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Technical Report

Improved Sample Pretreatment Using Offline Supercritical Fluid Extraction

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Abstract:

Separation analysis using HPLC, SFC or GC requires a pretreatment step to efficiently extract a target constituent from the sample in various forms (e.g., solid). Constituents are usually extracted from solid samples using dissolution or solid-liquid extraction methods. Dissolution methods can only be used when the sample is soluble in a given solvent, and they are difficult to optimize depending on the analytical conditions. Solid-liquid extraction methods (e.g., Soxhlet extraction) are not suited to the pretreatment of multiple samples as they require considerable time for extraction and non-extraction (e.g., cleaning, preparation) operations. However, extraction methods involving supercritical fluids can utilize the characteristics of supercritical fluids (e.g., high solubility, permeability) to achieve the elution of a target constituent from a solid sample with high efficiency and also allow automation during the extraction process. This article describes the utilization of the Nexera UC SFE pretreatment system, which increases the efficiency of sample pretreatment for analysis.

Keywords: supercritical fluid extraction, SFE, offline SFE

1. What Is Supercritical Fluid Extraction?

Supercritical fluid refers to the state of any substance at temperature and pressure conditions above its critical point. Supercritical fluids combine the ability of liquids to dissolve materials with high diffusivity and low viscosity properties of gases. Supercritical fluid extraction (SFE) with carbon dioxide is widely employed as a pretreatment method for analysis owing to its low critical points (critical temperature: 31.1 °C, critical pressure: 7.38 MPa), which makes it easy to handle, along with its incombustibility, inertness, and low cost. Some advantages of SFE are shown below:

• Supercritical fluids have high permeability and diffusion coefficients and can therefore be used for highly efficient extraction.

• Supercritical fluids achieve extraction at mild temperatures at which target constituents are unlikely to oxidize.

• Carbon dioxide evaporates, which simplifies sample treatment after extraction.

• Solvent costs are low compared with solvent extraction and other methods.

• The extraction process can be automated.

While supercritical carbon dioxide is as hydrophobic as hexane and is suited for the extraction of fat-soluble compounds, it can also be used for the extraction of compounds with a wide range of polarities by adding modifier substances such as methanol and ethanol.

Fig. 1   The Nexera UC SFE Pretreatment System

2. The Nexera UC SFE Pretreatment System

The Nexera UC SFE pretreatment system (Fig. 1) is a dedicated pretreatment system that performs offline SFE. Solid samples are placed in dedicated extraction vessels (Fig. 2) and introduced into the system, after which the system automatically performs the extraction. The extraction vessels are available in volumes of 0.2 mL and 5 mL such that they can be selected based on the sample to be analyzed. The system allows accommodation of a maximum of 48 extraction vessels, which are mounted into a rack changer (Fig. 3). This setup enables extraction pretreatment of multiple samples via automated sample transfer and cycling. The automation of the multi-sample extraction pretreatment using the Nexera UC SFE pretreatment system substantially reduces the time and labor required for the pretreatment operations while also preventing human error.

Fig. 2   Extraction Vessels

Fig. 3   Rack Changer

The extraction conditions can be configured such that the pretreatment operations can be run from the same LabSolutions workstation used for the analysis. Thus, the extraction conditions and pretreatment can be intuitively controlled in the same way as sample analysis.

The material extracted by the supercritical fluid is collected in a trap column, subsequently eluted by an organic solvent, and finally recovered using a fraction collector before performing the analysis by LC (LC/MS), GC (GC/MS), or NMR. Comprehensive and complementary sample analysis can be achieved by combining the results from several of these analysis methods.

Compared with Soxhlet extraction, SFE uses a much lower quantity of organic solvent during the pretreatment, thereby reducing costs and allowing a more environmentally friendly pretreatment step.

3. Operating Principles of the Nexera UC SFE Pretreatment System

The schematic diagrams of the Nexera UC SFE pretreatment system showing material flow and principle of operation are shown in Fig. 4. The extraction process can be roughly divided into four operations:

(1) Extraction vessel delivery and temperature control

An extraction vessel is moved from the rack changer to the SFE unit, and the extraction vessel is subsequently heated to the set temperature (40–80 °C).

(2) Static extraction

Once the extraction vessel has reached the set temperature, the supercritical fluid is introduced into the vessel and static extraction occurs. Parameters such as the extraction vessel temperature, pressure and duration of the extraction, and type and quantity of the modifier can be independently controlled during extraction depending on the sample and target constituent to be extracted.

(3) Dynamic extraction

After the static extraction, a dynamic extraction is performed by delivering the fluid through the extraction vessel. This operation allows the extraction of the target material from the extraction vessel and subsequent collection in a trap column located downstream of a back pressure regulator. Downstream of the back pressure regulator is held at close to atmospheric pressure such that carbon dioxide is in a gaseous state while collecting the extraction material in the trap column. ODS and other columns used for HPLC can be used as trap columns. Similar to static extraction, the extraction vessel temperature, pressure and duration of the extraction, and type and quantity of the modifier can be adjusted depending on the sample and target constituent to be extracted.

(4) Elution from the trap column and recovery of the extraction material

Once the dynamic extraction has finished, the fluid delivery is stopped and the back pressure regulator is opened, thereby allowing the system pressure to drop to atmospheric pressure. The delivery pump is then switched from the modifier to the eluent, which is passed through the trap column to elute the extraction material. The eluate is then recovered into collection tubes using a fraction collector. An organic solvent is used as the eluent to simplify concentration and post-treatment steps of the eluate.

(1) Extraction vessel delivery and temperature control

A specified extraction vessel is transferred to the SFE unit and heated to the set temperature.

(2) Static extraction

When the temperature of the extraction vessel has reached the set temperature, the supercritical fluid is introduced and static extraction (i.e., in the absence of fluid flow) is allowed.

(3) Dynamic extraction

The extraction is dynamically performed by passing the supercritical fluid through the extraction vessel. The extraction material is taken from the extraction vessel and collected at atmospheric pressure after evaporation of CO_2 in the trap column downstream the back pressure regulator.

(4) Elution from the trap column and recovery of the extraction material

The delivery pump is used to deliver the eluent through the trap column, thereby eluting the extraction material, which is then recovered using a fraction collector.
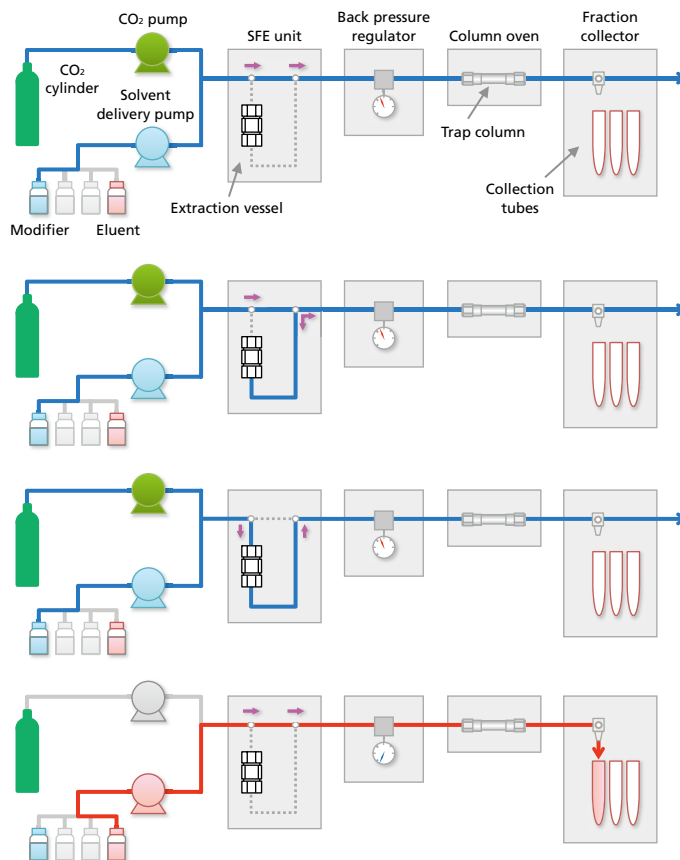

Fig. 4   Material Flow and Principle of Operation

Samples showing high water content complicate the extraction process (i.e., reduce both the extraction efficiency and the repeatability of the pretreatment) as supercritical carbon dioxide does not mix with water. In these cases, the extraction efficiency can be increased by mixing the sample with a dehydrating agent before enclosing it into the extraction vessel. Extraction efficiency can also be low when supercritical carbon dioxide is used for the extraction of highly polar constituents. In this case, the extraction efficiency can be increased by adding modifiers such as methanol during the extraction. In the case of samples with constituents showing ionic polar groups, acid (e.g., formic acid, acetic acid), salt (e.g., ammonium formate, ammonium acetate), and bases (e.g., ammonia, diethylamine) can be added during the extraction. Fine pulverization of the sample normally increases extraction efficiency. For polymer samples, a fine freeze-crushing treatment before the extraction often results in increased extraction efficiencies.

4. Using the Nexera UC SFE Pretreatment System for Extracting Fat-Soluble Vitamins

Vitamin E is a group of fat-soluble compounds widely used as antioxidants and for nutritional support in foods and medicinal products. We present an example of offline SFE using the Nexera UC SFE pretreatment system to extract d-α-tocopherol, a vitamin E compound, from a nutritional supplement. The sample used was a commercially available soft capsule supplement containing d-α-tocopherol. The soft capsule supplement contained a paste, which was mixed with a dehydrating agent before being enclosed into an extraction vessel. The extraction was exclusively performed with supercritical carbon dioxide, and hexane was used as the eluent after trapping. The detailed extraction conditions are shown in Table 1. The extraction liquid recovered by the fraction collector was diluted to 10 mL with hexane in a measuring flask. The sample extraction vessel contained 7.4 mg of d-α-tocopherol, and the theoretical concentration of d-α-tocopherol in the final SFE extraction liquid was 0.74 mg/mL.
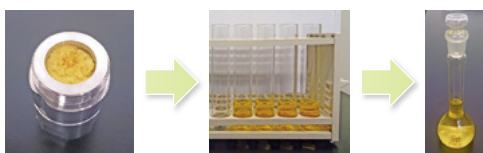

Fig. 5   Sample Before and After the SFE Process

Table 1   SFE Conditions

SFE	
Extraction vessel	: 5 mL
Extraction solvent	: CO_2
Flow rate	: 5 mL/min
Temperature	: 40°C
Back pressure	: 15 MPa
Extraction time	: 15 min (Static extraction → Dynamic extraction)
Trap & Pressure down conditions	
Trap column	: Shim-pack VP-ODS 4.6 mmI.D. × 50 mmL. 5 µm
Temperature	: 60°C
Pressure down time	: 10 min (15–25 min)
Recovery conditions	
Elution solvent	: Hexane
Flow rate	: 2 mL/min
Fraction time	: 3.5 min (25–28.5 min)

Six extraction vessels were prepared, each containing the same amount of supplement sample. Each extraction vessel was subjected to offline SFE after which the recovered liquid was analyzed by SFC at the conditions shown in Table 2. The recovery and repeatability of the process was confirmed.

The six chromatograms obtained are shown overlapping each other in Fig. 6.

Table 2   Conditions Used for the Analysis of the Pretreated Samples (SFC)

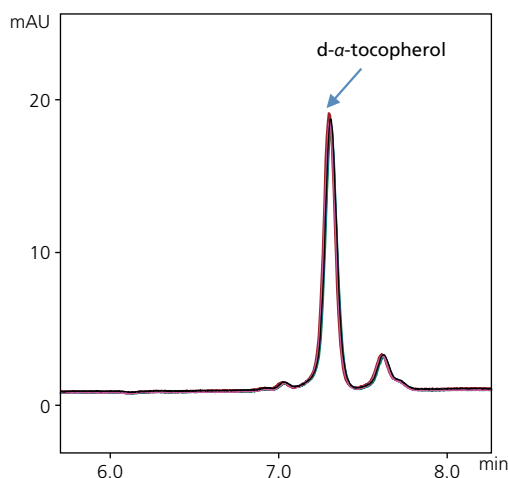|	
Column	: Nacalai COSMOSIL Cholester 4.6 mmI.D. × 250 mmL. 3 µm
Modifier	: IPA
Gradient	: 2% (0 min) → 20% (10 min) → 50% (10–12 min)
Flow rate	: 3 mL/min
Temperature	: 40°C
Back pressure	: 15 MPa
Injection volume	: 2 µL
Detector	: UV-VIS (@293 nm)

Fig. 6   Results for SFE Extraction Liquid Analyses (The Six Samples Are Shown Overlapping Each Other).

The concentration and recovery of d-α-tocopherol in the six SFE extraction liquid chromatograms (Fig. 6) are summarized in Table 3. The extraction pretreatment showed both high repeatability and high recovery, thereby revealing that the Nexera UC SFE pretreatment system can be used for the automated consecutive pretreatment of target constituents in a solid sample with good efficiency.

Table 3   Repeatability and Recovery of Vitamin E Extraction

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	

5. Using the Nexera UC SFE Pretreatment System for the Extraction of Residual Pesticides from Agriproducts

Over 800 pesticides are subjected to analysis for their residual presence in food products. Analytical methods that enable rapid and simple testing of a large number of pesticides involving any pretreatment operations required for analysis are needed. Conventional analysis for residual pesticides in food normally involves a solvent extraction method to extract the pesticides, followed by LC/MS or GC/MS analyses. The pretreatment operations employed in these analytical methods are highly labor and time consuming, and they use a large volume of organic solvent. We present an example of utilizing the Nexera UC SFE pretreatment system to extract residual pesticides before analyzing them using a GC/MS/MS system. 1 g of dehydrating agent was added to 1 g of pulverized brown rice*. This mixture was then enclosed into an extraction vessel, and an extraction pretreatment was performed using the conditions shown in Table 4. The extraction liquid recovered by the fraction collector was diluted to 2 mL with an acetone/hexane (1/1, V/V) mixture in a measuring flask and then analyzed using GC/MS/MS under the conditions shown in Table 5. The components included in pesticide standard mixture solutions for GC/MS (PL2005 Pesticide GC/MS Mix I to VI and Mix 7, Hayashi Pure Chemical Ind., Ltd.) were analyzed.

* "Miyazaki Hydro-Protect" Patent No. 3645552

Table 4   SFE Conditions

Offline SFE	
Extraction vessel	: 5 mL
Extraction solvent	: CO_2 + Methanol
Flow rate	: 5 mL/min
Temperature	: 40°C
Back pressure	: 15 MPa
Extraction time	: 8 min (Static extraction → Dynamic extraction)
Trap & Pressure down conditions	
Trap column	: Shim-pack VP-ODS 4.6 mmI.D. × 50 mmL. 5 µm
Temperature	: 60°C
Pressure down time	: 4 min (8–12 min)
Recovery conditions	
Elution solvent	: Acetone/Hexane = 1/1 (V/V)
Flow rate	: 2 mL/min
Fraction time	: 2 min (12–14 min)
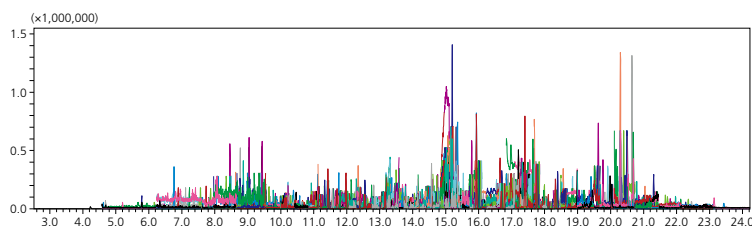
The pesticide standard solutions were added to an brown rice sample (pesticide concentrations of 100 ng/g). An MRM chromatogram of the extraction liquid obtained from this sample is shown in Fig. 7. The theoretical concentration of each pesticide in the extraction liquid used for GC/MS/MS analysis was 50 ng/mL.

The extraction was performed on six samples to which the abovementioned pesticide standard solutions were added; each pesticide was quantified using a matrix calibration curve created using the SFE extraction liquid obtained from a blank brown rice sample, after which repeatability and recovery were confirmed. Good repeatability (relative standard deviation of quantified concentration: <10 %) and good recovery (70 %–120 %) were obtained for the 301 pesticides studied. An excerpt from these results showing the repeatability and recovery for some representative pesticides is shown in Table 6. The Nexera UC SFE pretreatment system can be used for automated consecutive pretreatment of up to 48 samples while consuming low amounts of solvent.

Table 5   Conditions Used for the Analysis of the Pretreated Samples (GC/MS/MS)

Column	: Rxi-5Sil MS 30 m × 0.25 mmI.D., df = 0.25 µm
Column temp.	: 50°C (1 min) → (25°C/min) → 125°C
	→ (10°C/min) → 300°C (15 min)
Carrier gas	: He (Constant linear velocity mode)
Linear velocity	: 47.2 cm/sec
Injection mode	: Splitless (Sampling time 1.00 min)
High press inj.	: 250 kPa (1.5 min)
Injection volume	: 1 µL
Interface temp.	: 250°C
Ion source temp.	: 200°C
MS mode	: MRM
Loop time	: 0.3 sec

Table 6   Repeatability and Recovery of Representative Pesticide Extraction

Compounds	Repeatability (%RSD, n=6)	Recovery (%)
Cyhalofop-butyl	4.2	93
Etofenprox	3.8	90
Iprodione	2.5	93
Malathion	3.2	93
Piperonyl butoxide	3.8	89

Fig. 7   MRM Chromatogram of the Brown Rice Extraction Liquid

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