

TOCOPHEROL CONCENTRATE, MIXED

Prepared at the 30th JECFA (1986), published in FNP 37 (1986) and in FNP 52 (1992). Metals and arsenic specifications revised at the 61st JECFA (2003). A group ADI of 0.15-2 mg/kg bw for dl- α -tocopherol and d- α -tocopherol, concentrate, singly or in combination, was established at the 30th JECFA (1986).

SYNONYMS

Vitamin E, INS No 307b

DEFINITION

A form of Vitamin E obtained by the vacuum steam distillation of edible vegetable oil products, comprising concentrated tocopherols. It may contain an edible vegetable oil added to adjust the required amount of total tocopherols, and the tocopherol forms may be adjusted by suitable physical and chemical means.

Chemical names

Mixed Tocopherol Concentrate contains tocopherols such as d- α -, d- β -, d- γ -, d- δ -tocopherols

C.A.S. number

No single definite C.A.S. number is for this substance. No. 59-02-9 is for vitamin E, 1406-18-4 is for α -tocopherol, 2074-53-5 is for all-rac- α -tocopherol, and 10191-40-0 is for racemic- α -tocopherol synthesized from natural phytol or its derivative.

Assay

Not less than 34% of total tocopherols

DESCRIPTION

Brownish red to red, clear, viscous oil having a mild, characteristic odour; may show a slight separation of waxlike constituents in microcrystalline form

It oxidizes and darkens slowly in air and on exposure to light, particularly when in alkaline media.

FUNCTIONAL USES Antioxidant

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)

Insoluble in water; soluble in ethanol; miscible in ether

Chromatography

The retention time of the third major peak (i.e. the peak occurring just before that of the internal standard) in the chromatogram of the Assay Preparation is the same as that of the Standard Preparation, 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 (Vol. 4) $[\alpha]_{25, D}$: Not less than $+20^\circ$
See description under TESTS

Sulfated ash (Vol. 4) Not more than 0.1%
Test 1 g of the sample (Method II)

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 amount of 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 sodium hydroxide solution (1 in 125), 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

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 Preparations: 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.

Assay Preparation: 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 gum on 80- to 100-mesh acid-base washed silinized 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% column is used, or 30 to 32 min. when a 5% column is used. (NOTE: Cure and condition the column as necessary).

System Suitability

Chromatograph a suitable number of injections of the Assay Preparation, 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 (β-plus gamma-tocopheryl propionates), relative of hexadecyl hexadecanoate at 1.00, is not less than 2.5.

Calibration

Chromatograph successive 2- to 5-μl portions of each Standard Preparation 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 (I-tocopheryl propionate) and second (hexadecyl hexadecanoate) major peaks (excluding the solvent peak), 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 μl) 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} , $a_{\text{beta+gamma}}$, a_{alpha} and a_1 , corresponding to delta-tocopheryl propionate, β-plus 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:

$$\text{delta-tocopherol} = (10C_1/F) \times (a_{\text{delta}}/a_1);$$

$$\beta\text{-plus gamma-tocopherols} = (10C_1/F) \times (a_{\text{beta+gamma}}/a_1);$$

$$\text{alpha-tocopherol} = (10C_1/F) \times (a_{\text{alpha}}/a_1),$$

where

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

(NOTE: The relative response factor for delta-tocopheryl propionate and for β - plus gamma-tocopheryl propionates has been determined empirically to be the same as for alpha-tocopheryl propionate).