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Vitamin E Preparation

DEFINITION

Vitamin E Preparation is a combination of a single form of Vitamin E with one or more inert substances. It may be in a liquid or solid form. It contains NLT 95.0% and NMT 120.0% of the labeled amount of vitamin E. Vitamin E Preparation labeled to contain an *all-rac* form of Vitamin E also may contain a small amount of a *RRR* form of Vitamin E, occurring as a minor constituent of an added substance.

IDENTIFICATION

• **A.**

[Note—Use low-actinic glassware.]

Sample solution: [**Caution—**Wear safety goggles.] Transfer an amount of Vitamin E Preparation, 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 of ether, and adding the rinsings to the funnel. Shake vigorously, allow the layers to separate, and collect each of the two layers in individual separatory funnels. 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 dehydrated alcohol, transfer to a 250-mL volumetric flask, and dilute with dehydrated alcohol to volume.

Analysis: 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 solution: A volume of the *Sample solution* from *Identification A*, equivalent to 100 mg of Vitamin E Preparation

Analysis: Transfer the *Sample solution* 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. To the combined ether extracts, 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 about 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

For Vitamin E Preparation labeled to contain *RRR*-isomers: NLT +24°

For Vitamin E Preparation labeled to contain *all-rac* forms: -0.01° to +0.01°

• **C.** The retention time of the major peak of the *Sample solution* corresponds 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

For Vitamin E Preparation in liquid form: Dissolve a portion of Vitamin E Preparation in the *Internal standard solution* to prepare a vitamin E (*RRR*- or *all-rac*-alpha tocopherol) solution with a nominal concentration of 10 mg/mL.

For Vitamin E Preparation in solid form: Transfer a portion of Vitamin E Preparation, equivalent to 50 mg of alpha tocopherol, 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 separatory funnel 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 separatory funnels. 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 remain and complete the evaporation without the application of heat. Dissolve the residue in the *Internal standard solution* to prepare a vitamin E (*RRR*- or *all-rac*-alpha tocopherol) solution with a nominal concentration of 10 mg/mL.

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 the labeled amount of vitamin E (*RRR*- or *all-rac*-alpha tocopherol) in the portion of Vitamin E Preparation 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 = nominal concentration of vitamin E as *RRR*- or *all-rac*-alpha tocopherol in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–120.0% of the labeled amount of vitamin E as *RRR*- or *all-rac*-alpha tocopherol ($C_{29}H_{50}O_2$)

• **Alpha Tocopheryl Acetate:** Proceed as directed in the *Assay* for *Alpha Tocopherol* except as follows. For the *Standard solution* and *Sample solution*, substitute alpha tocopheryl acetate for alpha tocopherol, and substitute USP Alpha Tocopheryl Acetate RS for USP Alpha Tocopherol RS.

Acceptance criteria: 95.0%–120.0% of the labeled amount of vitamin E as *RRR*- or *all-rac*-alpha tocopheryl acetate ($C_{31}H_{52}O_3$)

• **Alpha Tocopheryl Acid Succinate**

Internal standard solution, System suitability solution, Chromatographic system, System suitability, and **Analysis:** Proceed as directed in the *Assay* 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

For Vitamin E Preparation in liquid form: Transfer a portion of Vitamin E Preparation, equivalent to 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.

For Vitamin E Preparation in solid form: Transfer a portion of Vitamin E Preparation, equivalent to 30 mg of vitamin E (*RRR*- or *all-rac*-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 separatory funnel 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 separatory funnels. 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 remain. 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 the *Internal standard solution* and mix on a vortex mixer to dissolve.

Acceptance criteria: 95.0%–120.0% of the labeled amount of vitamin E as *RRR*- or *all-rac*-alpha tocopheryl acid succinate ($C_{33}H_{54}O_5$)

SPECIFIC TESTS

• **Acidity** (for Vitamin E Preparation in liquid form)

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

Sample solution: Dissolve 1 g of Vitamin E Preparation in 25 mL of *Diluent*.

Analysis: To 25 mL of the *Sample solution* 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: NMT 1.0 mL of 0.10 N sodium hydroxide is required.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers, protected from light. Protect Vitamin E Preparation containing *RRR*- or *all-rac*-alpha tocopherol with a blanket of inert gas.

Change to read:

• **Labeling:** Label it to indicate the chemical form of vitamin E present, and to indicate whether the *RRR*- or *all-rac* form is present, excluding any different forms that may be introduced as a minor constituent of the vehicle. ▲Express Vitamin E content in terms of alpha tocopherol equivalent in mg/g.[1]▲ (USP 1-May-2024)

• **USP Reference Standards** ⟨11⟩

USP Alpha Tocopherol RS
USP Alpha Tocopheryl Acetate RS
USP Alpha Tocopheryl Acid Succinate RS

[1] 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.

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
VITAMIN E PREPARATION	Natalia Davydova Scientific Liaison	NBDS2020 Non-botanical Dietary Supplements
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	NBDS2020 Non-botanical Dietary Supplements

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