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Application News

Supercritical Fluid Chromatograph - Nexera™ UC

Development of Vitamin E Analysis in Palm Oil

Kosuke Nakajima[1,2] Mei hui[3]
[1]Shimadzu Corporation, [2]Shimadzu (Asia Pacific) Pte Ltd., [3] Shimadzu Malaysia Sdn. Bhd.

User Benefits

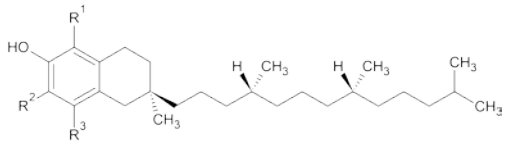
- Significant reduction of analytical time compared with normal phase HPLC analysis.
- Reduction of organic solvent consumption compared with normal phase HPLC analysis.

■ Introduction

Palm oil is made from oil palm and mainly produced in Asia. Palm oil contains various functional natural compounds, which include tocopherol homologues known as Vitamin E. Currently, normal phase HPLC is widely used for quantitative analysis of tocopherol homologues in palm oil. However, this separation mode requires a long time more than 15 min and consumes much organic solvent. Here, we introduce the analysis method for tocopherol in palm oil using Nexera UC, which is Shimadzu supercritical fluid chromatograph (SFC), and compare the analytical time and organic solvents consumptions against conventional HPLC analysis.

■ Analytical method of tocopherol homologues by normal phase HPLC

Fig. 1 shows the chemical structure of tocopherol homologues. Tocopherol has main four types of homologues, and they are required to measure for quality control in palm oil manufacturing. Tocopherol homologues in palm oil are typically analyzed in normal phase HPLC that uses much *n*-hexane. Table 1 and Fig.2 show analytical conditions and chromatogram of tocopherol homologues by normal phase HPLC analysis.



	alpha	beta	gamma	delta
R1	CH3	CH3	H	H
R2	CH3	H	CH3	H
R3	CH3	CH3	CH3	CH3

Fig. 1 Chemical structures of tocopherol homologues

Table 1 Analytical Conditions（HPLC）

Column	: Shim-pack™ CLC-SIL *1 (250 mm × 4.6 mm I.D., 5 µm)
Mobile phase	: *n*-hexane/2-propanol=100:0.5
Flow rate	: 2 mL/mn
Column temp.	: 40℃
Injection Vol.	: 20 µL
Vial	: SHIMADZU LabTotal™ for LC 1.5 mL, Glass *2
Detection	: PDA 292 nm

1* P/N: 228-17872-92 2* P/N: 227-34001-01

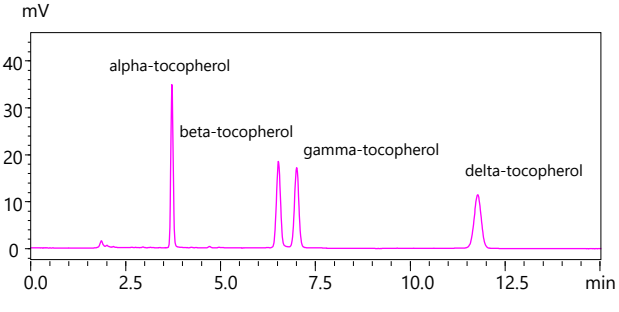


Fig. 2 Chromatogram of tocopherols by normal phase HPLC

■ About Supercritical fluid chromatography

Supercritical Fluid Chromatography (SFC) is one of the chromatography systems with supercritical fluid (i.e., supercritical carbon dioxide) as mobile phase. The low cost of carbon dioxide (purity: 99.9 %) can significantly trim the total running cost of analyses. Generally, SFC shows high separation than typical HPLC and shortens analytical time. Furthermore, SFC can replace normal phase HPLC because supercritical carbon dioxide is similar to *n*-hexane.

■ Method development of analytical conditions of tocopherol homologues by SFC

Table 2 shows the analytical conditions for SFC, and fig 3 shows the acquired chromatogram. We created the calibration curves for alpha-tocopherol in the range from 0.8 mg/L to 500 mg/L. The calibration curves for other tocopherol homologues were created in the range from 0.8 mg/L to 100 mg/L. Repeatability, linearity of the calibration curves, and LOD (limit of detection) are summarized in Table 3.

Table 2 Analytical Conditions（SFC）

Column	: Shim-pack UC-X NH2 *3 (250 mm × 2.1 mm I.D., 5 µm)
Mobile phase	: A: CO2 B: Methanol
Flow rate	: 4 mL/mn
Time program	: B conc. 1.5 %(0-2.5 min)→ 20 % (2.51-2.9 min) →1.5 %(2.91-3.0 min)
Column temp.	: 40℃
Injection Vol.	: 5 µL
Vial	: SHIMADZU LabTotal™ for LC 1.5 mL, Glass *2
BPR setting	: 15 MPa, 50℃
Detection	: PDA 292 nm (reference 500 nm)

*3 P/N: 227-30522-02

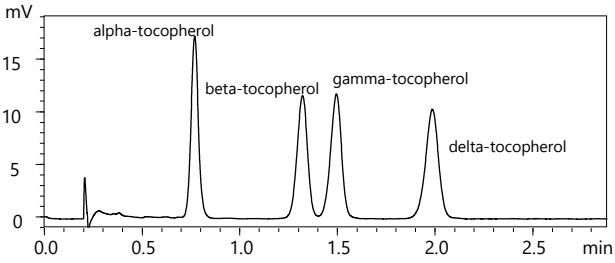


Fig. 3 Chromatogram of tocopherol homologues by SFC

Table 3. Repeatability, linearity, and LOD of tocopherol homologues

	Tocopherol			
	alpha	beta	gamma	delta
%RSD (peak area, n=6 (100 mg/L))	0.24	0.37	0.21	0.65
Linearity of calibration curve	0.9999	0.9999	0.9999	0.9999
LOD (mg/L, calculated values)	0.4	0.6	0.6	0.7

■ Analysis of tocopherol homologues in palm oil

We analyzed the concentration of tocopherol homologues for several types of palm oils. Fig. 4 shows the information of palm oils and the pretreatment method for them. Fig 5 shows acquired chromatograms of palm oils by SFC, and table 4 shows the quantitative results for the concentration of tocopherol homologues in each palm oil. These results show that all palm oils contains high concentrate alpha-tocopherol, and the crude palm oil contained the most significant amount of tocopherol homologues than other palm oils.

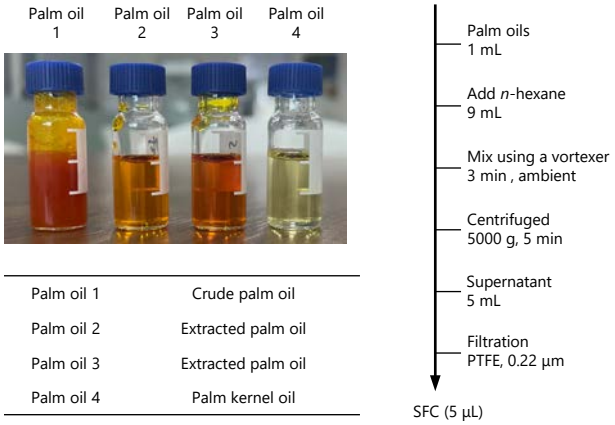


Fig. 4 Palm oils information and pretreatment method

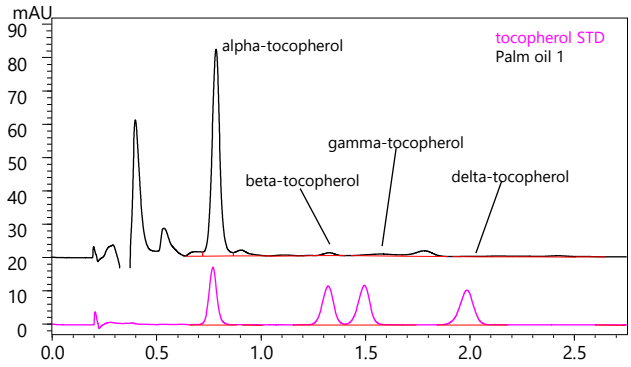


Fig. 5 Chromatogram of tocopherol homologues by SFC

Table 4 Quantitative results of tocopherol homologues in Palm oils

	Tocopherol (mg/L)			
	alpha	beta	gamma	delta
Palm oil 1	358.6	7.1	10.4	1.1
Palm oil 2	17.0	0.6*	0.6*	N.D.
Palm oil 3	20.1	0.7*	1.0	1.0
Palm oil 4	11.1	1.0	0.6*	N.D

* Should be considered reference values, as they were obtained by extrapolation from the calibration curve.

■ Comparison of analysis time and mobile phase consumptions

Fig. 6 shows the comparison chromatogram between normal phase HPLC analysis and SFC analysis. The normal phase analysis required 15 min. However, the SFC method was completed in about 3 minutes. Shorten five times or less analytical time than the typical normal phase HPLC method.

A comparison of mobile phase consumption is shown in Table 5. The SFC method significantly reduced the organic solvent consumption compared to normal phase HPLC. In addition, SFC method reduced the total running cost owing to the low cost of carbon dioxide used for SFC.

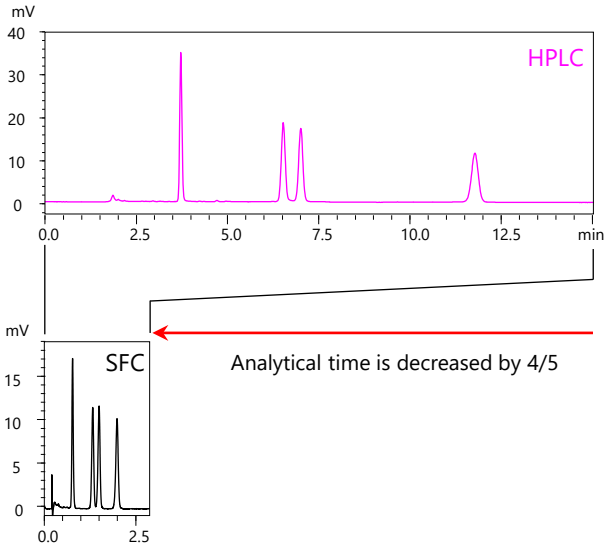


Fig. 6 Comparison of chromatograms and analytical time
(upper HPLC, lower SFC)

Table 5 Comparison of organic solvents consumption

	Mobile Phase	Usage Volume	Organic solvent consumptions
Preparative HPLC	*n*-hexane	29.8 mL	30 mL
	2-propanol	0.2 mL	
SFC	CO_2	6.6 mL	0.5 mL
	Methanol	0.5 mL	

■ Conclusion

A novel SFC method was developed for quantitative analysis of tocopherol homologues in palm oil using Nexera UC system. SFC significantly reduces the running time of analysis time and organic solvent consumption than typical HPLC. Moreover, SFC is possible to connect a mass spectrometer or FRC-40 SF as a fraction collector. Nexera UC system is expected to be used widely from R&D to quality control in the food industry.

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