⟨551⟩ VITAMIN E ASSAY

INTRODUCTION

The following liquid chromatographic procedures are provided for the determination of vitamin E as an active pharmaceutical ingredient, as a dietary supplement ingredient, or as a component in compendial dosage forms in the forms of alpha tocopherol ($C_{29}H_{50}O_2$), alpha tocopheryl acetate ($C_{31}H_{52}O_3$), or alpha tocopheryl acid succinate ($C_{33}H_{54}O_5$).

Throughout this assay, protect solutions containing, and derived from, the test specimen and the Reference Standard from the atmosphere and light, preferably by the use of a blanket of inert gas and low-actinic glassware.

Where vitamin E (alpha tocopherol, alpha tocopheryl acetate, or alpha tocopheryl acid succinate) is specified in the following procedure, use the chemical form present in the formulation and the relevant USP Reference Standard.

ASSAY

• **PROCEDURE 1**

- This procedure can be used to determine vitamin E in:
  - Oil-Soluble Vitamins Tablets
  - Oil-Soluble Vitamins Capsules
  - Oil-Soluble Vitamins with Minerals Tablets
  - Oil-Soluble Vitamins with Minerals Capsules
  - Oil- and Water-Soluble Vitamins Tablets
  - Oil- and Water-Soluble Vitamins Capsules
  - Oil- and Water-Soluble Vitamins with Minerals Tablets
  - Oil- and Water-Soluble Vitamins with Minerals Capsules
- This is a neutral procedure that involves the use of dimethyl sulfoxide to dissolve the excipients in the sample, followed by a liquid–liquid extraction of vitamin E with hexane. The hexane extract is then evaporated in vacuum to dryness, and the residue is reconstituted in methanol prior injection into the chromatograph.
- Unless specified in the individual monographs, the *System suitability solution*, *Standard solution*, *Sample solutions*, and reagent solutions are prepared as follows.

Solution A: Phosphoric acid solution (1 in 100) in water

Mobile phase: Methanol and *Solution A* (19:1)

System suitability solution: Prepare a 0.65-mg/mL solution of USP Ergocalciferol RS in methanol. Transfer 1.0 mL of this solution to a 100-mL volumetric flask containing 100 mg of USP Alpha Tocopheryl Acetate RS. Dissolve in 30 mL of methanol, with the aid of sonication if necessary, and dilute with methanol to volume. Store this solution in a refrigerator.

Standard solution: 2 mg/mL of USP Alpha Tocopherol RS, USP Alpha Tocopheryl Acetate RS, or USP Alpha Tocopheryl Acid Succinate RS in methanol

Sample solution for Tablets: Finely powder NLT 20 Tablets. Transfer a portion of the powder typically equivalent to 20 mg of the vitamin E form under testing but not exceeding 7.5 g of the powder, to a centrifuge tube having a polytef-lined screw cap. Add about 2 mL of dimethyl sulfoxide per each g of powdered Tablets, and about 3 mL of *n*-hexane each per g of powdered Tablets, and shake for 45 min on a shaker in a water bath maintained at 60°. [NOTE—Set up the shaker to ensure that the contents of the container are mixed vigorously and thoroughly.] Centrifuge at 3000 rpm for 10 min, and transfer the hexane layer by means of a pipet to a volumetric flask. [NOTE—Volumetric flask size: NLT 20 mL.] Add 3 mL of *n*-hexane per each g of powdered Tablets to the dimethyl sulfoxide layer, shake thoroughly for 5 min, and transfer the hexane layer by means of a pipet to the same volumetric flask. Repeat this extraction with three additional portions of *n*-hexane. Dilute the extracts in the volumetric flask with *n*-hexane to volume. Transfer NLT 20 mL of this solution to a suitable container, and evaporate in vacuum at room temperature to dryness. Transfer the residue with the aid of methanol to a suitable volumetric flask, and dilute with methanol to volume to obtain a concentration of 2 mg/mL of alpha tocopherol, alpha tocopheryl acetate, or alpha tocopheryl acid succinate.

Sample solution for Capsules: Transfer the contents of NLT 20 Capsules to a suitable container, mix, and weigh. Transfer a portion of the mixture, typically equivalent to 20 mg of the vitamin E form under testing but not exceeding 7.5 g of the mixture, to a centrifuge tube having a polytef-lined screw cap. [NOTE—For hard gelatin Capsules, remove, as completely as possible, the contents of NLT 20 Capsules by cutting open the Capsule shells, transferring the shells and their contents to a suitable container, and triturating to a homogeneous mass. Transfer a portion of the mass, typically equivalent to 20 mg of the vitamin E form under testing but not exceeding 7.5 g of the mixture to a centrifuge tube having a polytef-lined screw cap.] Add about 2 mL of dimethyl sulfoxide per each g of Capsule contents, and about 3 mL of *n*-hexane per each g of Capsule contents, and shake for 45 min on a shaker in a water bath maintained at 60°. [NOTE—Set up the shaker to ensure that the contents of the container are mixed vigorously and thoroughly.] Centrifuge at 3000 rpm for 10 min, and transfer the hexane layer by means of a pipet to a volumetric flask. [NOTE—Volumetric flask size: NLT 20 mL.] Add 3 mL of *n*-hexane per each g of Capsule contents to the dimethyl sulfoxide layer, shake thoroughly for 5 min, and transfer the hexane layer by means of a pipet to the same volumetric flask. Repeat this extraction with three additional portions of *n*-hexane. Dilute the extracts in the volumetric flask with *n*-hexane to volume. Transfer NLT 20 mL of this solution to a suitable container, and evaporate in vacuum at room temperature to dryness. Transfer the residue with the aid of methanol to a suitable volumetric flask, and dilute with methanol to volume to obtain a concentration of 2 mg/mL of alpha tocopherol, alpha tocopheryl acetate, or alpha tocopheryl acid succinate.

Chromatographic system

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

Mode: LC

Detector: UV 254 nm

Column: 8-mm × 10-cm; 5-µm packing L1

Flow rate: 2 mL/min
Injection volume: 100 µL

System suitability

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

[NOTE—The relative retention times for ergocalciferol and alpha tocopheryl acetate are about 0.5 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 12 between ergocalciferol and alpha tocopheryl acetate, *System suitability solution*
Tailing factor: 0.8–1.2, *System suitability solution*
Relative standard deviation: NMT 3.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of alpha tocopherol, alpha tocopheryl acetate, or alpha tocopheryl acid succinate in the portion of the sample taken:

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

r_U = peak response of the relevant vitamin E form from the *Sample solution*
r_S = peak response of the relevant vitamin E form from the *Standard solution*
C_S = concentration of the relevant vitamin E form of the corresponding USP Reference Standard in the *Standard solution* (mg/mL)
C_U = nominal concentration of the corresponding form of vitamin E in the *Sample solution* (mg/mL)

- **PROCEDURE 2**
  - This procedure can be used for the formulations containing vitamins A, D, and E. Application includes:
    - Oil-Soluble Vitamins Tablets
    - Oil-Soluble Vitamins Capsules
    - Oil-Soluble Vitamins with Minerals Tablets
    - Oil-Soluble Vitamins with Minerals Capsules
    - Oil- and Water-Soluble Vitamins Tablets
    - Oil- and Water-Soluble Vitamins Capsules
    - Oil- and Water-Soluble Vitamins with Minerals Tablets
    - Oil- and Water-Soluble Vitamins with Minerals Capsules
  - It involves the treatment of sample with methanolic sulfuric acid, followed by extraction with 2,2,4-trimethylpentane.
  - Unless specified in the individual monographs, the *System suitability solution*, *Standard solution*, *Sample solutions*, and reagent solutions are prepared as follows.

Mobile phase: Mix 240 mL of methanol with 10 mL of water, followed by 0.5 mL of 50% phosphoric acid, and dilute with acetonitrile to 1000 mL.

3 N methanolic sulfuric acid solution: Cautiously add 9 mL of sulfuric acid to 80 mL of methanol in a 100-mL volumetric flask. Cool, and dilute with methanol to volume.

Sodium ascorbate–pyrogallol solution: Transfer 10 g of sodium ascorbate and 5 g of pyrogallol to a 100-mL volumetric flask, and add sufficient water to dissolve. Add 1.7 mL of sulfuric acid, and dilute with water to volume.

Lecithin solution: 5 mg/mL of lecithin in 2,2,4-trimethylpentane

System suitability solution: 2 mg/mL each of USP Alpha Tocopherol RS, USP Alpha Tocopheryl Acetate RS, and USP Alpha Tocopheryl Acid Succinate RS in methanol

Standard solution: 2 mg/mL of USP Alpha Tocopherol RS, USP Alpha Tocopheryl Acetate RS, or USP Alpha Tocopheryl Acid Succinate RS in methanol

Sample solution for Tablets: [NOTE—This preparation is suitable for the determination of vitamin A, vitamin D, and vitamin E when present in the formulation. The sample amount may be adjusted depending on the presence or absence of the appropriate vitamins.] Finely powder NLT 20 Tablets. Use a portion of the powder nominally equivalent to an amount 90 mg of alpha tocopherol, alpha tocopheryl acetate, or alpha tocopheryl acid succinate. Add 0.5 g of sodium bicarbonate, 1.5 mL of *Lecithin solution*, and 12.5 mL of 2,2,4-trimethylpentane, and disperse on a vortex mixer. Add 6 mL of *Sodium ascorbate–pyrogallol solution*, shake slowly, and allow the solution to degas. Continue shaking until the evolution of gas has ceased, and then shake for an additional 12 min. Add 6 mL of dimethyl sulfoxide, mix on a vortex mixer to form a suspension, and shake for 12 min. Add 6 mL of *3 N methanolic sulfuric acid solution*, mix on a vortex mixer to form a suspension, and shake for 12 min. Add 12.5 mL of 2,2,4-trimethylpentane, mix on a vortex mixer to form a suspension, and shake for 10 min. Centrifuge for 10 min to break up the emulsion and to clarify the supernatant. Transfer a volume of the supernatant 2,2,4-trimethylpentane layer to a suitable volumetric flask, the volume of the specimen withdrawn from the 2,2,4-trimethylpentane layer and the size of the volumetric flask being such that the final concentration of the *Sample solution* is equivalent to that of the *Standard solution*. Evaporate nearly to dryness, add several mL of methanol, and evaporate the remaining 2,2,4-trimethylpentane. Dilute with methanol to volume.

Sample solution for Capsules: [NOTE—This preparation is suitable for the determination of vitamin A, vitamin D, and vitamin E when present in the formulation. The sample amount may be adjusted depending on the presence or absence of the appropriate vitamins.] Weigh NLT 20 Capsules in a tared weighing bottle. Using a sharp blade if necessary, carefully open the Capsules, without loss of shell material, and transfer the contents to a 100-mL beaker. Remove any contents adhering to the empty shells by washing with several portions of ether. Discard the washings, and dry the Capsule shells with the aid of a current of dry air. Weigh the empty Capsule shells in the tared weighing bottle, and calculate the net

weight of the Capsule contents. Transfer a portion of the Capsule contents, equivalent to 55 mg of vitamin E, to a container having a polytef-lined screw cap. Add 0.5 g of sodium bicarbonate, 1.5 mL of *Lecithin solution*, and 12.5 mL of 2,2,4-trimethylpentane, and disperse on a vortex mixer. Add 6 mL of *Sodium ascorbate–pyrogallol solution*, shake slowly, and allow the solution to degas. Continue shaking until the evolution of gas has ceased, and then shake for an additional 12 min. Add 6 mL of dimethyl sulfoxide, mix on a vortex mixer to form a suspension, and shake for 12 min. Add 6 mL of *3 N methanolic sulfuric acid solution*, mix on a vortex mixer to form a suspension, and shake for 12 min. Add 12.5 mL of 2,2,4-trimethylpentane, mix on a vortex mixer to form a suspension, and shake for 10 min. Centrifuge for 10 min to break up the emulsion and to clarify the supernatant layer. Transfer a volume of the supernatant 2,2,4-trimethylpentane layer to a suitable volumetric flask, the volume of the specimen withdrawn from the 2,2,4-trimethylpentane layer and the size of the volumetric flask being such that the final concentration of the *Sample solution* is equivalent to that of the *Standard solution*. Evaporate nearly to dryness, add several mL of methanol, and evaporate the remaining 2,2,4-trimethylpentane. Dilute with methanol to volume.

Chromatographic system

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

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1.5 mL/min

Injection volume: 25 µL

System suitability

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

[NOTE—The relative retention times for alpha tocopheryl acid succinate, alpha tocopherol, and alpha tocopheryl acetate are about 0.6, 0.8, and 1.0, respectively]

Suitability requirements

Resolution: NLT 4.0 between alpha tocopheryl acid succinate and alpha tocopherol; NLT 3.0 between alpha tocopherol and alpha tocopheryl acetate, *System suitability solution*

Relative standard deviation: NMT 3.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of alpha tocopherol, alpha tocopheryl acetate, alpha tocopheryl acid succinate in the portion of the sample taken:

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

r_U = peak response of the relevant vitamin E form from the *Sample solution*

r_S = peak response of the relevant vitamin E form from the *Standard solution*

C_S = concentration of the relevant vitamin E form of the corresponding USP Reference Standard in the *Standard solution* (mg/mL)

C_U = nominal concentration of the corresponding form of vitamin E in the *Sample solution* (mg/mL)

[NOTE—Account for the initial extraction volume of 26.5 mL of 2,2,4-trimethylpentane and the dilution factor to exchange the solvent from 2,2,4-trimethylpentane to methanol to calculate the nominal concentration.]

- **PROCEDURE 3**
  - This procedure can be used for the determination of vitamin E in:
    - Oil-Soluble Vitamins Tablets
    - Oil-Soluble Vitamins Capsules
    - Oil-Soluble Vitamins with Minerals Tablets
    - Oil-Soluble Vitamins with Minerals Capsules
    - Oil- and Water-Soluble Vitamins Tablets
    - Oil- and Water-Soluble Vitamins Capsules
    - Oil- and Water-Soluble Vitamins with Minerals Tablets
    - Oil- and Water-Soluble Vitamins with Minerals Capsules
    - Oil- and Water-Soluble Vitamins Oral Solution
    - Oil- and Water-Soluble Vitamins with Minerals Oral Solution
  - It involves the saponification of the sample, followed by a liquid–liquid extraction of vitamin E from the sample with *n*-hexane. Evaporate the hexane extract to dryness, and reconstitute the residue in a mixture of acetonitrile and ethyl acetate (1:1).
  - Unless specified in the individual monographs, the *Standard solution*, *Sample solutions*, and reagent solutions are prepared as follows.

Mobile phase: Methanol, acetonitrile, and *n*-hexane (46.5: 46.5: 7.0)

Diluent: Acetonitrile and ethyl acetate (1:1)

Potassium hydroxide solution: [NOTE—Used for the Oral Solution sample.] Transfer 90 g of potassium hydroxide pellets to a 100-mL volumetric flask containing 60 mL of water. Mix to dissolve, cool, and dilute with water to volume.

Standard solution: 0.3 mg/mL of USP Alpha Tocopherol RS in *Diluent*

Sample solution for Tablets: Finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 8 mg of alpha tocopherol, to a 125-mL flask fitted with a ground-glass joint. Add 25.0 mL of water, 25.0 mL of dehydrated alcohol, and 3.5 g of potassium hydroxide pellets. Shake for 1 h in a water bath maintained at 55°. Cool, and transfer with the aid of a

minimum volume of water to a 125-mL separatory funnel. Rinse the flask with 50 mL of *n*-hexane, and add the rinsing to the separatory funnel. Insert the stopper, shake vigorously for 60 s, and allow the layers to separate. Drain the aqueous layer into a second 250-mL separatory funnel, and repeat the extraction with 50 mL of *n*-hexane. Discard the aqueous layer, and combine the hexane extracts. Wash the combined extracts with 25 mL of water, allow the layers to separate, and discard the aqueous layer. Add 3 drops of glacial acetic acid, and repeat the washing procedure two more times. Filter the washed hexane layer through anhydrous sodium sulfate into a 250-mL round-bottom flask. Rinse the funnel and sodium sulfate with a few mL of *n*-hexane, and add the rinsing to the hexane solution in the flask. Place the flask in a water bath maintained at 50°, and evaporate the hexane solution with the aid of a rotary evaporator to dryness. Immediately add 25.0 mL of *Diluent*, and swirl to dissolve the residue.

Sample solution for Capsules: Weigh NLT 20 Capsules in a tared weighing bottle. Open the Capsules, without loss of shell material, and transfer the contents to a 100-mL beaker. Remove any contents adhering to the empty shells by washing with several portions of ether. Discard the washings, and dry the Capsule shells with the aid of a current of dry air. Weigh the empty Capsule shells in the tared weighing bottle, and calculate the net weight of the Capsule contents. Transfer a portion of the Capsule contents, equivalent to an amount of 8.0 mg of alpha tocopherol, to a glass-stoppered conical flask. Add 25.0 mL of water, 25.0 mL of dehydrated alcohol, and 3.5 g of potassium hydroxide pellets. Shake for 1 h in a water bath maintained at 55°. Cool, and transfer with the aid of a minimum volume of water to a 125-mL separatory funnel. Rinse the flask with 50 mL of *n*-hexane, and add the rinsing to the separatory funnel. Insert the stopper, shake vigorously for 60 s, and allow the layers to separate. Drain the aqueous layer into a second 250-mL separatory funnel, and repeat the extraction with 50 mL of *n*-hexane. Discard the aqueous layer, and combine the hexane extracts. Wash the combined extracts with 25 mL of water, allow the layers to separate, and discard the aqueous layer. Add 3 drops of glacial acetic acid, and repeat the washing procedure two more times. Filter the washed hexane layer through anhydrous sodium sulfate into a 250-mL round-bottom flask. Rinse the funnel and sodium sulfate with a few mL of *n*-hexane, and add the rinsing to the hexane solution in the flask. Place the flask in a water bath maintained at 50°, and evaporate the hexane solution with the aid of a rotary evaporator to dryness. Immediately add 25.0 mL of *Diluent*, and swirl to dissolve the residue.

Sample solution for Oral Solution: Transfer an amount of Oral Solution equivalent to 1.5 mg of alpha tocopherol to a 125-mL conical flask fitted with a ground-glass joint, and add 25.0 mL of dehydrated alcohol. Attach a reflux condenser, and reflux in a boiling water bath for 1 min. Cautiously add 3 mL of *Potassium hydroxide solution* through the condenser, and continue to reflux for 30 min. Remove the flask from the bath, and rinse the condenser with about 15 mL of water. Cool, and transfer with a minimum volume of water to a 250-mL separatory funnel. Rinse the flask with 50 mL of *n*-hexane, and add the rinsings to the separatory funnel. Insert the stopper, shake vigorously for 1 min, and allow the layers to separate. Drain the aqueous layer into a second 250-mL separatory funnel, and repeat the extraction with 50 mL of *n*-hexane. Discard the aqueous layer, and combine the hexane extracts. Wash the combined extracts with 25 mL of water, allow the layers to separate, and discard the aqueous layer. Add 3 drops of glacial acetic acid, and repeat the washing procedure two more times. Filter the washed hexane layer through anhydrous sodium sulfate into a 250-mL round-bottom flask. Rinse the funnel and sodium sulfate with *n*-hexane, and add the rinsing to the hexane solution in the flask. Evaporate the hexane solution to dryness with the aid of a rotary evaporator over a water bath maintained at about 50°. Immediately add 5.0 mL of *Diluent*, and swirl to dissolve the residue.

Chromatographic system

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

Mode: LC

Detector: UV 291 nm

Column: 4.6-mm × 25-cm; packing L1

Column temperature: 40°

Flow rate: 3 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 0.5%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of vitamin E, as alpha tocopherol, in the portion of the sample taken:

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

r_U = peak response of alpha tocopherol from the *Sample solution*

r_S = peak response of alpha tocopherol from the *Standard solution*

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

C_U = nominal concentration of vitamin E, as alpha tocopherol, in the *Sample solution* (mg/mL)

[NOTE—Calculate the alpha tocopherol equivalent of alpha tocopheryl acetate, or alpha tocopheryl acid succinate by multiplying their contents by the factors 0.91 or 0.81, respectively.]

- **PROCEDURE 4**
  - This gas chromatographic procedure is provided for the determination of vitamin E as an active pharmaceutical ingredient, as a dietary supplement ingredient, or as a component in compendial dosage forms. It can be used for:
    - Vitamin E
    - Vitamin E Capsules

- Vitamin E Preparation
- Unless specified in the individual monographs, the *Standard solutions*, *Sample solutions*, and reagent solutions are prepared and used appropriately as follows.

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 1: 10 mg/mL of USP Alpha Tocopherol RS in *Internal standard solution*

Standard solution 2: 10 mg/mL of USP Alpha Tocopheryl Acetate RS in *Internal standard solution*

Standard solution 3: 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 solutions for Active Pharmaceutical Ingredient

Sample solution 1 (vitamin E as alpha tocopherol or alpha tocopheryl acetate)**:** 10 mg/mL of Vitamin E (*d*- or *dl*-alpha tocopherol or *d*- or *dl*-alpha tocopheryl acetate) in *Internal standard solution*

Sample solution 2 (vitamin E as alpha tocopheryl acid succinate)**:** Transfer 30.0 mg of Vitamin E (*d*- or *dl*-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 *Internal standard solution*, and mix on a vortex mixer to dissolve.

Sample solutions for Vitamin E Preparation

Sample solution 1 (vitamin E as alpha tocopherol or alpha tocopheryl acetate in liquid form)**:** Dissolve a portion of Preparation in *Internal standard solution* to prepare a Vitamin E (*d*- or *dl*-alpha tocopherol or *d*- or *dl*-alpha tocopheryl acetate) solution with nominal concentration of 10 mg/mL.

Sample solution 2 (vitamin E as alpha tocopheryl acid succinate in liquid form)**:** Transfer a portion of Preparation, equivalent to 30.0 mg of Vitamin E (*d*- or *dl*-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 *Internal standard solution*, and mix on a vortex mixer to dissolve.

Sample solution 3 (vitamin E as alpha tocopherol or alpha tocopheryl acetate in solid form)**:** Transfer a portion of Preparation, equivalent to 50 mg of alpha tocopherol or alpha tocopheryl acetate, into a flask suitable for refluxing. Add 5 mL of water, and heat on a water bath at 60° for 10 min. Add 25 mL of alcohol, and reflux for 30 min. Cool, and transfer to a separator with the aid of 50 mL of water and 50 mL of ether. Shake vigorously, allow the layers to separate, and collect each layer in individual separators. Extract the aqueous layer with two 25-mL portions of ether, combining the extracts with the original ether layer. Wash the combined extract with one 25-mL portion of water, filter the ether solutions through 1 g of anhydrous sodium sulfate, and with the aid of a stream of nitrogen evaporate the ether solution on a water bath, controlled at a temperature that will not cause the ether solution to boil over. Remove the container from the water bath when 5 mL remains, and complete the evaporation without the application of heat. Dissolve the residue in *Internal standard solution* to prepare a Vitamin E (*d*- or *dl*-alpha tocopherol or *d*- or *dl*-alpha tocopheryl acetate) solution with nominal concentration of 10 mg/mL.

Sample solution 4 (vitamin E as alpha tocopheryl acid succinate in solid form)**:** Transfer a portion of Preparation, equivalent to 30 mg of Vitamin E (*d*- or *dl*-alpha tocopheryl acid succinate), into a flask suitable for refluxing. Add 5 mL of water, and heat on a water bath at 60° for 10 min. Add 25 mL of alcohol, and reflux for 30 min. Cool, and transfer to a separator with the aid of 50 mL of water and 50 mL of ether. Shake vigorously, allow the layers to separate, and collect each layer in individual separators. Extract the aqueous layer with two 25-mL portions of ether, combining the extracts with the original ether layer. Wash the combined extract with one 25-mL portion of water, filter the ether solutions through 1 g of anhydrous sodium sulfate, and with the aid of a stream of nitrogen evaporate the ether solution on a water bath, controlled at a temperature that will not cause the ether solution to boil over. Remove the container from the water bath when 5 mL remains. Quantitatively transfer the remains into a 20-mL vial, and complete the evaporation without the application of heat. 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 solutions for Vitamin E Capsules

Sample solution 1 (vitamin E as alpha tocopherol or alpha tocopheryl acetate)**:** Weigh NLT 10 Capsules in a tared weighing bottle. With a sharp knife or by other appropriate means, carefully open the Capsules, without loss of the shell material, and transfer the combined Capsule content to a 100-mL beaker. Remove any adhering substance from the emptied Capsules by washing with several small portions of *n*-hexane. Discard the washings, and allow the empty Capsules to dry in a current of dry air until the odor of *n*-hexane is no longer perceptible. Weigh the empty Capsules in the original tared weighing bottle, and calculate the average net weight per Capsule. Dissolve a portion of the combined Capsule contents in *Internal standard solution* to prepare a Vitamin E (*d*- or *dl*-alpha tocopherol or *d*- or *dl*-alpha tocopheryl acetate) solution with a nominal concentration of 10 mg/mL.

Sample solution 2 (vitamin E as alpha tocopheryl acid succinate)**:** Weigh NLT 10 Capsules in a tared weighing bottle. With a sharp knife or by other appropriate means, carefully open the Capsules, without loss of the shell material, and transfer the combined Capsule content to a 100-mL beaker. Remove any adhering substance from the emptied Capsules by washing with several small portions of *n*-hexane. Discard the washings, and allow the empty Capsules to dry in a current of dry air until the odor of *n*-hexane is no longer perceptible. Weigh the empty Capsules in the original tared weighing bottle, and calculate the average net weight per Capsule. Transfer a portion of the combined Capsule contents, equivalent to 30.0 mg of Vitamin E (*d*- or *dl*-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 *Internal standard solution*, and mix on a vortex mixer to dissolve.

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
Column: 280°
Injection port: 290°
Detector: 290°
Carrier gas: Helium
Flow rate: 1 mL/min
Split ratio: 100:1
Injection volume: 1 µL

System suitability
Samples: *System suitability solution* and appropriate *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 relevant vitamin E form to internal standard peak responses from replicate injections, appropriate *Standard solution*

Analysis
Samples: Appropriate *Standard solution* and appropriate *Sample solution*
Calculate the percentage of vitamin E in terms of alpha tocopherol, alpha tocopheryl acetate, or alpha tocopheryl acid succinate in the portion of the sample taken:

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

R_U = internal standard ratio (peak response of relevant vitamin E form/peak response of the internal standard) from the appropriate *Sample solution*
R_S = internal standard ratio (peak response of relevant vitamin E form/peak response of the internal standard) from the appropriate *Standard solution*
C_S = concentration of the corresponding USP Reference Standard in the appropriate *Standard solution* (mg/mL)
C_U = nominal concentration of the corresponding form of vitamin E in the appropriate *Sample solution* (mg/mL)

- **PROCEDURE 5**
  - This gas chromatographic procedure is provided for the determination of Vitamin E (as *d*- or *dl*-alpha tocopherol) in:
    - Vitamin E Polyethylene Glycol Succinate (Vitamin E polyethylene glycol succinate is a mixture formed by the esterification of *d*-alpha tocopheryl acid succinate and polyethylene glycol).
  - Unless specified in the individual monographs, the *Standard solutions*, *Sample solutions*, and reagent solutions are prepared as follows.

Solvent: 0.25 mL of phenolphthalein TS in 1 L of alcohol
Internal standard solution: 12 mg/mL of ethyl arachidate in isooctane
Standard solution: Transfer 32.5 mg of USP Alpha Tocopherol RS to a suitable reaction flask. Add 2 mL of pyridine and 0.5 mL of *N,O*-bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane, and heat the flask at 100° for 10 min. Cool the flask, add 5.0 mL of *Internal standard solution* followed by 20 mL of isooctane, and shake.
Sample solution: Transfer a quantity equivalent to 0.100–0.160 g of Vitamin E Polyethylene Glycol Succinate molten at 60° to a culture tube (about 20 cm long and 2.5 cm in diameter) equipped with a screw cap. Add 40–50 mg of ascorbic acid and a few boiling chips, followed by 20 mL of *Solvent*. [NOTE—Reflux the solution gently without emission of contents. Place the tube in a heating block set at 100°–150°.] When the sample is fully dissolved, add 0.25 g of potassium hydroxide, and continue to reflux for 30 min. Remove the tube from heat, and while contents are still hot, add 1–2 mL of hydrochloric acid dropwise until the pink coloration disappears. [**CAUTION—**Exothermic reaction. Allow the acid to trickle down the inside of the tube to prevent splashing.] Cool the tube, then wash the sides of the tube with 20 mL of water. Add 5.0 mL of *Internal standard solution*, cap, and shake to ensure thorough mixing. Allow the tube to stand until two distinct layers are formed. Transfer 2.5–3.5 mL of the upper layer into a suitable reaction flask, and add 2.0 mL of pyridine followed by 2.5 mL of *N,O*-bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane. Heat the flask at 100° for 10 min. Cool, and then add 12 mL of isooctane.

Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: GC
Detector: Flame ionization
Column: 0.25-mm × 15-m fused-silica capillary, bonded with a 0.25-µm film of phase G27
Temperatures
Injection port: 280°
Detector: 345°
Column: See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
260	20	340	1

Carrier gas: Helium
Flow rate: 1.5 mL/min
Split ratio: 200:1
Injection volume: 1 µL

System suitability
Sample: *Standard solution*
Suitability requirements
Tailing factor: NMT 2.0 for the alpha tocopherol peak
Relative standard deviation: NMT 2.0% for the ratio of the alpha tocopherol peak response to the internal standard peak response from replicate injections

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of vitamin E as *d*-alpha tocopherol in the portion of the sample taken:

$$\text{Result} = (R_U/R_S) \times (W_S/W_U) \times 100$$

R_U = internal standard ratio (peak response of alpha tocopherol/peak response of the internal standard) from the *Sample solution*
R_S = internal standard ratio (peak response of alpha tocopherol/peak response of the internal standard) from the *Standard solution*
W_S = weight of USP Alpha Tocopherol RS used to prepare the *Standard solution* (mg)
W_U = weight of Vitamin E Polyethylene Glycol Succinate taken to prepare the *Sample solution* (mg)

ADDITIONAL REQUIREMENTS

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Alpha Tocopherol RS
USP Alpha Tocopheryl Acetate RS
USP Alpha Tocopheryl Acid Succinate RS
USP Ergocalciferol RS