Mode: LC
Detector: UV 325 nm
Column: 4.6-mm × 10-cm; 3-µm packing L1
Flow rate: 1.0 mL/min
Injection size: 50 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 1.5 for retinyl acetate and NMT 2.0 for retinyl palmitate
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of retinyl acetate or retinyl palmitate dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S \times V/L) \times 100$$

r_U = peak area of the all-*trans*-retinyl ester from the *Sample solution*
r_S = peak area of the all-*trans*-retinyl ester from the appropriate *Standard solution*
C_S = concentration of retinol in the appropriate *Standard solution* (µg/mL)
V = volume of *Medium*, 900 mL
L = label claim of vitamin A, as retinol (µg/Tablet)
Tolerances: NLT 75% (Q) of the labeled amount of retinyl acetate or retinyl palmitate is dissolved.

- **UNIFORMITY OF DOSAGE UNITS ⟨905⟩:** Meet the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at room temperature. Protect Tablets from light.
- **LABELING:** Label it to indicate the chemical form of vitamin A present, and to indicate the vitamin A activity in terms of the equivalent amount of retinol. Vitamin A activity may be stated also in USP units. USP units of activity for vitamins, where such exist or formerly existed, are equivalent to the corresponding international units, where such formerly existed. International units (IU) for vitamins also have been discontinued; however, the use of IU on the labels of vitamin products continues. Where articles are labeled in terms of Units in addition to the required labeling, the relationship of the USP Units or IU to mass is as follows. One USP Vitamin A Unit = 0.3 µg of all-*trans*-retinol (vitamin A alcohol) or 0.344 µg of all-*trans*-retinyl acetate (vitamin A acetate) or 0.55 µg of all-*trans*-retinyl palmitate (vitamin A palmitate), and 1 µg of retinol (3.3 USP Vitamin A Units) = 1 retinol equivalent (RE).
- **USP REFERENCE STANDARDS ⟨11⟩**
  USP Retinyl Acetate RS
  USP Retinyl Palmitate RS

Vitamin E

DEFINITION

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

IDENTIFICATION

- **A.**
  [NOTE—Use low-actinic glassware.]
  Sample solutions
  Alpha tocopherol: 1 mg/mL in dehydrated alcohol
  Alpha tocopheryl acetate: Transfer 220 mg of *d*- or *dl*-alpha tocopheryl acetate to a round-bottom, glass-stoppered, 150-mL flask, and dissolve in 25 mL of dehydrated alcohol. Add 20 mL of dilute 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 dilute sulfuric acid in alcohol (1 in 72) to volume.
  Alpha tocopheryl acid succinate: 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.
  [**CAUTION—** Wear safety goggles.]
  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 separator, rinsing the flask with 100 mL each of water and of ether, and adding the rinsings to the separator. 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 or 8 mL remain. Complete the evaporation, removing the last traces of ether without the application of heat. Immediately dissolve the residue in dilute 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.*
  Add 2 mL of nitric acid with swirling to 10 mL of *Sample solution*, and heat at about 75° for 15 min.
  Acceptance criteria: A bright red or orange color develops.
- **B. OPTICAL ROTATION ⟨781⟩**
  Sample solutions
  Alpha tocopherol: Dissolve 100 mg of alpha tocopherol in 50 mL of ether.
  Alpha tocopheryl acetate: Transfer a volume of sample solution for *Alpha tocopheryl acetate* from *Identification* test *A*, equivalent to 100 mg of the test article, to a separator, and add 200 mL of water. Extract first with 75 mL, then with 25 mL, of ether, and combine the ether extracts in another separator.
  Alpha tocopheryl acid succinate: Transfer a volume of sample solution for *Alpha tocopheryl acid succinate* from *Identification* test *A*, equivalent to 100 mg of the test article, to a separator, and add 200 mL of water. Extract first with 75 mL, then with 25 mL, of ether, and combine the ether extracts in another separator.
  Analysis
  Sample: Use the appropriate *Sample solution.*
  To the entire volume of a *Sample solution*, as prepared above, 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 isooctane, and determine the optical rotation using as *c* the number of g of total tocopherols, determined in the *Assay*, in each 100 mL of solution employed for the test.

Acceptance criteria

d-**Isomers:** NLT +24°

dl-**Forms:** Show no optical rotation activity

- **C.** The retention time of the major peak for alpha tocopherol 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 (*d*- or *dl*-alpha tocopherol) in *Internal standard solution*

Chromatographic system

(See *Chromatography* ⟨621⟩*, System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.25-mm x 30-m fused silica capillary, bonded with a 0.25-µm film of phase G2

Temperature

Column: 280°

Injection port: 290°

Detector: 290°

Carrier gas: Helium

Flow rate: 1 mL/min

Split ratio: 100:1

Injection size: 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 ratios of alpha tocopherol to internal standard peak responses from replicate injections, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of *d*- or *dl*-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 = ratio of alpha tocopherol peak response to internal standard peak response from the *Sample solution*

R_S = ratio of alpha tocopherol peak response to internal standard peak response 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 *d*- or *dl*-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*. For the *Standard solution*, *Sample solution*, and *Relative standard deviation*, substitute alpha tocopheryl acetate for alpha tocopherol and USP Alpha Tocopheryl Acetate RS for USP Alpha Tocopherol RS.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of *d*- or *dl*-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 = ratio of alpha tocopheryl acetate peak response to internal standard peak response from the *Sample solution*

R_S = ratio of alpha tocopheryl acetate peak response to internal standard peak response 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 *d*- or *dl*-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*. 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 *Internal standard solution*, and mix on a vortex mixer to dissolve.

Sample solution: Transfer 30.0 mg of Vitamin E (*d*- or *dl*-alpha tocoopheryl 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 *Internal standard solution*, and mix on a vortex mixer to dissolve.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of *d*- or *dl*-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 = ratio of alpha tocopheryl acid succinate peak response to internal standard peak response from the *Sample solution*

R_S = ratio of alpha tocopheryl acid succinate peak response to internal standard peak response 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 *d*- or *dl*-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: 40 mg

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 *d*- or *dl*-alpha tocopherol with a blanket of an inert gas.
- **LABELING:** Label Vitamin E to indicate the chemical form and to indicate whether it is the *d*- or the *dl*-form. The Vitamin E activity may be expressed in terms of the equivalent amount of *d*-alpha tocopherol, in mg/g, based on the following relationship between the former USP Units[1] (equal to the former International Units) and mass.
- **USP REFERENCE STANDARDS** ⟨11⟩
  USP Alpha Tocopherol RS
  USP Alpha Tocopheryl Acetate RS
  USP Alpha Tocopheryl Acid Succinate RS

Vitamin E Capsules

DEFINITION

Vitamin E Capsules contain Vitamin E or Vitamin E Preparation, where Vitamin E is a form of alpha tocopherol ($C_{29}H_{50}O_2$) that includes *d*- or *dl*-alpha tocopherol ($C_{29}H_{50}O_2$), *d*- or *dl*-alpha tocopheryl acetate ($C_{31}H_{52}O_3$), and *d*- or *dl*-alpha tocopheryl acid succinate ($C_{33}H_{54}O_5$); and Vitamin E Preparation is a combination of a single form of Vitamin E with one or more inert substances. Vitamin E Capsules contain NLT 95.0% and NMT 120.0% of the labeled amount of Vitamin E.

IDENTIFICATION

- **A.**

  [NOTE—Use low-actinic glassware.]

  Sample solutions

  Alpha tocopherol: Solution with a nominal concentration of 1 mg/mL in dehydrated alcohol

  Alpha tocopheryl acetate: Transfer 220 mg of *d*- or *dl*-alpha tocopheryl acetate from the Capsule contents to a round-bottomed, glass-stoppered, 150-mL flask, and dissolve in 25 mL of dehydrated alcohol. Add 20 mL of dilute 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 dilute sulfuric acid in alcohol (1 in 72) to volume.

  Alpha tocopheryl acid succinate: [**CAUTION—** Wear safety goggles.] Transfer an amount of Capsule contents, equivalent to 200 mg of alpha tocopherol, to a round-bottomed, 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 separator, rinsing the flask with 100 mL each of water and of ether, and adding the rinsings to the separator. 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 dilute sulfuric acid in alcohol (1 in 72), transfer to a 200-mL volumetric flask, and dilute with dilute sulfuric acid in alcohol (1 in 72) to volume.

  Analysis

  Sample: Use the appropriate *Sample solution*.

  Add 2 mL of nitric acid with swirling to 10 mL of *Sample solution*, and heat at about 75° for 15 min.

  Acceptance criteria: A bright red or orange color develops.

- **B. OPTICAL ROTATION** ⟨781⟩

  Sample solutions

  Alpha tocopherol: Dissolve an amount of the sample equivalent to 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* test *A*, equivalent to 100 mg of the test article, to a separator, and add 200 mL of water. Extract with ether, first with 75 mL, then with 25 mL; and combine the ether extracts in another separator.

  Alpha tocopheryl acid succinate: Transfer a volume of the *Sample solution* for *Alpha tocopheryl acid succinate* from *Identification* test *A*, equivalent to 100 mg of the test article, to a separator, and add 200 mL of water. Extract with ether, first with 75 mL, then with 25 mL; and combine the ether extracts in another separator.

  Analysis

  Sample: Use the appropriate *Sample solution*.

  To the entire volume of a *Sample solution*, as prepared above, 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 isooctane, and determine the optical rotation using as *c* the number of g of total tocopherols, determined in the *Assay*, in each 100 mL of solution used for the test.

  Acceptance criteria

  For Capsules labeled to contain *d*-isomers: NLT +24°

  For Capsules labeled to contain *dl*-forms: Show essentially no optical rotation

- **C.** The retention time of the major peak for alpha tocopherol of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

Change to read:

- **ALPHA TOCOPHEROL**

  ▲[NOTE—Use low-actinic glassware.]

  Internal standard solution: 10 mg/mL of squalane in cyclohexane

[1] In terms of USP Units, 1 mg of *dl*-alpha tocopherol = 1.1 former USP Vitamin E Units; 1 mg of *dl*-alpha tocopheryl acetate = 1 former USP Vitamin E unit; 1 mg of *dl*-alpha tocopheryl acid succinate = 0.89 former USP Vitamin E Unit; 1 mg of *d*-alpha tocopherol = 1.49 former USP Vitamin E Units; 1 mg of *d*-alpha tocopheryl acetate = 1.36 former USP Vitamin E Units; and 1 mg of *d*-alpha tocopheryl acid succinate = 1.21 former USP Vitamin E Units. In terms of *d*-alpha tocopherol equivalents, 1 mg of *d*-alpha tocopheryl acetate = 0.91; 1 mg of *d*-alpha tocopheryl acid succinate = 0.81; 1 mg of *dl*-alpha tocopherol = 0.74; 1 mg of *dl*-alpha tocopheryl acetate = 0.67; and 1 mg of *dl*-alpha tocopheryl acid succinate = 0.60.