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Application News

No.L496

Supercritical Fluid Extraction / Chromatography

Analysis of Unstable Compounds Using Online SFE-SFC

Supercritical fluids have characteristics of both gas and liquid; low viscosity, high diffusivity and solubility. In particular, carbon dioxide becomes a supercritical fluid at a relatively modest critical point (31.1 °C and 7.38 MPa). Due to its low toxicity, inertness, easy availability, and low cost, supercritical carbon dioxide fluid is used in a wide variety of fields. Analytical applications using it include supercritical fluid extraction (SFE) and supercritical fluid chromatography (SFC).

Previously SFE and SFC were offline operations for pretreatment or analysis, respectively, and treated as completely separate workflows. However, now SFE and SFC can be connected online using the Nexera UC system, which allows integration of all the processes from pretreatment to data acquisition into a single workflow. This article describes using the Nexera UC system for online SFE-SFC analysis.

Online SFE-SFC

A flow diagram of online SFE-SFC analysis is shown in Fig. 1. Online SFE-SFC involves online introduction of components extracted from an extraction vessel using supercritical fluid to an SFC analytical column, where they are separated and then detected accordingly. The entire process, from extraction to data acquisition, is performed by switching flow lines using a valve inside the SFE unit. Two types of extraction operations are involved. After supercritical fluid is introduced to the extraction vessel, static extraction is performed where components are extracted while fluid flow is stopped. Then dynamic extraction is done to extract components while pumping fluid through the extraction vessel. In the case of online SFE-SFC, the sample is transported through the analytical column during dynamic extraction.
Consequently, the entire online SFE-SFC process, from extraction to separation and detection, can be completed within a single system, which eliminates the need for any complicated pretreatment processes and enables automation. That can significantly reduce the time and effort required for the various operations involved in the analysis.
It also means that the entire process, from extraction to separation and detection, can be performed without exposure to light, without oxidation, and in a moisture-free environment. Therefore, the method is extremely useful for analyzing unstable compounds, such as compounds with components easily decomposed by light, easily oxidized, or easily hydrolyzed. Unlike offline SFE, online SFE-SFC eliminates need for preparing sample solutions, which means it eliminates possibility of dilution of target components by the sample solvent, thus providing an easy way of increasing sensitivity.

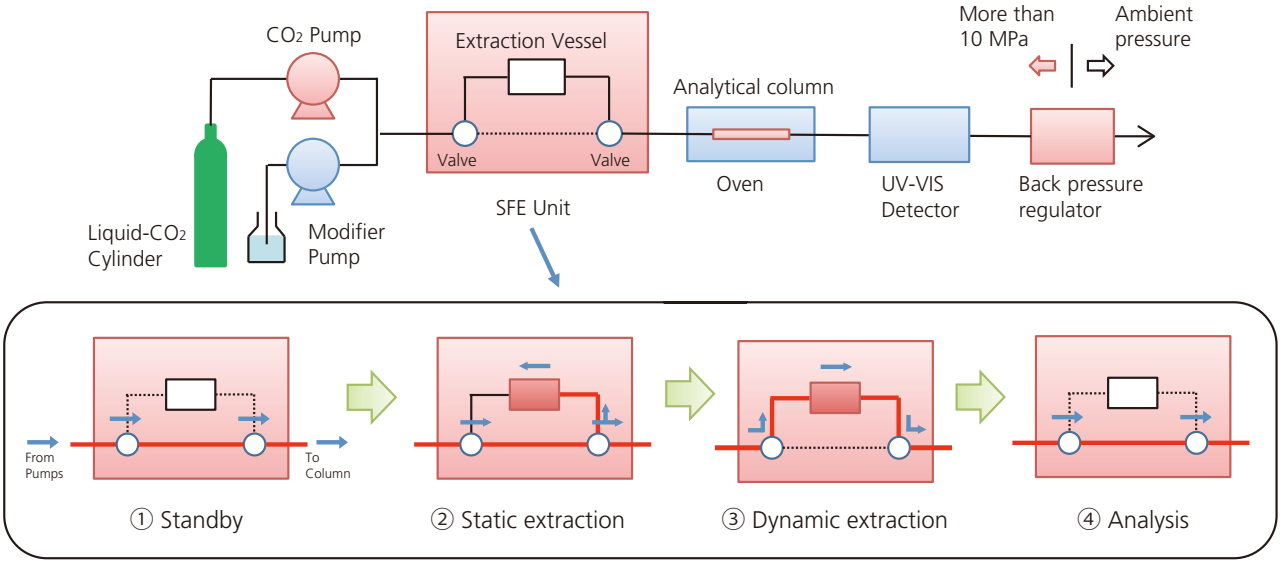

Fig. 1 Process Flow Diagram of Online SFE-SFC System

Online SFE-SFC Analysis of Reduced Coenzyme Q10

Fig. 2 shows the structure of the reduced coenzyme Q10 (ubiquinol). It is easily oxidized to form oxidized coenzyme Q10 (ubiquinone). In this case, both solvent extraction-SFC and online SFE-SFC were used to analyze the reduced coenzyme Q10 contained in a supplement capsule.
Pretreatment operations and analytical conditions for the solvent extraction-SFC analysis are indicated in Fig. 3 and Table 1.
Chromatograms from analyzing the supplement and the oxidized coenzyme Q10 standard sample are shown in Fig. 4.

Table 1 Analytical Conditions for Solvent Extraction-SFC

System	: Nexera UC SFC-UV System
Column	: Shim-pack UC-RP (150 mm L. × 4.6 mm I.D., 3 µm)
Column Temp.	: 40 ˚C
Modifier	: MeOH
Flowrate	: 3 mL/min
Time Program	: 5 % (0 min) → 50 % (5 - 8 min)
BPR	: 10 MPa
Detector	: UV-VIS (220 nm)
Inj. Vol.	: 1 µL

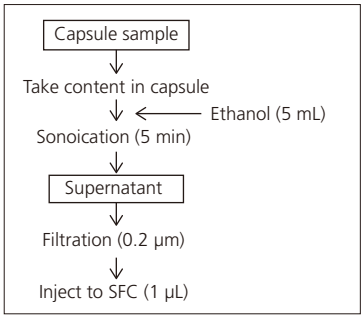

Fig. 3 Pretreatment

Analytical conditions for online SFE-SFC are indicated in Table 2.
About 5 µL each of the liquid sealed inside the supplement capsule and the standard sample of oxidized coenzyme Q10 were dripped onto filter paper. Then a portion of the filter paper was cut with a punch-out device and placed in the extraction vessel for analysis by online SFE-SFC. Chromatograms from analyzing the supplement and the oxidized coenzyme Q10 standard sample are shown in Fig. 5.

Table 2 Analytical Conditions for Online SFE-SFC

System	: Nexera UC Online SFE-SFC-UV System	
SFE		
Extraction Vessel	: 0.2 mL	
Static Extraction	: Time	; 0 - 2 min,
	: B.Conc.	; 5 %
	: BPR	; 10 MPa
	: Flowrate ; 3 mL/min	
Dynamic Extraction	: Time	; 2 - 4 min,
	: B.Conc.	; 5 %
	: BPR	; 10 MPa
	: Flowrate ; 3 mL/min	
SFC		
Column	: Shim-pack UC-RP (150 mm L. × 4.6 mm I.D., 3 µm)	
Column Temp.	: 40 ˚C	
Mobile Phase	: A; CO_2	
	: B; MeOH	
Flowrate	: 3 mL/min	
Time Program	: 5 % (4 min) → 50 % (9 - 13 min)	
BPR	: 10 MPa	
Detector	: UV-VIS (220 nm)	

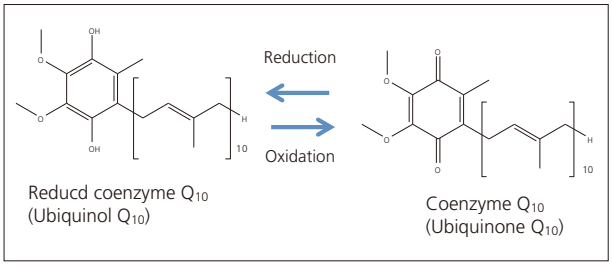

Fig. 2 Structural Formulas

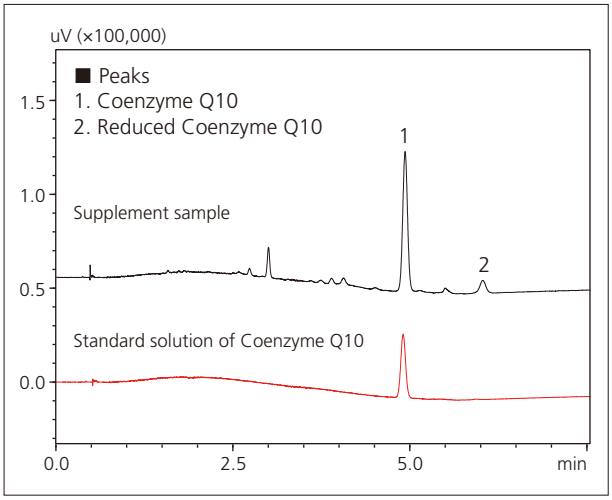

Fig. 4 Chromatograms Obtained by Solvent Extraction-SFC

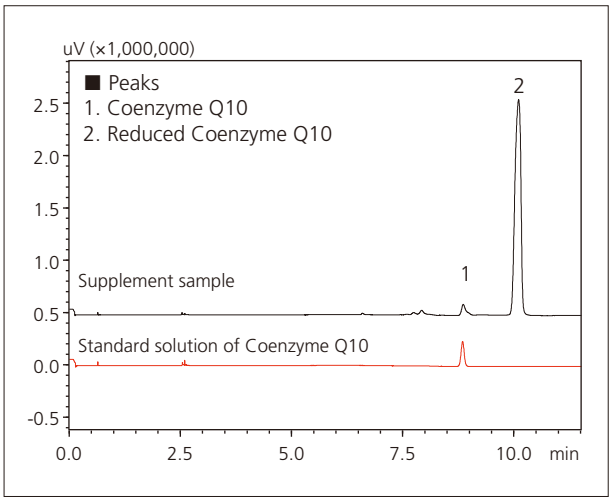

Fig. 5 Chromatograms Obtained by Online SFE-SFC

The results show that the coenzyme Q10 was oxidized during extraction with solvent extraction-SFC, but not oxidized and remained as the reduced coenzyme Q10 form throughout extraction, separation, and detection steps with online SFE-SFC. This shows how online SFE-SFC is an extremely unique analytical technique that can be used to analyze unstable compounds without altering their original form.

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