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# Vitamin E

## DEFINITION

Vitamin E is a form of alpha tocopherol ( $C_{29}H_{50}O_2$ ). It includes the following: *RRR*- or *all-rac*-alpha tocopherol ( $C_{29}H_{50}O_2$ ), *RRR*- or *all-rac*-alpha tocopheryl acetate ( $C_{31}H_{52}O_3$ ), and *RRR*- or *all-rac*-alpha tocopheryl acid succinate ( $C_{33}H_{54}O_5$ ). It contains NLT 96.0% and NMT 102.0% of *RRR*- or *all-rac*-alpha tocopherol, *RRR*- or *all-rac*-alpha tocopheryl acetate, or *RRR*- or *all-rac*-alpha tocopheryl acid succinate.

## IDENTIFICATION

### • A.

[NOTE—Use low-actinic glassware.]

#### Sample solutions

**Alpha tocopherol:** 1 mg/mL in [dehydrated alcohol](#)

**Alpha tocopheryl acetate:** Transfer 220 mg of *RRR*- or *all-rac*-alpha tocopheryl acetate to a round-bottom, glass-stoppered, 150-mL flask, and dissolve in 25 mL of [dehydrated alcohol](#). Add 20 mL of [diluted sulfuric acid](#) in [alcohol](#) (1 in 7), and reflux in an all-glass apparatus for 3 h, protected from sunlight. Cool, transfer to a 200-mL volumetric flask, and add [diluted sulfuric acid](#) in [alcohol](#) (1 in 72) to volume.

**Alpha tocopheryl acid succinate:** [CAUTION—Wear safety goggles.] Transfer an amount of the sample, equivalent to 200 mg of alpha tocopherol, to a round-bottom, glass-stoppered, 250-mL flask; dissolve in 50 mL of [dehydrated alcohol](#); and reflux for 1 min. While the solution is boiling, add, through the condenser, 1 g of [potassium hydroxide](#) pellets one at a time to avoid overheating. Continue refluxing for 20 min and, without cooling, add 2 mL of [hydrochloric acid](#) dropwise through the condenser. [NOTE—This technique is essential to prevent oxidative action by air while the sample is in an alkaline medium.] Cool, and transfer the contents of the flask to a 500-mL separatory funnel, rinsing the flask with 100 mL each of [water](#) and [ether](#), and adding the rinsings to the separatory funnel. Shake vigorously, allow the layers to separate, and collect each of the two layers in individual separators. Extract the aqueous layer with two 50-mL portions of [ether](#) and add these extracts to the main ether extract. Wash the combined ether extracts with four 100-mL portions of [water](#), then evaporate the ether solution on a water bath under reduced pressure or in an atmosphere of nitrogen until about 7–8 mL remain. Complete the evaporation, removing the last traces of ether without the application of heat. Immediately dissolve the residue in [diluted sulfuric acid](#) in [alcohol](#) (1 in 72), transfer to a 200-mL volumetric flask, and dilute with the alcoholic sulfuric acid to volume.

#### Analysis

**Sample:** Use the appropriate *Sample solution*.

To 10 mL of the *Sample solution* add 2 mL of [nitric acid](#), with swirling, and heat at about 75° for 15 min.

**Acceptance criteria:** A bright red or orange color develops.

#### Change to read:

- B. ▲[OPTICAL ROTATION \(781S\)](#), [PROCEDURES](#), [SPECIFIC ROTATION](#)▲ (USP 1-MAY-2024)

#### Sample solutions

**Alpha tocopherol:** Dissolve 100 mg of alpha tocopherol in 50 mL of [ether](#).

**Alpha tocopheryl acetate:** Transfer a volume of the *Sample solution* for *Alpha tocopheryl acetate* from *Identification A*, equivalent to 100 mg of alpha tocopheryl acetate, to a separatory funnel, and add 200 mL of [water](#). Extract with [ether](#), first with 75 mL, then with 25 mL, and combine the ether extracts in another separatory funnel.

**Alpha tocopheryl acid succinate:** Transfer a volume of the *Sample solution* for *Alpha tocopheryl acid succinate* from *Identification A*, equivalent to 100 mg of alpha tocopheryl acid succinate, to a separatory funnel, and add 200 mL of [water](#). Extract with [ether](#), first with 75 mL, then with 25 mL, and combine the ether extracts in another separatory funnel.

#### Analysis

**Sample:** Use the appropriate *Sample solution*.

To the entire volume of a *Sample solution*▲ (USP 1-May-2024) add 20 mL of a solution (1 in 10) 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 7–8 mL remain, then complete the evaporation, removing the last traces of ether without the application of heat.

Immediately dissolve the residue in 5.0 mL of [2,2,4-trimethylpentane](#), ▲ transfer into a sample cell, and record the observed rotation in degrees (°). For *RRR*-isomers, calculate the specific rotation using *c* as the concentration of alpha tocopherol determined in the appropriate Assay. ▲ (USP 1-May-2024)

#### Acceptance criteria

***RRR*-Isomers:** NLT +24°

***all-rac* Forms:** -0.01° to +0.01°

#### Change to read:

- **C.** The retention time of the major peak ▲ (USP 1-May-2024) of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

#### ASSAY

##### • ALPHA TOCOPHEROL

[NOTE—Use low-actinic glassware.]

**Internal standard solution:** 10 mg/mL of squalane in [cyclohexane](#)

**System suitability solution:** 0.1 mg/mL each of [USP Alpha Tocopherol RS](#) and [USP Alpha Tocopheryl Acetate RS](#) in [cyclohexane](#)

**Standard solution:** 10 mg/mL of [USP Alpha Tocopherol RS](#) in *Internal standard solution*

**Sample solution:** 10 mg/mL of Vitamin E (*RRR*- or *all-rac*-alpha tocopherol) in *Internal standard solution*

#### Chromatographic system

(See [Chromatography \(621\)](#), [System Suitability](#).)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 0.25-mm × 30-m fused silica capillary; bonded with a 0.25-μm film of phase [G2](#)

#### Temperatures

**Injection port:** 290°

**Column:** 280°

**Detector:** 290°

**Carrier gas:** Helium

**Flow rate:** 1 mL/min

**Injection type:** Split, split ratio 100:1

**Injection volume:** 1 μL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 3.5 between alpha tocopherol and alpha tocopheryl acetate, *System suitability solution*

**Relative standard deviation:** NMT 2.0% for the peak response ratios of alpha tocopherol to the internal standard from replicate injections, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of *RRR*- or *all-rac*-alpha tocopherol ( $C_{29}H_{50}O_2$ ) in the portion of Vitamin E taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of alpha tocopherol to the internal standard from the *Sample solution*

$R_S$  = peak response ratio of alpha tocopherol to the internal standard from the *Standard solution*

$C_S$  = concentration of [USP Alpha Tocopherol RS](#) in the *Standard solution* (mg/mL)

$C_U$  = concentration of Vitamin E in the *Sample solution* (mg/mL)

**Acceptance criteria:** 96.0%–102.0% of *RRR*- or *all-rac*-alpha tocopherol ( $C_{29}H_{50}O_2$ )

##### • ALPHA TOCOPHERYL ACETATE

[NOTE—Use low-actinic glassware.]

**Internal standard solution, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System**

**suitability:** Proceed as directed in the Assay for *Alpha Tocopherol* except as follows. For the *Standard solution*, *Sample solution*, and *Relative*

standard deviation, substitute alpha tocopheryl acetate for alpha tocopherol, and substitute [USP Alpha Tocopheryl Acetate RS](#) for [USP Alpha Tocopherol RS](#).

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of *RRR*- or *all-rac*-alpha tocopheryl acetate ( $C_{31}H_{52}O_3$ ) in the portion of Vitamin E taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of alpha tocopheryl acetate to the internal standard from the *Sample solution*

$R_S$  = peak response ratio of alpha tocopheryl acetate to the internal standard from the *Standard solution*

$C_S$  = concentration of [USP Alpha Tocopheryl Acetate RS](#) in the *Standard solution* (mg/mL)

$C_U$  = concentration of Vitamin E in the *Sample solution* (mg/mL)

**Acceptance criteria:** 96.0%–102.0% of *RRR*- or *all-rac*-alpha tocopheryl acetate ( $C_{31}H_{52}O_3$ )

#### • ALPHA TOCOPHERYL ACID SUCCINATE

[NOTE—Use low-actinic glassware.]

**Internal standard solution, System suitability solution, Chromatographic system, and System suitability:** Proceed as directed in the Assay for *Alpha Tocopherol* except as follows. For the *Relative standard deviation*, substitute alpha tocopheryl acid succinate for alpha tocopherol.

**Standard solution:** Transfer 30.0 mg of [USP Alpha Tocopheryl Acid Succinate RS](#) into a 20-mL vial. Add 2.0 mL of [methanol](#), 1.0 mL of 2,2-dimethoxypropane, and 0.1 mL of [hydrochloric acid](#) to the vial. Cap tightly and sonicate. Allow to stand in the dark for 1 h ± 5 min. Remove from the dark, uncap, and evaporate just to dryness on a steam bath with the aid of a stream of nitrogen. Add 3.0 mL of the *Internal standard solution* and mix on a vortex mixer to dissolve.

**Sample solution:** Transfer 30.0 mg of Vitamin E (*RRR*- or *all-rac*-alpha tocopheryl acid succinate) into a 20-mL vial. Add 2.0 mL of [methanol](#), 1.0 mL of 2,2-dimethoxypropane, and 0.1 mL of [hydrochloric acid](#) to the vial. Cap tightly and sonicate. Allow to stand in the dark for 1 h ± 5 min. Remove from the dark, uncap, and evaporate just to dryness on a steam bath with the aid of a stream of nitrogen. Add 3.0 mL of the *Internal standard solution* and mix on a vortex mixer to dissolve.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of *RRR*- or *all-rac*-alpha tocopheryl acid succinate ( $C_{33}H_{54}O_5$ ) in the portion of Vitamin E taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of alpha tocopheryl acid succinate to the internal standard from the *Sample solution*

$R_S$  = peak response ratio of alpha tocopheryl acid succinate to the internal standard from the *Standard solution*

$C_S$  = concentration of [USP Alpha Tocopheryl Acid Succinate RS](#) in the *Standard solution* (mg/mL)

$C_U$  = concentration of Vitamin E in the *Sample solution* (mg/mL)

**Acceptance criteria:** 96.0%–102.0% of *RRR*- or *all-rac*-alpha tocopheryl acid succinate ( $C_{33}H_{54}O_5$ )

#### SPECIFIC TESTS

##### • ACIDITY

**Diluent:** [Alcohol](#) and [ether](#) (1:1), neutralized to phenolphthalein with 0.1 N [sodium hydroxide](#)

**Sample:** 1.0 g

**Analysis:** Dissolve the *Sample* in 25 mL of *Diluent*, add 0.5 mL of [phenolphthalein TS](#), and titrate with 0.10 N [sodium hydroxide](#) until the solution remains faintly pink after shaking for 30 s.

**Acceptance criteria:** Alpha tocopheryl acid succinate requires 18.0–19.3 mL of 0.10 N sodium hydroxide; the other forms of Vitamin E require NMT 1.0 mL of 0.10 N sodium hydroxide.

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light. Protect *RRR*- or *all-rac*-alpha tocopherol with a blanket of an inert gas, and store at room temperature.

**Change to read:**

- **LABELING:** Label Vitamin E to indicate the chemical form and to indicate whether it is the *RRR* or the *all-rac* form. ▲Express Vitamin E content in terms of alpha tocopherol equivalent in mg/g.▲ (USP 1-May-2024) <sup>1</sup>
- **USP REFERENCE STANDARDS** (11).
  - [USP Alpha Tocopherol RS](#)
  - [USP Alpha Tocopheryl Acetate RS](#)
  - [USP Alpha Tocopheryl Acid Succinate RS](#)

▲<sup>1</sup> 1 mg of vitamin E (alpha tocopherol) = 1 mg of *RRR*-alpha tocopherol = 2 mg of *all-rac*-alpha tocopherol; 1 mg of *RRR*-alpha tocopheryl acetate = 0.91 mg of alpha tocopherol equivalent; 1 mg of *RRR*-alpha tocopheryl acid succinate = 0.81 mg of alpha-tocopherol equivalent. To convert IU to mg: 1 IU of *RRR*-alpha tocopherol = 0.67 mg of alpha tocopherol; 1 IU of *all-rac*-alpha tocopherol = 0.45 mg of alpha tocopherol.▲  
(USP 1-May-2024)

**Auxiliary Information** - Please [check for your question in the FAQs](#) before contacting USP.

Topic/Question	Contact	Expert Committee
VITAMIN E	<a href="#">Natalia Davydova</a> Scientific Liaison	NBDS2020 Non-botanical Dietary Supplements
REFERENCE STANDARD SUPPORT	RS Technical Services <a href="mailto:RSTECH@usp.org">RSTECH@usp.org</a>	NBDS2020 Non-botanical Dietary Supplements

**Chromatographic Database Information:** [Chromatographic Database](#)

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