

## d- $\alpha$ -TOCOPHEROL, CONCENTRATE

*Prepared at the 55th JECFA (2000) and published in FNP 52 Add 8 (2000), superseding tentative specifications prepared at the 30th JECFA (1986) and published in FNP 37 (1986) and in FNP 52 (1992). A group ADI of 0.15-2 mg/kg bw for dl- $\alpha$ -tocopherol and d- $\alpha$ -tocopherol, concentrate, singly or in combination, was established at the 30th JECFA (1986).*

### SYNONYMS

Vitamin E, RRR-alpha -tocopherol, 5,7,8-trimethyltolcol, (+)-alpha-Tocopherol; INS No. 307a

### DEFINITION

d-alpha-Tocopherol, concentrate is a form of Vitamin E obtained by the vacuum steam distillation of edible vegetable oil products, comprising a concentrated form of d-alpha-tocopherol. It may contain an edible vegetable oil added to adjust the required amount of total tocopherols, and the content of d-alpha-tocopherol may be adjusted by suitable physical and chemical means.

### Chemical names

(2R,4'R,8'R)-2,5,7,8-tetramethyl-2-(4',8',12'-trimethyltridecyl)-chroman-6-ol

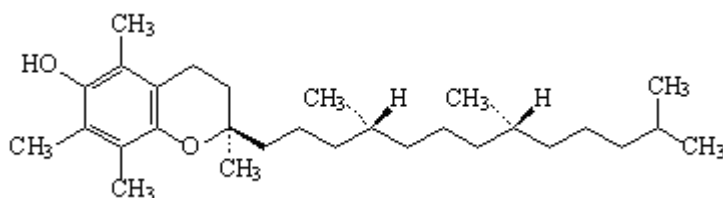
### C.A.S. number

No. 59-02-9 (Vitamin E)

### Chemical formula

C<sub>29</sub>H<sub>50</sub>O<sub>2</sub>

### Structural formula



### Formula weight

430.71

### Assay

Not less than 40% of total tocopherols, of which not less than 95% consists of d-alpha-tocopherol

### DESCRIPTION

Brownish red to light yellow, nearly odourless, clear viscous oil, which oxidizes and darkens slowly in air and on exposure to light

### FUNCTIONAL USES

 Antioxidant, nutrient

### CHARACTERISTICS

#### IDENTIFICATION

#### Solubility (Vol. 4)

Insoluble in water; soluble in ethanol; miscible with ether

#### Chromatography

The retention time of the major peak in the chromatogram of the sample solution is the same as that of the standard solution, both relative to the

internal standard, as obtained in the Assay.

Colour reaction Dissolve about 0.05 g of the sample in 10 ml of absolute ethanol. Add, with swirling, 2 ml of nitric acid and heat at about 75° for 15 min. A bright red to orange colour develops

PURITY

Specific rotation alpha (25, D): Not less than +24°  
See description under TESTS

Acidity Dissolve 1 g of the sample in 25 ml of a mixture of equal volumes of ethanol and ether that has been neutralized to phenolphthalein TS with 0.1 N sodium hydroxide, add 0.5 ml of phenolphthalein TS, and titrate with 0.1 N sodium hydroxide until the solution remains faintly pink after shaking for 30 sec. Not more than 1.0 ml of 0.1 N sodium hydroxide is required.

Lead (Vol. 4) Not more than 2 mg/kg  
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental methods".

## TESTS

PURITY TESTS

Specific rotation Transfer an accurately weighed sample, equivalent to about 100 mg of total tocopherols, to a separator, and dissolve it in 50 ml of ether. To the separator add 20 ml of a 10% solution of potassium ferricyanide in 0.8% sodium hydroxide solution, and shake for 3 min. Wash the ether solution with four 50-ml portions of water, discard the washings, and dry over anhydrous sodium sulfate.

Evaporate the dried ether solution on a water bath under reduced pressure or in an atmosphere of nitrogen until about 7 or 8 ml remain, and then complete the evaporation, removing the last traces of ether without the application of heat. Immediately dissolve the residue in 5 ml of isooctane, and determine the optical rotation. Calculate the specific rotation, using as *c* the concentration expressed as the number of g of total tocopherols, determined in the Assay, in 100 ml of the solution.

## METHOD OF ASSAY

Gas Liquid Chromatographic Method (Volume 4)

### Reagents and solutions

Internal Standard Solution: Transfer about 600 mg of hexadecyl hexadecanoate, accurately weighed, to a 200-ml volumetric flask, dissolve in a solution containing 2 parts of pyridine and 1 part of propionic anhydride, dilute to volume with the solution, and mix.

### Standard solution

Transfer 12-, 25-, 37-, and 50-mg portions of USP Alpha Tocopherol Reference Standard, accurately weighed, to separate 50-ml Erlenmeyer flasks having 19/38 standard-taper ground-glass necks. Pipet 25.0 ml of the Internal Standard Solution into each flask, mix, and reflux for 10 min. under water-cooled condensers.

### Sample solution

Transfer about 60 mg of the sample, accurately weighed, to another 50-ml Erlenmeyer flask, pipet 10.0 ml of the Internal Standard Solution into the flask, mix, and reflux for 10 min. under a water-cooled condenser.

### Chromatographic System

Use a gas chromatograph equipped with a flame-ionization detector and a glass-lined sample-introduction system or on-column injection. Under typical conditions, the instrument contains a 2-m x 4-mm borosilicate glass column packed with 2% to 5% methylpolysiloxane on 80- to 100-mesh acid-base washed siliconized chromatographic diatomaceous earth. The column is maintained isothermally between 240° and 260°, the injection port at about 290°, and the detector block at about 300°. The flow rate of dry carrier gas is adjusted to obtain a hexadecyl-hexadecanoate peak approximately 18 to 20 min after sample introduction when a 2% stationary phase is used, or 30 to 32 min when a 5% stationary phase is used. (Note: Cure and condition the column as necessary).

### System Suitability

Chromatograph a suitable number of injections of the sample solution, as directed under Calibration, to assure that the resolution factor R, between the major peaks occurring at retention times of approximately 0.50 (delta-tocopherol propionate) and 0.63 (beta- and gamma-tocopheryl propionates), relative of hexadecyl hexadecanoate at 1.00, is not less than 2.5.

### Calibration

Chromatograph successive 2- to 5- $\mu$ l portions of each standard solution until the relative response factor F, for each is constant (i.e. within a range of approximately 2%) for three consecutive injections. If graphic integration is used, adjust the instrument to obtain at least 70% maximum recorder response for the hexadecyl hexadecanoate peak. Measure the areas under the first (alpha-tocopheryl propionate) and second (hexadecyl hexadecanoate) major peaks, and record the values as  $A_s$  and  $A_1$ , respectively. Calculate the factor F, for each concentration of  $(A_s/A_1) \times (C_1/C_s)$ , in which  $C_1$  and  $C_s$  are the exact concentrations, in mg per ml, of hexadecyl hexadecanoate and of USP Alpha Tocopherol Reference Standard in the Standard Preparation, respectively. Prepare a relative response factor curve by plotting area of alpha-tocopheryl propionate versus relative response factor.

### Procedure

Inject a suitable portion (2 to 5  $\mu$ l) of the Assay Preparation into the chromatograph, and record the chromatogram. Measure the areas under the four major peaks occurring at relative retention times of 0.50, 0.63, 0.76, and 1.00, and record the values as  $A_\delta$ ,  $A_{\beta+\gamma}$ ,  $A_\alpha$  and  $A_1$ , corresponding

to delta-tocopherol propionate, beta- and gamma-tocopheryl propionates, alpha-tocopheryl propionate, and hexadecyl hexadecanoate, respectively.

Calculate the weight, in mg, of each tocopherol form in the sample by the following formulas.

$$\text{delta-tocopherol} = (10C_1/F) \times (A_\delta/A_1)$$

$$\text{beta- and gamma-tocopherols} = (10C_1/F) \times (A_{\beta+\gamma}/A_1)$$

$$\text{alpha-tocopherol} = (10C_1/F) \times (A_\alpha/A_1)$$

where

F is obtained from the relative response factor curve (see Calibration) for each of the corresponding areas under the delta-, beta- and gamma-, and alpha-tocopheryl propionate peaks produced by the Assay Preparation.

(NOTE: The relative response factors for delta-tocopheryl propionate and for beta- and gamma-tocopheryl propionates have been determined empirically to be the same as for alpha-tocopheryl propionate).