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Quantitative Analysis of Tocopherols in Vegetable Oils Using Supercritical Fluid Chromatography

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1. Introduction

Tocopherols are a class of structurally related compounds that partly constitute vitamin E. They are important natural fat-soluble compounds with outstanding antioxidant properties, which assist with neurotransmission, keep the muscles working well, prevent blood clots, and boost immune system performance. Plant-based oils, nuts, seeds, fruits, and vegetables are rich in vitamin E.

CO_2, which is used in supercritical fluid chromatography (SFC), is mainly used to analyze compounds subject to normal phase separation in liquid chromatography (NPLC). In addition, supercritical fluid is unique in terms of diffusivity and viscosity, with diffusivity about 100 times that of liquids, and viscosity approximately 1/10 that of liquids.

Tocopherols consist of four analogous forms, alpha-, beta-, gamma-, and delta-tocopherol, with slight structural differences. Quantitative tocopherol analysis can be performed using NPLC but transferring from LC to SFC reduces the consumption of organic solvents. Furthermore, analysis time can be decreased while maintaining identical peak resolution by increasing the flowrate.

This report introduces examples of the simultaneous quantitative analysis of tocopherols in vegetable oils using the Nexera™ UC, an SFC system.

Figure1. Nexera UC system

2. Materials and Methods

2-1. Analytical Conditions

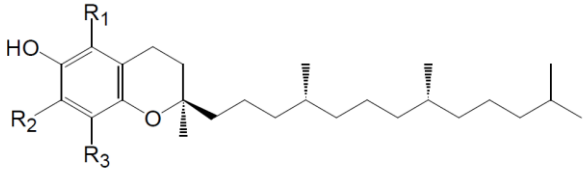
Quantitative analysis of tocopherols was performed by Nexera™ UC system (Shimadzu Corporation, Japan, Figure 1). The analytical condition is described as below.

SFC (Nexera UC system)

Column	Shim-pack™ UC-NH_2 (250 mm x 4.6 mm I.D., 5 µm)
Mobile Phase	A: CO_2
	B: Methanol
Time Program	B conc. 3 % (0 - 4 min) → 50 % (4 - 5 min)
	→50 % (5 - 7 min) → 3 % (7.01 - 9 min)
Flow rate:	5.0 mL/min
Column temp.	40 °C
Injection vol.	20 µL
BPR Pressure	15 MPa
BPR Temp.	50 °C
Detection	295 nm (Photo Diode Array Detector)

2-2. Analysis of a Mixed Standard Solution of Tocopherols

Figure 2 shows the structural formula for tocopherol. There are four forms of tocopherol, α, β, γ, and δ, which differ depending on the number and position of methyl groups in the chromanol ring. Figure 3 shows the chromatogram of a mixed standard solution of tocopherols (containing 50 mg/L of each standard in *n*-hexane.



	R_1	R_2	R_3
α	CH_3	CH_3	CH_3
β	CH_3	H	CH_3
γ	H	CH_3	CH_3
δ	H	H	CH_3

Figure 2. Chemical Structure of Tocopherol

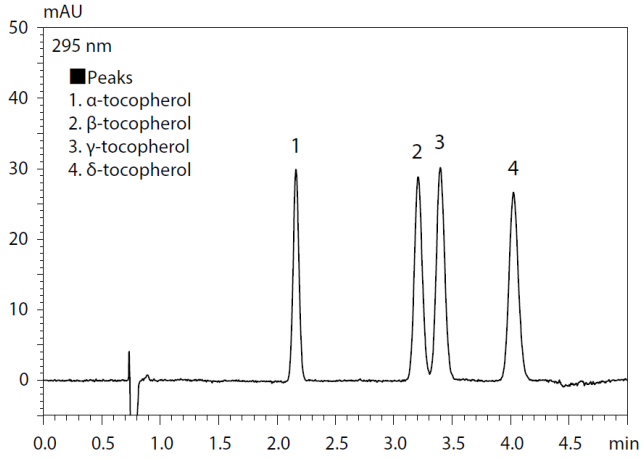


Fig.3 Chromatogram of a Mixed Standard Solution of Tocopherols at 50 mg/L

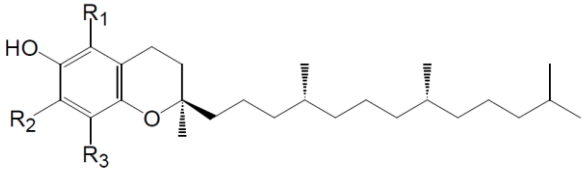
2. Materials and Methods

2-3. Calibration Curves

For each of the tocopherols, a calibration curve was created with the range shown in Table 1. Table 1 shows the calibration ranges adjusted based on the actual content of the respective forms and the linearity of the respective calibration curves. All calibration curves yielded favorable linearity, with coefficients of determination of 0.9995 or higher.

Table 1 Linear range and coefficient of determination (r^2)

Compound	Calibration range (mg/L)	r^2
α-tocopherol	10 - 400	0.9995
β-tocopherol	1 - 20	0.9995
γ-tocopherol	10 - 400	0.9996
δ-tocopherol	2 – 100	0.9995



	R_1	R_2	R_3
α	CH_3	CH_3	CH_3
β	CH_3	H	CH_3
γ	H	CH_3	CH_3
δ	H	H	CH_3

Figure 2. Chemical Structure of Tocopherol

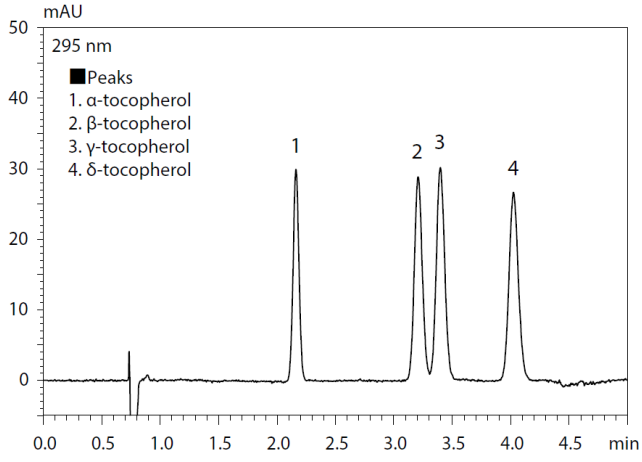


Fig.3 Chromatogram of a Mixed Standard Solution of Tocopherols at 50 mg/L

3. Results

Quantitative analyses of tocopherols were performed using commercially available sunflower oil, olive oil, palm oil, and sesame oil. 500 µL of each oil was diluted with n-hexane to prepare the respective oil samples. The prepared samples were subjected to vortex mixing before SFC analysis.

The chromatograms of the vegetable oils are shown in Figure. 4, 5, 6, and 7. Table 2 shows the concentrations of tocopherols in the vegetable oils calculated at a specific gravity of 0.9 g/cm^3.

Table 2 Concentrations of Tocopherols in the Vegetable Oils

Compound	Concentration (mg/100 g)			
	Sunflower oil	Olive oil	Palm oil	Sesame oil
α-tocopherol	53.4	13.1	8.2	9.6
β-tocopherol	1.3	N.D.	N.D.	1.2
γ-tocopherol	N.D.	N.D.	4.9	32.6
δ-tocopherol	N.D.	N.D.	7.6	13.3

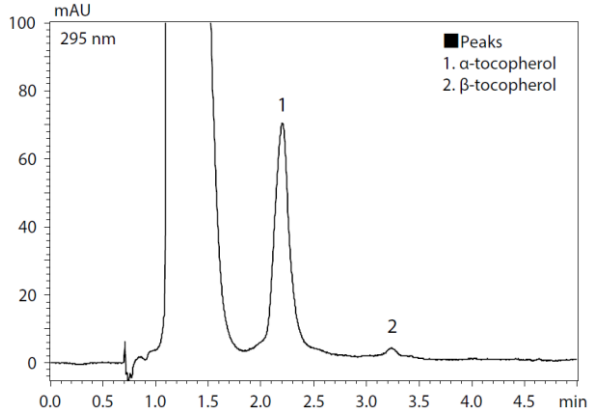


Figure 4. Chromatogram of Sunflower Oil

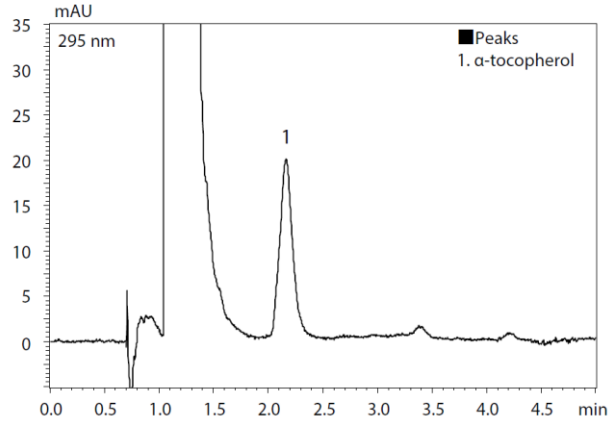


Figure 5. Chromatogram of Olive Oil

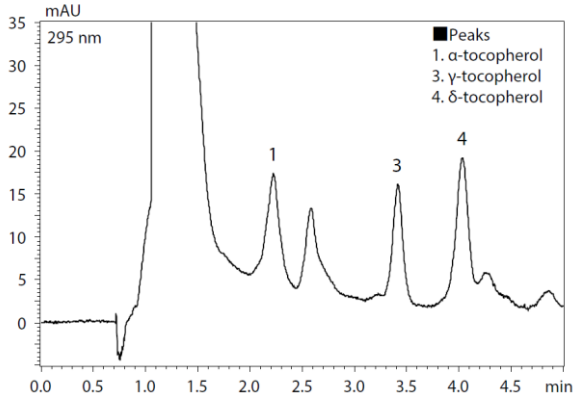


Figure 6. Chromatogram of Palm Oil

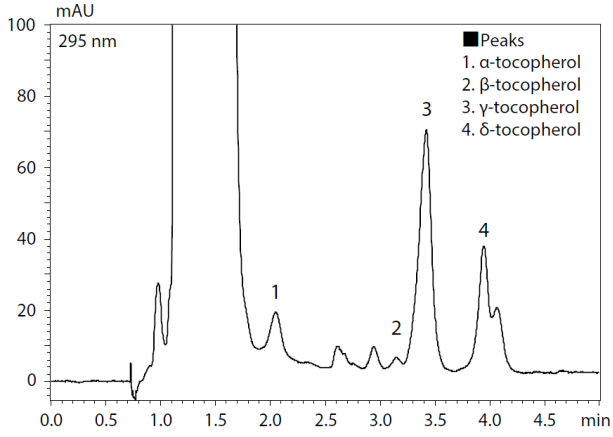


Figure 7. Chromatogram of Sesame Oil

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4. Conclusion

This report introduced examples of the quantitative analysis of tocopherols in vegetable oils using the SFC system. The elution of compounds takes at least ten minutes in tocopherol analysis using NPLC, but only four minutes in SFC analysis. Furthermore, the CO2 used for SFC analysis is less expensive than the n-hexane and other organic solvents used in NPLC, and waste disposal is also cheaper.

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