GB

NATIONAL STANDARD OF THE

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National Food Safety Standard - Food Additive - Vitamin E

食品安全国家标准 食品添加剂 维生素 E

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National Food Safety Standard - Food Additive - Vitamin E

1 Application Scope

This standard applies to natural vitamin E extracted from by-products of natural edible vegetable oil or synthetic vitamin E, and products prepared from edible vegetable oil. Vitamin E applicable to this standard includes the following tocopherol types: d-α-tocopherol, dl-α-tocopherol, d-α-tocopheryl acetate, dl-α-tocopheryl acetate, d-α-tocopheryl acid succinate, dl-α-tocopheryl acid succinate, d-α-tocopheryl acetate concentrate and mixed tocopherols concentrate.

2 Chemical Name, Molecular Formula, Structural Formula and Relative Molecular Mass

2.1 Chemical name

d-α-tocopherol: (+) 2,5,7,8-tetramethyl 2-(4,8,12-trimethyltridecyl) 6-chromanone;

dl-α-tocopherol: (±) 2,5,7,8-tetramethyl 2-(4,8,12-trimethyltridecyl) 6-chromanol;

d-α-tocopheryl acetate: (+) 2,5,7,8-tetramethyl 2-(4,8,12-trimethyltridecyl) benzopyran-6-acetate;

dl-α-tocopheryl acetate: (±) 2,5,7,8-tetramethyl 2-(4,8,12-trimethyltridecyl) 6-benzohydropyranol acetate;

d-α-tocopheryl acid succinate: (+) 2,5,7,8-tetramethyl 2-(4,8,12-trimethyltridecyl) chromool 6-succinate;

dl-α-tocopheryl acid succinate: (±) 2,5,7,8-tetramethyl 2-(4,8,12-trimethyltridecyl) chromanol 6-succinate.

2.2 Molecular formula

d-α-tocopherol: $C_{29}H_{50}O_2$

dl-α-tocopherol: $C_{29}H_{50}O_2$

d-α-tocopheryl acetate: $C_{31}H_{52}O_3$

Appendix A

Test Method

A.1 General provisions

Unless otherwise specified in this standard, the purity of reagents used shall be above the grade of analytical reagent; the used standard titration solutions, the standard solutions for the determination of impurities, the preparations and products shall be prepared in accordance with the provisions of GB/T 601, GB/T 602 and GB/T 603; the test water should meet the requirements of grade-3 water specified by GB/T 6682. The solution used in the test, if not indicated which solvent is used, refers to aqueous solution.

A.2 Identification test

A.2.1 Reagents and materials

A.2.1.1 Absolute ethanol.

A.2.1.2 Nitric acid.

A.2.2 Identification method

A.2.2.1 Take about 50 mg of sample; add 10 mL of absolute ethanol to dissolve; shake; add 2 mL of nitric acid; heat at 75°C for 15 min; then the color is shown light red to orange.

A.2.2.2 On the gas chromatogram of content determination, the main peak of the sample solution (mixed tocopherol should be the third main peak) should be consistent with the retention time of the main peak of the reference solution (except solvent peak and internal standard peak).

A.3 Content determination

A.3.1 Reagents and materials

A.3.1.1 Cetyl palmitate.

A.3.1.2 α-tocopherol reference substance.

A.3.1.3 α-tocopheryl acetate reference substance.

A.3.1.4 α-tocopheryl acid succinate reference substance.

A.3.1.5 n-hexane.

Where:

A_{int1} -- internal standard peak area;

$\frac{m_{ref\,1}}{10}$ -- the concentration of α-tocopherol reference substance, in milligrams per milliliter (mg/mL);

A_{ref1} -- α-tocopherol peak area;

ρ_{int1} -- internal standard concentration of the internal standard solution, in milligrams per milliliter (mg/mL).

A.3.3.1.4 Determination

Inject 1 μL of the sample solution into the gas chromatograph; record the chromatogram; calculate the peak area of α-tocopherol, β- and γ-tocopherol, and δ-tocopherol ($A_{\alpha 2}$, $A_{\beta,\gamma 2}$, $A_{\delta 2}$); calculate the sum of the first three items (A_{t2}) and the internal standard peak area A_{int2}; calculate the content according to Formula (A.2) and Formula (A.3):

Total tocopherol content $$w_{T2} = f_1 \times \frac{A_{t2} \times \rho_{int1}}{A_{int2} \times \frac{m_{sample2}}{10}} \times w_{ref} \times 100\% \quad \cdots\cdots (A.2)$$

Relevant content of d-α-tocopherol: $$w_{a3} = \frac{A_{a2}}{A_{t2}} \times 100\% \quad \cdots\cdots (A.3)$$

Where:

f_1 -- correction factor;

A_{t2} -- total peak area of tocopherol peak;

ρ_{int1} -- internal standard solution concentration, in milligrams per milliliter (mg/mL);

A_{int2} -- internal standard peak area;

$\frac{m_{sample2}}{10}$ -- concentration of the sample solution, in milligrams per milliliter (mg/mL);

w_{ref} - content of α-tocopherol reference substance, %.

A_{a2} -- α-tocopherol peak area.

A.3.3.2 Determination of mixed tocopherols concentrate content

$A_{\beta, \gamma 4}$ -- β- and γ-tocopherol peak area;

$A_{\delta 4}$ -- δ-tocopherol peak area.

A.3.3.3 Determination of the content of d-α-tocopheryl acetate and d-α-tocopheryl acetate concentrate

A.3.3.3.1 Solution preparation

The internal standard solution is the same as A.3.3.1.1.

Reference solution: weigh 15 mg of d-α-tocopherol reference substance; accurate to 0.02 mg; place it in a brown volumetric flask; add internal standard solution dilute to 10.0 mL; cover the cork closely; shake to dissolve, then it's done.

Sample solution: weigh an appropriate amount of sample; accurate to 0.02 mg; place it in a brown volumetric flask; add internal standard solution to dilute to 10.0 mL; shake to dissolve to gain a solution containing about d-α-tocopheryl acetate of 1.5 mg/mL.

A.3.3.3.2 Reference chromatographic conditions

SE-30 capillary column; hydrogen flame ion detector; keep the oven temperature at 250°C; the vaporization temperature and detection temperature are 300°C. The carrier gas is nitrogen; adjust the carrier gas flow rate to make the internal standard peak retention time at 18 min ~ 20 min. Inject 1 μL of the reference solution into the chromatograph; record the chromatogram. The theoretical plate number should be larger than 1 500 according to the α-tocopheryl acetate peak; repeat the injections; the relative standard deviation of the correction factor should be no larger than 2.0%. Inject 1 μL of the sample solution into the chromatograph; record the chromatogram. Make the relative retention time of the internal standard peak at 1.0, and the relative retention time of the α-tocopheryl acetate peak is about 0.6.

A.3.3.3.3 Correction factor determination

Inject 1 μL of reference solution into the gas chromatograph; record the chromatogram; measure A_{ref6}, the peak area of the α-tocopheryl acetate peak, and A_{int6}, the internal standard peak area; calculate the correction factor f_6 according to Formula (A.6)

$$f_6 = \frac{A_{int6} \times \frac{m_{ref6}}{10}}{A_{ref6} \times \rho_{int6}} \qquad \cdots\cdots (A.6)$$

Where:

A_{int6} -- internal standard peak area;

Sample solution: weigh 15 g of sample; accurate to 0.02 mg; place it in a brown volumetric flask; add internal standard solution to dilute to 10.0 mL; shake to dissolve to gain the solution.

A.3.3.4.2 Reference chromatographic conditions

SE-30 capillary column; hydrogen flame ion detector; keep the oven temperature at 250°C; the vaporization temperature and detection temperature are 300°C. The carrier gas is nitrogen; adjust the carrier gas flow rate to make the internal standard peak retention time at 18 min ~ 20 min. Inject 1 μL of the reference solution into the chromatograph; record the chromatogram. The theoretical plate number should be larger than 1 500 according to the α-tocopheryl acid succinate peak; repeat the injections; the relative standard deviation of the correction factor should be no larger than 2.0%. Inject 1 μL of the sample solution into the chromatograph; record the chromatogram. Make the relative retention time of the internal standard peak at 1.0, and the relative retention time of the α-tocopheryl acid succinate peak is about 0.6.

A.3.3.4.3 Correction factor determination

Inject 1 μL of reference solution into the gas chromatograph; record the chromatogram; measure A_{ref8}, the peak area of the α-tocopheryl acid succinate, and A_{int8}, the peak area of the internal standard peak; calculate the correction factor f_8 according to Formula (A.8):

$$f_8 = \frac{A_{int8} \times \frac{m_{ref8}}{10}}{A_{ref8} \times \rho_{int8}} \quad \text{.....(A.8)}$$

Where:

A_{int8} -- internal standard peak area;

$\frac{m_{ref8}}{10}$ -- the concentration of α-tocopheryl acid succinate reference substance, in milligrams per milliliter (mg/mL);

A_{ref8} -- α-tocopheryl acid succinate peak area;

ρ_{int8} -- internal standard solution concentration, in milligrams per milliliter (mg/mL).

A.3.3.4.4 Determination

keep for 30 s without fading; record the volume of the sodium hydroxide standard titration solution.

A.5 Specific rotation

A.5.1 Reagents and materials

A.5.1.1 Anhydrous sodium sulfate.

A.5.1.2 Isooctane.

A.5.1.3 Potassium hydroxide.

A.5.1.4 Hydrochloric acid.

A.5.1.5 Sodium hydroxide solution: weigh 1 g of sodium hydroxide; use water to dissolve; add water to dilute to 125 mL.

A.5.1.6 Sodium hydroxide solution containing 10% of potassium ferricyanide: take 10.0 g of potassium ferricyanide; add sodium hydroxide solution (1 → 125); shake to dissolve; dilute to 100 mL;

A.5.1.7 Ethyl sulfate solution 1: Mix concentrated sulfuric acid and ethanol at a volume ratio of 1:6.

2: Mix 1 mol/L of sulfuric acid and ethanol at a volume ratio of 1:71.

A.5.2 Instruments and apparatuses

Use the instrument device specified in GB/T 613.

A.5.3 Analysis steps

A.5.3.1 d-α-tocopherol, mixed tocopherols concentrate

Weigh an appropriate amount of sample (equivalent to about 400 mg of tocopherol in total); accurate to 0.2 mg; set in a separating funnel; use 50 mL of ether to dissolve; add 50 mL of sodium hydroxide solution containing 10% of potassium ferricyanide; shake for 3 min; use water to wash the ether solution for four times, with 50 mL of water each time. Use anhydrous sodium sulfate to dry the ether solution; place the dried diethyl ether solution in a water bath to reduce pressure or under the nitrogen at atmospheric pressure to evaporate and concentrate to about 7 mL ~ 8 mL; then stop heating; let the ether evaporate to dryness. Use 25.0 mL of isooctane to dissolve the residue immediately; at 25°C ± 0.5°C, use the method specified in GB/T 613 to measure the optical rotation; and calculate the specific rotation according to Formula (A.10).

A.5.3.3 d-α-tocopheryl acid succinate

Weigh an appropriate amount of sample (equivalent to about 400 mg of α-tocopherol); accurate to 0.2 mg; place in a 250 mL round bottom flask; add 50 mL of absolute ethanol to dissolve; reflux for 1 min; when the solution is boiled, slowly add 1 g of granular potassium hydroxide from the condenser; avoid overheating; continue to reflux for 20 min; add 2 mL of hydrochloric acid through the condenser; let it cool; transfer the solution to a 500 mL separating funnel; use 100 mL of water and 100 mL of diethyl ether to wash the flask; place the washing solution in a separating funnel; shake vigorously; put it aside for layering; for the aqueous layer, use diethyl ether to extract twice, 50 mL each time; combine the ether extracts; then use water to wash for 4 times, 100 mL each time; place the ether solution in a water bath to reduce pressure or under a nitrogen stream to concentrate the volume to about 7 mL ~ 8 mL; then stop heating; continue to evaporate ether to dryness; immediately add 220 mL of ethyl sulfate solution to dissolve the residue; transfer to a separating funnel; use a small amount of ethyl sulfate solution to wash the container twice; then transfer to the separating funnel; add 200 mL of water; use diethyl ether to extract, for 3 times, for 75 mL the first time, and 25 mL the second and the third times respectively; combine the ether solutions; add 50 mL of sodium hydroxide solution containing 10% of potassium ferricyanide; shake for 3 min; carry out the following procedure in the same manner as in A.5.3.1. Calculate the specific rotation according to Formula (A.12).

$$[\alpha]_{D3}^{25\,℃} = \frac{\alpha}{L \times X \times \frac{m}{25} \times 0.811} \qquad \text{(A.12)}$$

Where:

α -- the measured optical rotation;

L -- length of the measuring tube, in decimeters (dm);

X -- content of tocopheryl acid succinate in the sample, %;

m -- sample quality, in grams (g);

$\frac{m}{25}$ -- concentration of the measuring solution, in grams per milliliter (g/mL);

0.811 -- unit conversion index relative to d-α-tocopherol.

A.6 Determination of absorbance coefficient

A.6.1 Reagents and materials

Absolute ethanol.

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