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Application News

No.L501A

Supercritical Fluid Extraction / Chromatography

Analysis of Vitamin E in a Commercial Supplement by Offline SFE-SFC-PDA

Vitamin E, also called tocopherol, is a fat-soluble vitamin and an important chemical substance that exhibits an antioxidant effect, particularly in the human body. There are four tocopherols (α, β, γ and δ) that differ based on the number and position of methyl groups. The α-tocopherol exhibits the strongest antioxidant activity, and this is the tocopherol form found in most commercial supplements as vitamin E. Since it is highly fat-soluble, a quick and simple extraction method using supercritical fluid is expected to be applicable. In this article, we introduce a procedure for α-tocopherol pretreatment that uses supercritical fluid extraction (SFE).

■ Offline SFE System

While the online SFE-SFC system has already been described in several Application News articles, many have expressed the desire to combine SFE with existing analytical methods other than SFC, and SFE has gained attention for its flexibility in terms of sample handling. The advantages of SFE are as follows.

1. Quick and highly efficient extraction using supercritical fluid that is highly permeable and has a high diffusion rate.
2. Extraction of unstable compounds under mild temperature conditions with light-shielding.
3. Low cost compared to solvent extraction.
4. Complete automation of the extraction procedure.
5. Easy handling of the extraction sample.
6. Compatible with various analysis methods.

Fig. 1 shows a flow diagram for an offline SFE system. A supercritical state is present upstream of the BPR back-pressure control unit. Valves inside the SFE unit are controlled to switch between static extraction via enclosure of supercritical fluid in the vessel and dynamic extraction via passage of supercritical fluid through the vessel, which enables quick and highly efficient extraction of the target compounds.

A HPLC pump with a low-pressure GE valve installed is used in the solvent delivery system, and the extraction conditions can be optimized by changing the type of modifier (maximum of four types, including eluent from the trap column) and the concentration relative to carbon dioxide. Extract is retained in the trap column, and the low-pressure GE valve on the solvent delivery pump is switched to the solvent suitable for elution from the trap column. Then the eluent is collected in test tubes with a fraction collector.

■ SFE Treatment for α-Tocopherol

The commercial supplement used as an actual sample may be present as a paste inside the capsule and may be moisture absorbent. As shown in Fig. 2, we mixed 275 mg of paste supplement with 1 g of Miyazaki Hydro-Protect, which is a dehydrating agent for SFE sold by Shimadzu, and transferred this mixture to the SFE extraction vessel.
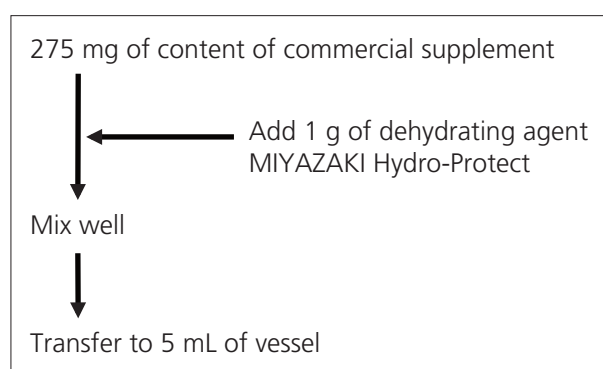

275 mg of content of commercial supplement

↓ ← Add 1 g of dehydrating agent MIYAZAKI Hydro-Protect

Mix well

↓

Transfer to 5 mL of vessel

Fig. 2 Preliminary Pretreatment for Supplement Sample Before SFE
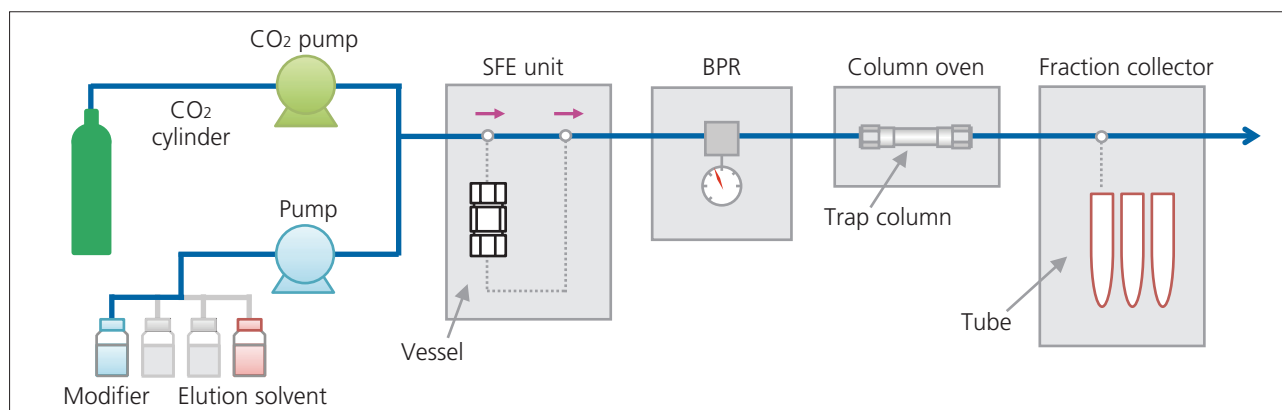

Fig. 1 Flow Diagram of Supercritical Fluid Extraction (SFE) System

The conditions used for SFE are shown in Table 1. We investigated column selection, chose the Shim-pack^{TM} UCX-SIL analytical column, optimized each analytical condition for online SFE-SFC analysis, then performed analysis using the conditions shown in Table 2.

Table 1 SFE Conditions for α-Tocopherol

Offline SFE:
Extraction Vessel	: 5 mL
Extraction Solvent	: CO_2
Flowrate	: 5 mL/min
Temperature	: 40 ℃
Back Pressure	: 15 MPa
Extraction Time	: 15 min
	(Static 2 min → Dynamic 3 min) × 3 times

Trap & Pressure Down Conditions
Trap Column	: Shim-pack VP-ODS (50 mm L. × 4.6 mm I.D.)
Temperature	: 60 ℃
Pressure Down Time	: 10 min (15 - 25 min)

Recovery Conditions
Elution Solvent	: n-Hexane
Flowrate	: 2 mL/min
Temperature	: 60 ℃
Fraction Time	: 3.5 min (25 - 28.5 min)

■ SFE Evaluation of α-Tocopherol in a Commercial Supplement

For the α-tocopherol extract obtained through offline SFE, we performed SFC under the conditions shown in Table 2 then evaluated the extraction procedure. Extract was mixed with hexane to make up 10 mL before being used for SFC analysis. A representative SFC chromatogram is shown in Fig. 3.

Table 2 SFC Conditions for α-Tocopherol

SFC Conditions:
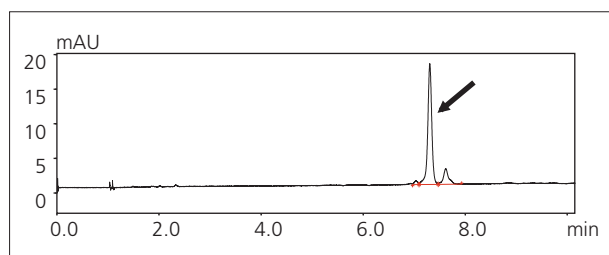|	
Column	: Shim-pack UC-Choles
	(250 mm L. × 4.6 mm I.D., 5 µm)
Flowrate	: 3 mL/min
Modifier	: 2-propanol
Gradient	: 2 % (0 min) - 20 % (10 min) - 50 % (10 - 12 min)
Temperature	: 40 ℃
Back Pressure	: 15 MPa
Injection Volume	: 2 µL

Fig. 3 SFC Analysis of α-Tocopherol Obtained by SFE from a Commercial Supplement

First, we used a standard product to evaluate the suitability of the α-tocopherol SFC conditions used for evaluation of offline SFE. Fig. 4 shows the linearity in the sample concentration range of 0.5 µg/L to 2.0 µg/L, and Table 3 shows the repeatability at a concentration of 1.0 µg/L. Good linearity and sufficient repeatability in terms of retention time, peak area and peak height were obtained.
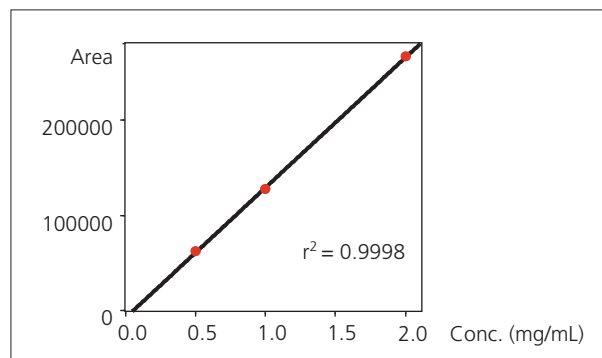

Fig. 4 Linearity for Standard α-Tocopherol Obtained by SFC

Table 3 Repeatability for Standard α-Tocopherol Obtained by SFC (n=6)

No	Retention Time (min)	Area	Height
Average	7.242	127,338	19,682
RSD (%)	0.057	0.573	0.274

Table 4 shows the repeatability of the quantitative α-tocopherol result obtained by repeated SFE treatment, and α-tocopherol recovery relative to the theoretical value (7.4 mg). Fig. 5 shows the overlaid chromatograms for α-tocopherol. Good recovery and repeatability was confirmed after just one extraction, showing that offline SFE is effective for vitamin E compound extraction.

Table 4 Repeatability and Recovery of α-Tocopherol in a Commercial Supplement Using SFE

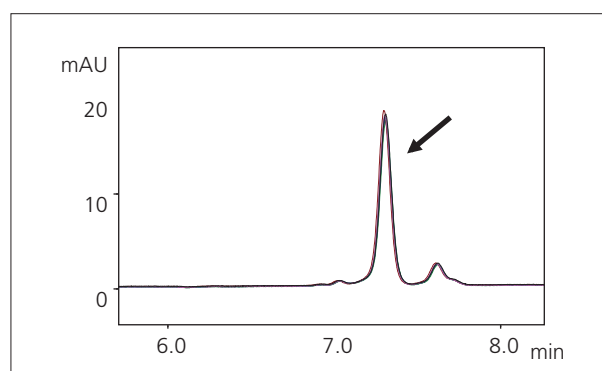| No	Conc. (mg/mL)	Recovery (%)
1	0.776	104.46
2	0.780	105.00
3	0.772	103.92
4	0.790	106.35
5	0.761	102.44
6	0.758	102.04
Average	0.773	
RSD (%)	1.549	

Fig. 5 Overlaid Chromatograms for α-Tocopherol After SFE

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