

(wavelength: 284 nm).

Column: A stainless steel column about 4 mm in inside diameter and 15 to 30 cm in length, packed with octadecylsilanized silica gel (5 to 10 μm in particle diameter).

Column temperature: Room temperature.

Mobile phase: A mixture of methanol and water (49:1).

Flow rate: Adjust the flow rate so that the retention time of tocopherol acetate is about 12 minutes.

Selection of column: Dissolve 50 mg each of Tocopherol Acetate and tocopherol in 50 mL of ethanol (99.5). Proceed with 20 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of tocopherol and tocopherol acetate in this order with the resolution between these peaks being not less than 2.6.

System repeatability: Repeat the test five times with the standard solution under the above operating conditions: the relative standard deviation of the peak height of tocopherol acetate is not more than 0.8%.

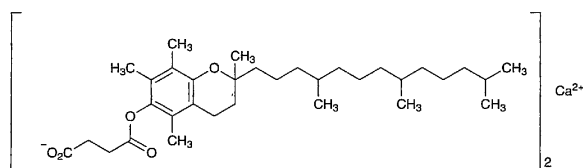
Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Tocopherol Calcium Succinate

Vitamin E Calcium Succinate

コハク酸トコフェロールカルシウム



$\text{C}_{66}\text{H}_{106}\text{CaO}_{10}$: 1099.62

Monocalcium bis{3-[2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)chroman-6-yloxy]propanoate} [14638-18-7]

Tocopherol Calcium Succinate, when dried, contains not less than 96.0% and not more than 102.0% of $\text{C}_{66}\text{H}_{106}\text{CaO}_{10}$.

Description Tocopherol Calcium Succinate occurs as a white to yellowish white powder. It is odorless.

It is freely soluble in chloroform and in carbon tetrachloride, and practically insoluble in water, in ethanol (95) and in acetone.

Shake 1 g of Tocopherol Calcium Succinate with 7 mL of acetic acid (100): it dissolves, and produces a turbidity after being allowed to stand for a while.

It dissolves in acetic acid (100).

It is optically inactive.

Identification (1) Dissolve 0.05 g of Tocopherol Calcium Succinate in 1 mL of glacial acetic acid, add 9 mL of ethanol (99.5), and mix. To this solution add 2 mL of fuming nitric acid, and heat at 75°C for 15 minutes: a red to orange color develops.

(2) Dissolve 0.08 g of Tocopherol Calcium Succinate, previously dried, in 0.2 mL of carbon tetrachloride. Deter-

mine the infrared absorption spectrum of the solution as directed in the liquid film method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 5 g of Tocopherol Calcium Succinate in 30 mL of chloroform, add 10 mL of hydrochloric acid, shake for 10 minutes, then draw off the water layer, and neutralize with ammonia TS: the solution responds to the Qualitative Tests for calcium salt.

Absorbance $E_{1\text{cm}}^{1\%}$ (286 nm): 36.0–40.0 (0.01 g, chloroform, 100 mL).

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Tocopherol Calcium Succinate in 10 mL of chloroform: the solution is clear, and has no more color than the following control solution.

Control solution: To 0.5 mL of Ferric Chloride Colorimetric Stock Solution add 0.5 mol/L hydrochloric acid TS to make 100 mL.

(2) Alkali—To 0.20 g of Tocopherol Calcium Succinate add 10 mL of diethyl ether, 2 mL of water, 1 drop of phenolphthalein TS and 0.10 mL of 0.1 mol/L hydrochloric acid VS, and shake: no red color develops in the water layer.

(3) Chloride—Dissolve 0.10 g of Tocopherol Calcium Succinate in 4 mL of acetic acid (100), add 20 mL of water and 50 mL of diethyl ether, shake thoroughly, and collect the water layer. To the diethyl ether layer add 10 mL of water, shake, and collect the water layer. Combine the water layers, add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution in the same manner using 0.60 mL of 0.01 mol/L hydrochloric acid VS in place of Tocopherol Calcium Succinate (not more than 0.212%).

(4) Heavy metals—Proceed with 1.0 g of Tocopherol Calcium Succinate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(5) Arsenic—Prepare the test solution with 1.0 g of Tocopherol Calcium Succinate according to Method 3, and perform the test using Apparatus B (not more than 2 ppm).

(6) α -Tocopherol—Dissolve 0.10 g of Tocopherol Calcium Succinate in exactly 10 mL of chloroform, and use this solution as the sample solution. Separately, dissolve 0.050 g of Tocopherol Reference Standard in chloroform to make exactly 100 mL. Pipet 1 mL of this solution, add chloroform to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under the Thin-layer Chromatography. Spot 10 μL each of the sample solution and the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of toluene and acetic acid (100) (19:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of iron (III) chloride hexahydrate in ethanol (99.5) (1 in 500) on the plate, then spray evenly a solution of α - α' -dipyridyl in ethanol (99.5) (1 in 200) on the same plate, and allow to stand for 2 to 3 minutes: the spots from the sample solution corresponding to the spots from the standard solution is not larger than and not more intense than the spots from the standard solution.

Loss on drying Not more than 2.0% (1 g, in vacuum, phosphorus (V) oxide, 24 hours).

Assay Weigh accurately about 0.05 g each of Tocopherol Calcium Succinate and Tocopherol Succinate Reference Standard, previously dried, dissolve in a mixture of ethanol (99.5) and diluted acetic acid (100) (1 in 5) (9:1) to make exactly 50 mL, and use these solutions as the sample solution and the standard solution. Pipet 20 μ L each of the sample solution and the standard solution, and perform the test as directed under the Liquid Chromatography according to the following operating conditions. Determine the peak heights, H_T and H_S , of tocopherol succinate in these solutions, respectively.

$$\begin{aligned} & \text{Amount (mg) } C_{66}H_{106}CaO_{10} \\ & = \text{amount (mg) of Tocopherol Succinate} \\ & \quad \text{Reference Standard} \\ & \quad \times \frac{H_T}{H_S} \times \frac{1099.6}{1061.6} \end{aligned}$$

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 284 nm).

Column: A stainless steel column about 4 mm in inside diameter and 15 to 30 cm in length, packed with octadecylsilylanized silica gel (5 to 10 μ L in particle diameter).

Column temperature: Room temperature.

Mobile phase: A mixture of methanol, water and acetic acid (100) (97:2:1).

Flow rate: Adjust the flow rate so that the retention time of tocopherol succinate is about 8 minutes.

Selection of column: Dissolve 0.05 g each of tocopherol succinate and tocopherol in 50 mL of a mixture of ethanol (99.5) and diluted acetic acid (100) (1 in 5) (9:1). Proceed with 20 μ L of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of tocopherol succinate and tocopherol in this order with the resolution between these peaks being not less than 2.0.

System repeatability: Repeat the test five times with the standard solution under the above operating conditions: the relative standard deviation of the peak height of tocopherol succinate is not more than 0.8%.

Containers and storage Containers—Tight containers.

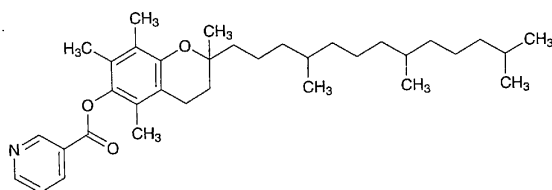
Storage—Light-resistant.

Tocopherol Nicotinate

Vitamin E Nicotinate

dl- α -Tocopherol Nicotinate

ニコチン酸トコフェロール



$C_{35}H_{53}NO_3$: 535.80

2,5,7,8-Tetramethyl-2-(4,8,12-trimethyltridecyl)chroman-6-yl nicotinate [51898-34-1]

Tocopherol Nicotinate contains not less than 96.0% of nicotinic acid *dl*- α -tocopherol ($C_{35}H_{53}NO_3$).

Description Tocopherol Nicotinate occurs as a yellow to orange-yellow liquid or solid.

It is freely soluble in ethanol (99.5), and practically insoluble in water.

A solution of Tocopherol Nicotinate in ethanol (99.5) (1 in 10) shows no optical rotation.

It is affected by light.

Identification (1) Determine the absorption spectrum of a solution of Tocopherol Nicotinate in ethanol (99.5) (1 in 20,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Tocopherol Nicotinate Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectrum of Tocopherol Nicotinate, if necessary melt by warming, as directed in the liquid film method under the Infrared Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of Tocopherol Nicotinate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Heavy metals—Proceed with 1.0 g of Tocopherol Nicotinate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic—Prepare the test solution with 1.0 g of Tocopherol Nicotinate according to Method 4, and perform the test using Apparatus B (not more than 2 ppm).

(3) Related substances—Dissolve 0.05 g of Tocopherol Nicotinate in 50 mL of ethanol (99.5), and use this solution as the sample solution. Pipet 7 mL of this solution, add ethanol (99.5) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with 10 μ L each of the sample solution and the standard solution as directed under the Liquid Chromatography according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than tocopherol nicotinate from the sample solution is not larger than the peak area of tocopherol nicotinate from the standard solution, and the area of a peak which has a retention time 0.8 to 0.9 times that of tocopherol nicotinate from the sample solution is not larger than 4/7 of the peak area of tocopherol nicotinate from the standard solution.

Operating conditions—

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay.

Mobile phase: A mixture of methanol and water (19:1).

Flow rate: Adjust the flow rate so that the retention time of tocopherol nicotinate is about 20 minutes.

Time span of measurement: About 1.5 times as long as the retention time of tocopherol nicotinate after the solvent peak.

System suitability—

Test for required detection: To exactly 1 mL of the sample solution add ethanol (99.5) to make exactly 100 mL, and use this solution as the test solution for system suitability. Pipet 1 mL of the test solution for system suitability, add ethanol (99.5) to make exactly 10 mL. Confirm that the peak