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Method of Test for Vitamin E in Edible Oils (2)

1. Scope

This method is applicable to the determination of vitamin E including tocopherols and tocotrienols (α-tocopherol, β-tocopherol, γ-tocopherol, δ-tocopherol, α-tocotrienol, β-tocotrienol, γ-tocotrienol, δ-tocotrienol), and α-tocopheryl acetate in edible oils.

2. Method

After extraction, tocopherols and tocotrienols are determined by high performance liquid chromatography (HPLC) coupled with a fluorescence detector (FLD), and α-tocopheryl acetate is determined by HPLC coupled with a tandem mass spectrometer (MS/MS).

2.1. Equipment

2.1.1. Liquid chromatograph/fluorescence detector/tandem mass spectrometer (HPLC-FLD-MS/MS).

2.1.1.1. Fluorescence detector: excitation wavelength, 298 nm; emission wavelength, 345 nm.

2.1.1.2. Tandem mass spectrometer.

2.1.1.2.1. Ion source: electrospray ionization, ESI.

2.1.1.3. Column: InfinityLab Poroshell 120 PFP, 1.9 μm, 3.0 mm i.d. × 10 cm, or an equivalent product.

2.1.1.4. Guard column: InfinityLab Poroshell 120 PFP, 1.9 μm, 3.0 mm i.d. × 5 mm, or an equivalent product.

2.1.2. Nitrogen evaporator.

2.1.3. Vortex mixer.

2.1.4. Solid phase extraction vacuum manifolds.

2.2. Chemicals

Formic acid, reagent grade;

Dibutyl hydroxyl toluene, BHT, reagent grade;

Methanol, HPLC grade;

Dichloromethane, HPLC grade;

n-Hexane, HPLC grade;

Deionized water, resistivity ≥ 18 MΩ・cm (at 25℃);

dl-α-tocopherol, dl-β-tocopherol, dl-γ-tocopherol, dl-δ-tocopherol, d-α-tocotrienol, d-β-tocotrienol, d-γ-tocotrienol, d-δ-tocotrienol, dl-α-tocopheryl acetate, reference standards.

2.3. Apparatus

2.3.1. Volumetric flask: 5 mL.

2.3.2. Solid phase extraction cartridge: Strata® SI-1 Silica (55 μm, 70 Å), 1 g, 6 mL, or an equivalent product.

2.3.3. Membrane filter: 0.22 μm, PVDF.

2.4. Reagents

2.4.1. 12.5 mg/L BHT in *n*-hexane:

Dissolve and dilute 12.5 mg of BHT with *n*-hexane to 1000 mL.

2.4.2. 12.5 mg/L BHT in *n*-hexane containing 3% dichloromethane:

Dilute 15 mL of dichloromethane with 12.5 mg/L BHT in *n*-hexane to 500 mL.**0

2.5. Mobile phase

2.5.1. Solvent A

Dilute 1 mL of formic acid with deionized water to 1000 mL, and filter with a membrane filter.

2.5.2. Solvent B

Dilute 1 mL of formic acid with methanol to 1000 mL, and filter with a membrane filter.

2.6. Standard solution preparation

2.6.1. Tocopherols and tocotrienols

Transfer about 50 mg of dl-α-tocopherol, dl-β-tocopherol, dl-γ-tocopherol, dl-δ-tocopherol, d-α-tocotrienol, d-β-tocotrienol, d-γ-tocotrienol, d-δ-tocotrienol reference standards accurately weighed to each 5-mL volumetric flask, dissolve and dilute with *n*-hexane to volume as the standard stock solutions. Store in the dark in the freezer. When to use, mix appropriate volume of each standard stock solution, evaporate to dryness by gently flushing with a stream of nitrogen, then dissolve and dilute with methanol to 0.04 ~ 1 μg/mL as the standard solutions.

2.6.2. α-Tocopheryl acetate

Transfer about 50 mg of α-tocopheryl acetate reference standard accurately weighed to a 5-mL volumetric flask, dissolve and dilute with *n*-hexane to volume as the standard stock solution. Store in the dark in the freezer. When to use, transfer appropriate volume of the standard stock solution, evaporate to dryness by gently flushing with a stream of nitrogen, then dissolve and dilute with methanol to 1 μg/mL as the standard solution.

2.7. Sample solution preparation

Transfer about 0.1 g of the well-mixed sample accurately weighed into a flask. Dissolve with 5 mL of 12.5 mg/L BHT in *n*-hexane. Transfer into a solid phase extraction cartridge prerinsed with 6 mL of *n*-hexane, and discard the eluent. Wash the cartridge with 6 mL of 12.5 mg/L BHT in *n*-hexane containing 3% dichloromethane, and discard the eluent. Add 9 mL of dichloromethane to the cartridge, and collect the eluent. Evaporate to dryness by gently flushing with a stream of nitrogen, dissolve and dilute the residue with methanol to 5 mL. Filter with a membrane filter, and dilute 5 times with methanol as the sample solution.

2.8. Standard curves of tocopherols and tocotrienols

Accurately inject 5 μL of the standard solutions into the HPLC-FLD-MS/MS separately, and operate according to the following conditions. Establish the standard curve of each tocopherol and tocotrienol by the peak areas of each tocopherol and tocotrienol vs. the added concentrations.

HPLC-FLD operating conditions(note)

Fluorescence detector: excitation wavelength, 298 nm; emission wavelength, 345 nm.

Column: InfinityLab Poroshell 120 PFP, 1.9 μm, 3.0 mm i.d. × 10 cm.

Guard column: InfinityLab Poroshell 120 PFP, 1.9 μm, 3.0 mm i.d. × 5 mm.

Column temperature: 40℃.

Sample oven temperature: 10℃.

Injection volume: 5 μL.

Mobile phase: a gradient program of solvent A and solvent B is as follows:

Time (min)	A (%)	B (%)
0.0 → 15.5	19 → 19	81 → 81
15.5 → 16.5	19 → 15	81 → 85
16.5 → 21.0	15 → 10	85 → 90
21.0 → 22.0	10 → 5	90 → 95
22.0 → 25.0	5 → 5	95 → 95
25.0 → 25.1	5 → 0	95 → 100
25.1 → 29.0	0 → 0	100 → 100
29.0 → 29.1	0 → 19	100 → 81
29.1 → 30.0	19 → 19	81 → 81

Flow rate: 0.27 mL/min.

2.9. Matrix-matched calibration curve of α-tocopheryl acetate

Take a blank sample, and follow the procedure described in section 2.7 to obtain the eluent from the cartridge. Evaporate to dryness by gently flushing with a stream of nitrogen, dissolve and dilute the residue with methanol to 5 mL. Filter with a membrane filter as the blank sample solution. Take several 0.2 mL of the blank sample solution, add 40-500 μL of the standard solution of α-tocopheryl acetate, dilute with methanol to 1 mL, and mix well as the matrix-matched standard solutions. Operate HPLC-FLD-MS/MS according to the following conditions. Establish the matrix-matched calibration curve of α-tocopheryl acetate by the peak areas of α-tocopheryl acetate vs. the added concentrations in the range of 0.04 ~ 5 μg/mL.

LC-MS/MS operating conditions(note):

Column, guard column, column temperature, sample oven temperature, inject volume, mobile phase, and flow rate are the same as those in section 2.8.

Ionization mode: ESI^+.

Capillary voltage: 3.0 kV.

Ion source temperature：150℃.

Heating temperature：400℃.

Cone gas flow: 50 L/hr.

Desolvation flow: 900 L/hr.

Detection mode: multiple reaction monitoring (MRM). Detection ion pair, cone voltage and collision energy are as follows:

Analyte	Ion pair: Precursor ion (*m/z*) > product ion (*m/z*)	Cone voltage (V)	Collision energy (eV)
α-Tocopheryl acetate	473 > 207*	32	28
	473 > 165	32	20

* Quantitative ion pair.

Note: 1. All the parameters can be adjusted depending on the instruments used if the above conditions are not applicable.

2. The high performance liquid chromatograph coupled with the fluorescence detector and the tandem mass spectrometer may increase background pressure, so it should be paid attention to the background pressure to prevent damage to the fluorescence detector.

2.10. Identification and quantification

Accurately inject 5 μL of the sample solution, the standard solutions of tocopherols and tocotrienols, and the matrix-matched standard solutions of α-tocopheryl acetate into HPLC-FLD-MS/MS separately. Operate according to the conditions in section 2.8 and 2.9. Identify each tocopherol, tocotrienol or α-tocopheryl acetate based on the retention time and the relative ion intensities(note). Calculate the amount of each tocopherol, tocotrienol or α-tocopheryl acetate in the sample by the following formula:

The amount of each tocopherol, tocotrienol or α-tocopheryl acetate in the sample (mg/100 g) = $\frac{C \times V \times D}{W \times 10}$

Where,

C: the concentration of each tocopherol and tocotrienol in the sample solution calculated by the standard curve, or that of α-tocopheryl acetate in the sample solution calculated by the matrix-matched calibration curve (μg/mL)

V: the final make-up volume of the sample (5 mL)

W: the weight of the sample (g)

D: dilution factor (5)

Note: Relative ion intensities are calculated by peak areas of qualitative ions divided by peak areas of quantitative ions (≤ 100%). Maximum permitted tolerances of relative ion intensities by LC-MS/MS are as follows:

Relative ion intensity (%)	Tolerance (%)
> 50	± 20
> 20 ~ 50	± 25
> 10 ~ 20	± 30
≤ 10	± 50

Remark

1. Limits of quantification (LOQs) for tocopherols, tocotrienols and α-tocopheryl acetate are all 1 mg/100 g.
2. Further validation should be performed when interfering compounds appear in samples.

Reference

1. Wong, Y. F., Makahleh, A., Saad, B., Ibrahim, M. N. M., Rahim, A. A. and Brosse N. 2014. UPLC method for the determination of vitamin E homologues and derivatives in vegetable oils, margarines and supplement capsules using pentafluorophenyl column. Talanta 130: 299-306.
2. Grigoriadou, D., Androulaki, A., Psomiadou, E. and Tsimidou, M. Z. 2007. Solid phase extraction in the analysis of squalene and tocopherols in olive oil. Food Chem. 105: 675-680.
3. Qin, C. H., Wu, P. W., Hou, J. H., Lin, N. C., Kao, Y. M., Tseng, S. H. and Wang, D. Y. Simultaneous quantification of vitamin E homologues and its derivative in edible oils. Ann. Rep. Food Drug Res. 10: 22-34.

Reference chromatogram

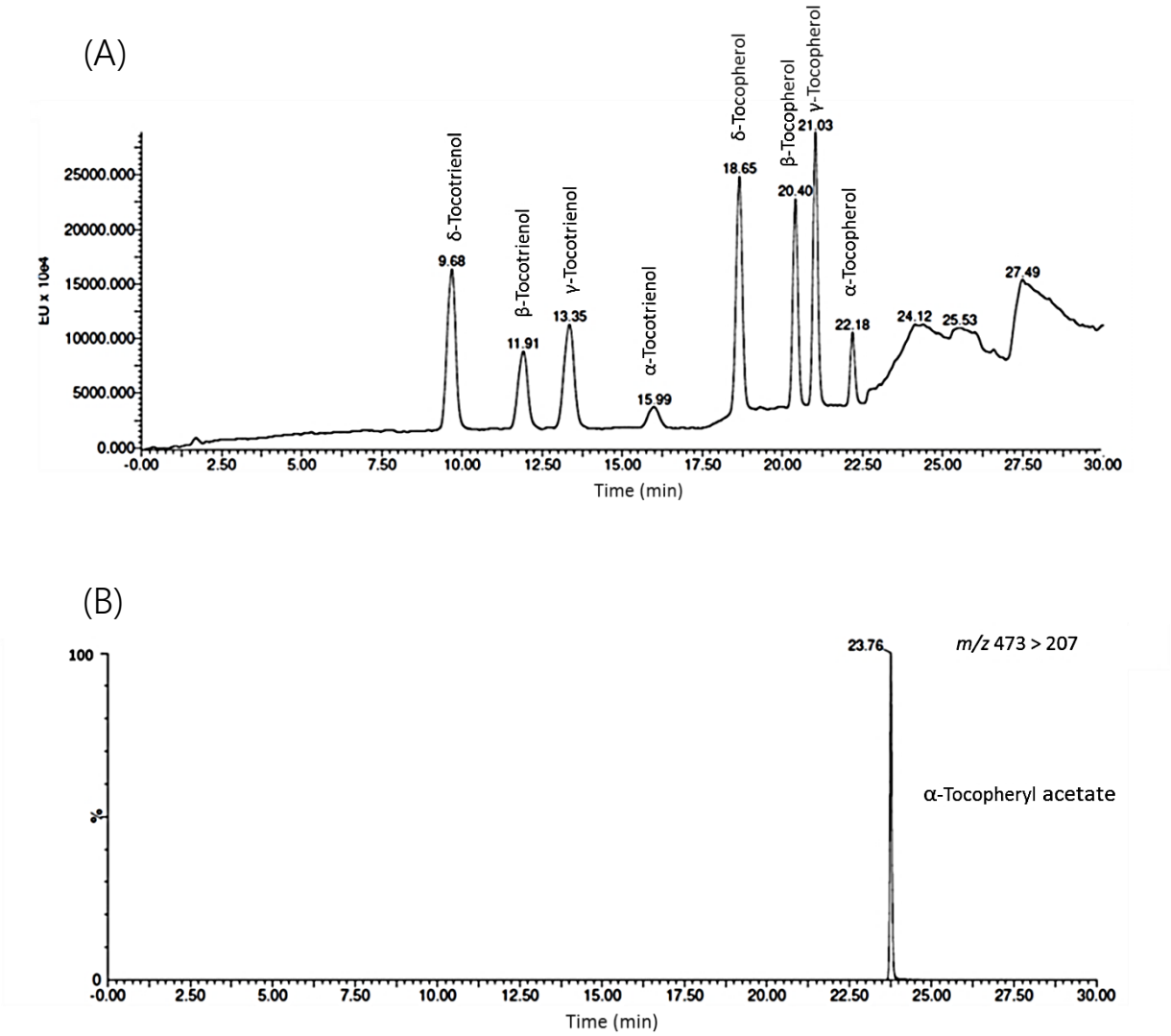

Figure. The HPLC chromatogram of tocopherols and tocotrienols standards (A) and the MRM chromatogram of α-tocopheryl acetate standard (B) analyzed by HPLC-FLD-MS/MS.