Vitamin E

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 (C31H52O3); d- or dl-alpha tocopheryl acid succinate ($C_{33}H_{54}O_5$). It contains not less than 96.0 percent and not more than 102.0 percent of $C_{29}H_{50}O_2$, $C_{31}H_{52}O_3$, or $C_{33}H_{54}O_5$, respectively.

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 per g, based on the following relationship between the former USP Units (equal to the former International Units) and mass.[*]

Identification—

Test solution for alpha tocopheryl acetate— [note—Use low-actinic glassware.] Transfer about 220 mg of d- or dl-alpha tocopheryl acetate, accurately weighed, 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 hours, protected from sunlight. Cool, transfer to a 200-mL volumetric flask, add dilute sulfuric acid in alcohol (1 in 72) to volume, and mix.

Test solution for alpha tocopheryl acid succinate— [note—Use low-actinic glassware.] Transfer an accurately weighed amount of the sample, equivalent to about 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 minute. 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 minutes 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, dilute with the alcoholic sulfuric acid to volume, and mix.

A: Prepare a solution in dehydrated alcohol containing 10 mg of unesterified alpha tocopherol in 10 mL, or use 10 mL of Test solution for alpha tocopheryl acetate or of Test solution for alpha tocopheryl acid succinate. Add, with swirling, 2 mL of nitric acid, and heat at about 75 for 15 minutes: a bright red or orange color develops.

B: Prepare a solution of about 100 mg, accurately weighed, of unesterified alpha tocopherol in 50 mL of ether, or in the case of esterified d-tocopherols, transfer an accurately measured volume of Test solution for alpha tocopheryl acetate or of Test solution for alpha tocopheryl acid succinate, equivalent to about 100 mg of the test specimen, 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. To the ether solution of unesterified or hydrolyzed alpha tocopherol, add 20 mL of a 1 in 10 solution of potassium ferricyanide in sodium hydroxide solution (1 in 125), and shake for 3 minutes. 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, 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. Calculate the specific rotation (see Optical Rotation 781), using as c the number of g of total tocopherols, determined in the Assay, in each 100 mL of solution employed for the test: the d-isomers have a specific rotation of not less than +24 . The dl-forms show essentially no optical rotation.

C: The retention time of the major peak 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.

Acidity— Dissolve 1.0 g of the test specimen in 25 mL of a mixture of equal volumes of alcohol and ether (which has been neutralized to phenolphthalein with 0.1 N sodium hydroxide), 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 seconds: alpha tocopheryl acid succinate requires between 18.0 and 19.3 mL of 0.10 N sodium hydroxide; the other forms of Vitamin E require not more than 1.0 mL of 0.10 N sodium hydroxide.

Assay for alpha tocopherol—

Internal standard solution— Dissolve an accurately weighed quantity of hexadecyl hexadecanoate in n-hexane to obtain a solution having a known concentration of about 1 mg per mL.

Standard preparation— [note—Use low-actinic glassware.] Dissolve in Internal standard solution a suitable quantity of USP Alpha Tocopherol RS, accurately weighed, to obtain a solution having a known concentration of about 1 mg of the Reference Standard in each mL.

Assay preparation— [note—Use low-actinic glassware.] Transfer about 50 mg of Vitamin E (d- or dl-alpha tocopherol), accurately weighed, to a 50-mL volumetric flask, dissolve in Internal standard solution, dilute with Internal standard solution to volume, and mix.

Chromatographic system (see Chromatography 621)—Under typical conditions, the instrument is equipped with a flame-ionization detector and contains a 4-mm × 2-m borosilicate glass column packed with 2% to 5% liquid phase G2 on 80- to 100-mesh support S1AB utilizing either a glass-lined sample introduction system or on-column injection. The column is maintained isothermally at a temperature between 245 and 265 , and the injection port and detector block are maintained at about 10 higher than the column temperature; the flow rate of dry carrier gas is adjusted to obtain a hexadecyl hexadecanoate peak approximately 18 to 20 minutes after sample introduction when a 2% column is used, or 30 to 32 minutes when a 5% column is used.

[note—Cure and condition the column as necessary (see Chromatography 621).]

Interference check— Dissolve an accurately weighed quantity of the specimen in n-hexane to obtain a solution having a known concentration of about 1 mg per mL. Chromatograph an accurately measured volume of this solution to obtain a chromatogram in which the principal peak exhibits not less than 50% of maximum recorder response. Similarly chromatograph an accurately measured volume of Internal standard solution. If a peak observed in the chromatogram for the specimen has the same retention time as that for hexadecyl hexadecanoate, make any necessary correction for factors of dilution or attenuation, and determine the area due to the interfering

component that must be subtracted from the area of the internal standard peak appearing in the chromatogram recorded for the Assay preparation as directed for Procedure.

System suitability— Chromatograph a sufficient number of injections of a mixture, in n-hexane, of 1 mg per mL each of USP Alpha Tocopherol RS and USP Alpha Tocopheryl Acetate RS as directed for Procedure to ensure that the resolution factor, R (see Chromatography 621), is not less than 1.0.

Calibration— Chromatograph a portion of the Standard preparation, and record peak areas as directed under Procedure. Calculate the relative response factor, F, for the Standard preparation taken by the formula:

$$(A_S / A_D)(C_D / C_S)$$

in which C_D and C_S are the concentrations, in mg per mL, of hexadecyl hexadecanoate and of USP Alpha Tocopherol RS, respectively, in the Standard preparation. Successively chromatograph a sufficient number of portions of the Standard preparation to ensure that the relative response factor, F, is constant within a range of 2.0%.

Procedure— Inject a suitable portion (2 to 5 µL) of the Assay preparation into a suitable gas chromatograph, and record the chromatogram so as to obtain at least 50% of maximum recorder response. Measure the areas under the first (alpha tocopherol) and second major (hexadecyl hexadecanoate) peaks, record the values as a_U and a_D, respectively. Calculate the quantity, in mg, of alpha tocopherol in the Vitamin E taken by the formula:

$$(50C_D / F)(a_U / a_D)$$

in which C_D is the concentration, in mg per mL, of hexadecyl hexadecanoate in the Standard preparation; and F is the relative response factor (see Calibration).