R* and dilute to 100 ml with the same solvent.

Test solution (a). Dilute 10.0 ml of solution A to 100.0 ml with *anhydrous ethanol R.*

Test solution (b). Dilute 20.0 ml of solution A to 50.0 ml with *anhydrous ethanol R.*

Absorption maximum: at 284 nm for test solution (a).

Absorption minimum: at 254 nm for test solution (b).

Specific absorbance at the absorption maximum: 35 to 38 for test solution (a).

Specific absorbance at the absorption minimum: 6.0 to 8.0 for test solution (b).

Acid value (*2.5.1*): 101 to 108, determined on 1.00 g.

Free tocopherol: maximum 1.0 per cent.

Dissolve 0.500 g in 100 ml of *0.25 M alcoholic sulphuric acid R.* Add 20 ml of *water R* and 0.1 ml of a 2.5 g/l solution of *diphenylamine R* in *sulphuric acid R.* Titrate with *0.01 M ammonium and cerium sulphate* until a blue colour is