

Technical Report

Online Supercritical Fluid Extraction-Supercritical Fluid Chromatography (Online SFE-SFC)

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Abstract:

Online supercritical fluid extraction-supercritical fluid chromatography (online SFE-SFC) system is that directly connects the supercritical fluid extraction (SFE) and supercritical fluid chromatography (SFC). By using the online SFE-SFC, pretreatment to analysis can be automated, creating advantages such as simplification of pretreatment, analysis of unstable compounds, and high-sensitivity analysis. Here, we introduce the basic principle of online SFE-SFC, the characteristics of the "Nexera UC Online SFE-SFC System," and examples of extraction analyses.

Keywords: supercritical fluid chromatography, SFC, supercritical fluid extraction, SFE, online SFE-SFC

1. What is Supercritical Fluid?

Supercritical fluid is a material for which the temperature and pressure are at or over the critical point, has high diffusivity and low viscosity like gas, and solubility like liquid. Carbon dioxide has a critical temperature of 31.1°C and a critical pressure of 7.38 MPa. Since its critical point is low, its handling is easy, and as it is non-flammable, inert, and low cost, it is widely used in industrial settings like in the decaffeination of coffee. In analytical fields, supercritical fluid extraction (SFE) that uses supercritical carbon dioxide as the extraction medium and supercritical fluid chromatography (SFC) that mainly uses supercritical carbon dioxide as the mobile phase are currently being used.

SFE can extract at high efficiency using the high permeability and diffusivity of the supercritical fluid. Since the critical temperature of carbon dioxide is low, allowing for extraction under mild conditions, the target component can be extracted in a condition in which it is unlikely to be decomposed.

SFC uses supercritical fluid characterized by high diffusivity and low viscosity as the mobile phase. Hence, compared to the traditional HPLC analysis, the pressure on the column decreases, and even under faster flow rate conditions, separation functioning does not decrease in turn. Therefore, high-speed analysis becomes possible. In addition, the polarity of supercritical carbon dioxide is generally said to be near the level seen with hexane, but by mixing with a polar solvent such as methanol, it can respond to a wide range of polarities. Therefore, it can be applied to the analysis of a wide range of compounds, including hydrophobic and hydrophilic ones.

2. What is Online SFE-SFC?

Online SFE-SFC is an extraction separation technique that introduces the component extracted via SFE directly into the column and separates with SFC. It can be actualized in the Nexera UC Online SFE-SFC-MS System shown in Fig. 1.

Online SFE-SFC only requires the user to fill the sample in the extraction vessel, and automatic operation from extraction to analysis is possible. Since analysis can be performed without the extract being exposed to light or air, it is useful for compounds that are unstable with light or that tend to oxidize. Since water is not used as the extraction medium, it is also employed for compounds that are susceptible to hydrolysis. Since all the extracted compounds are introduced to the detector, the load of the target component can be reduced. As it allows for highly sensitive analysis, it is useful for components for which sensitivity is insufficient and a higher concentration is required, or for a small amount of sample.

The Nexera UC system can load a maximum of 48 samples using the rackchanger, allowing for the continuous processing of multiple samples. There are two types of extraction vessels: a 0.2 mL vessel for a small amount of sample, such as dried blood spot (DBS), and a 5 mL vessel for a large amount of sample, such as agricultural products. It can handle a wide range of samples.

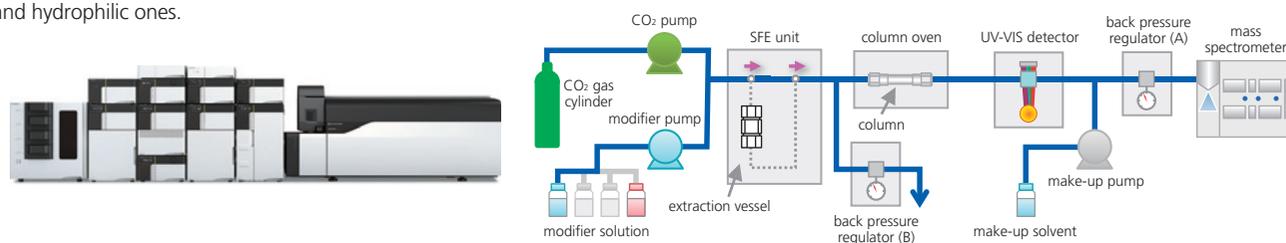


Fig. 1 Structure and Flow Path of Nexera UC Online SFE-SFC-MS System

3. Operating Principle of Online SFE-SFC

The operating principle of the Nexera UC Online SFE-SFC extraction separation analysis is shown in Fig. 2. These three processes follow:

(1) Extraction

(1)-1 Transportation and Temperature Control of the Extraction Vessel

Designated extraction vessel is transported to the SFE unit and its temperature is controlled until it reaches the set temperature. The extraction temperature can be set between 40 and 80°C.

(1)-2 Static Extraction

When the temperature of the extraction vessel reaches the designated temperature, the supercritical fluid is introduced into the extraction vessel. After the introduction, the fluid is not passed into the extraction vessel, and extraction is performed in a static state.

(1)-3 Dynamic Extraction

After the static extraction, extraction is performed while passing the supercritical fluid through the extraction vessel. The extract is removed from the vessel and introduced to the analytical column*.

* With the Nexera UC Online SFE-SFC System, when sample concentration is extremely high or when a sample with a high amount of matrix is extracted, part of the extract can be introduced into the column performing a split.

The ratio of the split can be adjusted by changing the pressure using two back pressure control valves.

(2) Separation

After completing the extraction, the extraction vessel is separated from the flow path, and by increasing the concentration of the modifier in the mobile phase, the extract is separated.

Regardless of the type of column and modifier, as the concentration of the modifier increases, the elution strength increases.

The modifier solvent can be changed so that it switches between separation and extraction.

(3) Detection

The eluted compound is detected by a photodiode array detector or mass spectrometer. When using a mass spectrometer and the main mobile phase is carbon dioxide, the ions to be used for ionization are insufficient and sensitivity is decreased; therefore, an organic solvent (make-up solvent) to support ionization is added with a make-up pump.

For the make-up solvent, a different solvent from the modifier can be used; therefore, one can select a solvent that allows for a highly sensitive analysis of the compound of choice.

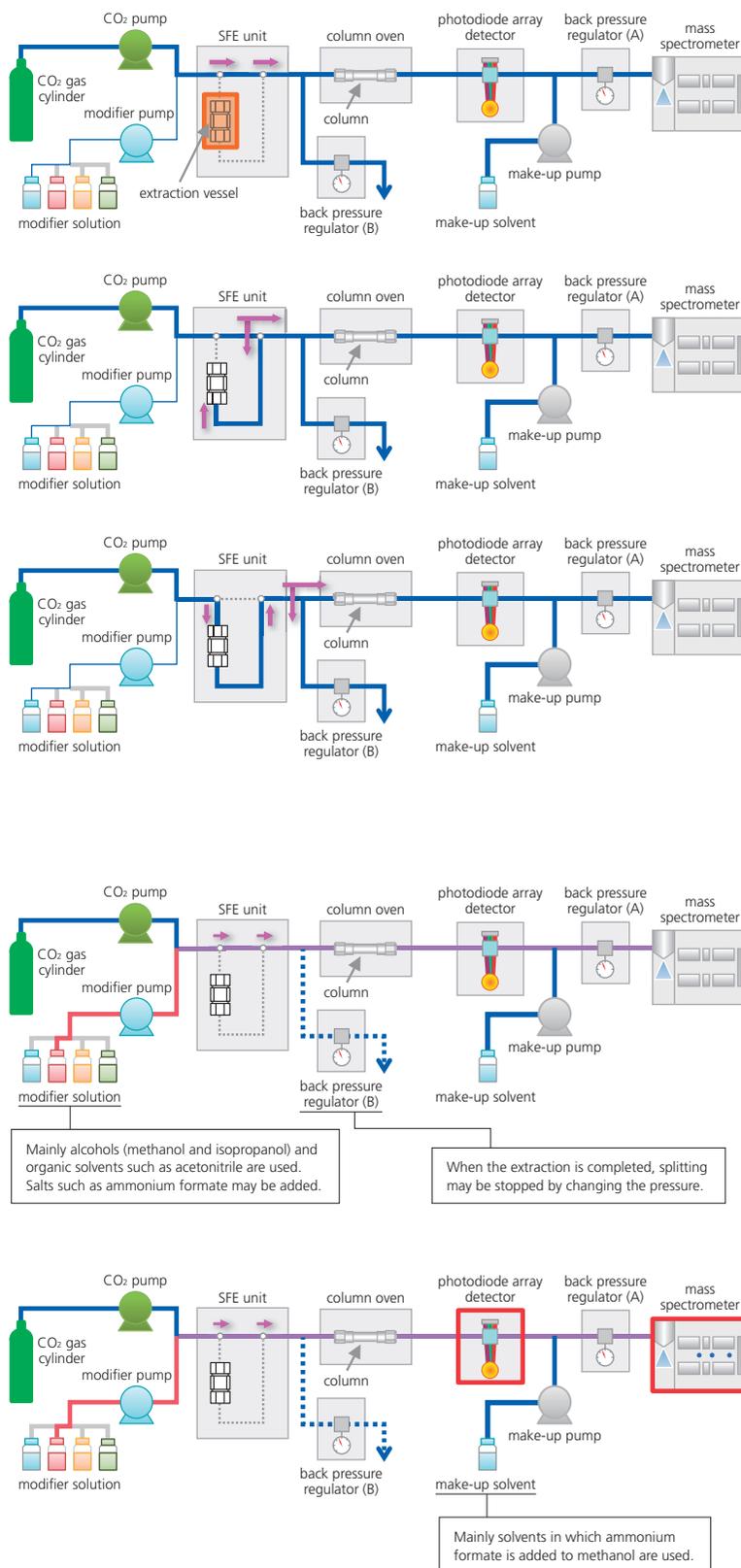


Fig. 2 Operating Principle of Nexera UC Online SFE-SFC System

4. Analytical Conditions

Using the Nexera UC Online SFE-SFC System, the following parameters can be employed to examine extraction conditions and separation conditions. By adjusting each parameter, the extraction efficiency and separation can be optimized.

Extraction conditions (static extraction and dynamic extraction)	Separation conditions
<ul style="list-style-type: none"> • Time • Temperature • Pressure • Type of modifier solvent • Adding a modifier 	<ul style="list-style-type: none"> • Type of column • Type of modifier solvent • Adding a modifier • Column oven temperature • BPR pressure

5. Extraction Vessels

With the Nexera UC, there are two types of extraction vessels, and the following characteristics are observed depending on the vessel:

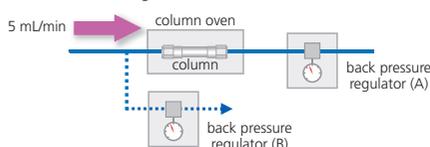
Capacity	Shape	Characteristics
0.2 mL		Since the capacity is small, peaks tend to be sharp in online SFE-SFC analysis.
5 mL		About 1 g of sample can be loaded. But since the capacity is large, this takes more time to remove the extracts from vessel.

6. Advantage of Introducing Splitting

In the Nexera UC Online SFE-SFC System, if the capacity of the extraction vessel is 5 mL, and the total volume of the extract is introduced into the column, the load on the column becomes too large, which causes peak broadening. Therefore, by introducing only a portion of the extract to the column using the split, the load can be reduced. Since the flow of the mobile phase in the column can be reduced, this creates a condition in which the target compound tends to remain at the top of the column (Fig. 3).

Without Splitting

- Excessive load
- The compound elutes even during extraction → Peaks broaden



With Splitting (split ratio 10%)

- Reduces load on the column
- Reduction of flow reduces elution during extraction → Improves the peak shape

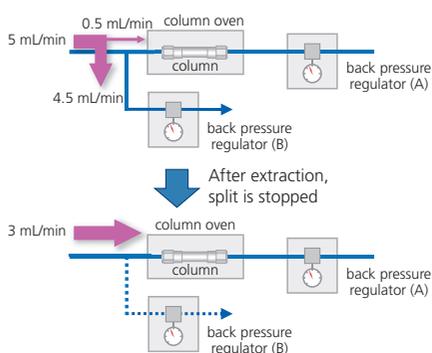
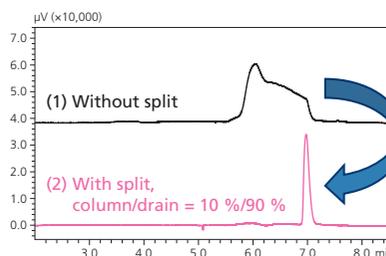


Fig. 3 Introducing the Split

By splitting, the elution of the extract can be suppressed; therefore, the shape of the peak can be improved. Fig. 4 shows an analytical example of the split using caffeine. (1) Without the split, the peak is quite broad, but (2) by introducing the split, the shape of the peak is improved.



Extraction vessel : 5 mL
 Sample : caffeine
 Modifier : MeOH
 Extraction time : 4 min (static 2 min, dynamic 2 min)
 Modifier ratio : 10% (0–4 min) → 50% (4–6 min)
 Flow rate : 5 mL/min (at extraction), 3.0 mL/min (at analysis)
 Back pressure : 15 MPa
 Column : Shim-pack UC-SIL 4.6 mm I.D. x 250 mm L., 5 μm
 Detection : photodiode array detector (at 272 nm)

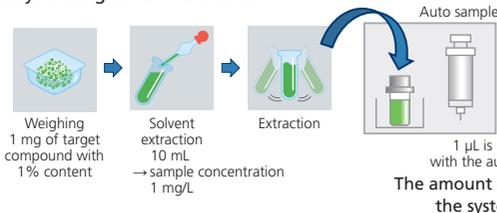
Fig. 4 An Example of Caffeine Analysis with Split

7. High-sensitivity Extraction Analysis

In the pretreatment with the traditional solvent extraction, a solvent was added to the sample for extraction. Therefore, the extract was diluted with the extraction solvent, decreasing the compound concentration in the extraction fluid. Furthermore, under analytical-scale conditions, since the inner diameter of the column is very narrow, only several microliters of the extraction fluid could be introduced. Therefore, sensitivity is insufficient when a sample with a small amount of extract is analyzed, and multiple steps of extraction and concentration become necessary. In contrast, since the extract can be directly introduced onto the column in the Nexera UC Online SFE-SFC System, the amount of sample introduced to the column can be significantly higher than that with the solvent extraction. An example of the comparison* is shown in Fig. 5. When the sample is directly introduced with the Nexera UC, compared to the 1 μL injection of 10 mL solvent in the solvent extraction, the amount introduced into the column is 10,000 times higher, allowing for a highly sensitive analysis.

* Assuming that the extraction efficiency is equivalent, and the target compound in the sample is 1%.

Analysis using solvent extraction



Analysis using the Nexera UC

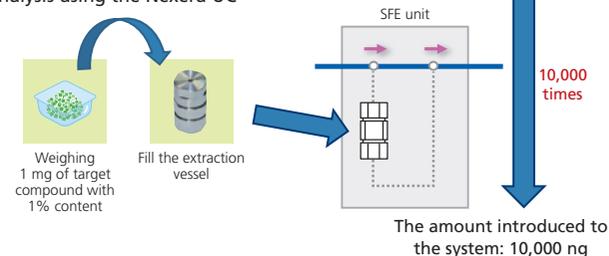


Fig. 5 High-sensitivity Analysis

8. Unstable Compound Extraction Analysis

With the Nexera UC Online SFE-SFC System, it is possible to use chemically stable carbon dioxide for extraction, and since the sample is not exposed to light or oxygen after being filled in the extraction vessel until detection, its extraction analysis of the unstable compound is effective. The reduced form of coenzyme Q₁₀ (CoQ₁₀) is easily oxidized, but by using online SFE-SFC, the reduced form can be accurately extracted and analyzed (Fig. 6).

Please refer to Application News No. L496 for details such as analytical conditions.

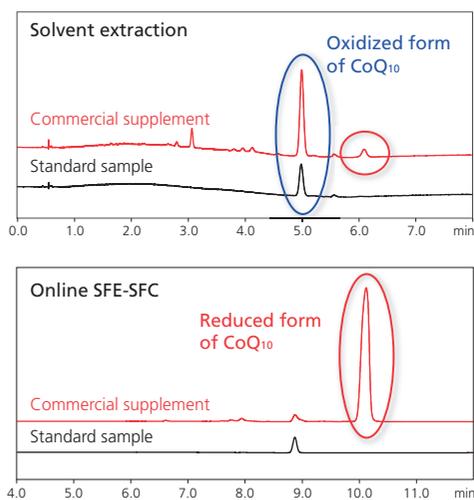
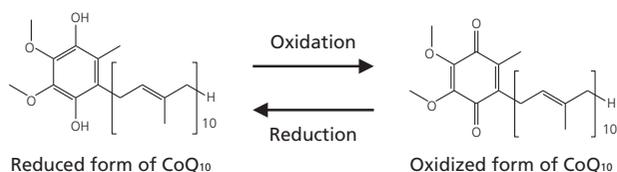


Fig. 6 Application of CoQ₁₀ to Online SFE-SFC

9. Simplifying the Pretreatment and Multi-sample Processing: Application of Residual Pesticide to the Nexera UC Extraction Analysis

Faster and simpler methods of analyzing pesticide residues in food are strongly desired due both to the large number of pesticides and to reduce the time required for testing. One of the existing methods is a pretreatment using organic solvent, but it involves many processes, which require much time and effort. For the Nexera UC pretreatment, a dehydrating agent is mixed in to remove moisture, and the pretreatment is completed by filling the extraction vessel (Fig. 7). This is because supercritical carbon dioxide does not mix with water, and if there is moisture, the extraction efficiency might decrease. The actual results of an online SFE-SFC-MS analysis of pesticides are shown in Table 1. These can be applied to pesticides with a wide range of polarity, from hydrophilic Log *P*_{ow} -1.4 compounds to hydrophobic Log *P*_{ow} 6.9 compounds.

In addition, by using a rack changer, 12 racks—each able to hold four extraction vessels—can be used. Therefore, a maximum of 48 samples can be analyzed consecutively.

With the use of online SFE-SFC, the complex pretreatment process can be simplified, improving the operation efficiency.

Please refer to Application News No. L497 for details such as analytical conditions for pesticide residue.

Table 1 Repeatability and Linearity for Representative Pesticides

Compounds	Log <i>P</i> _{ow}	Repeatability (%RSD, n=5)	Range (ng/g)	R ²
Ethofenprox	6.9	6.1	1–100	0.9991
Hexaflumuron	5.68	6.8	1–100	0.9992
Benzofenap	4.69	1.4	2–200	0.9990
Mepronil	3.66	4.6	1–100	0.9993
Prometryn	3.34	2.7	1–100	0.9994
Fenamidon	2.8	3.0	2–200	0.9991
Ethylchlozate	2.5	3.0	1–100	0.9996
Imazosulfuron	1.6	6.2	1–100	0.9998
Bensulfuron methyl	0.79	8.1	1–100	0.9996
Primisulfuron methyl	0.2	5.5	1–100	0.9994
Halosulfuron methyl	-0.02	5.5	1–100	0.9996
Azimsulfuron	-1.4	4.2	1–100	0.9998

Pretreatment in the traditional (QuEChERS) method



Pretreatment with the Nexera UC



Mixing an agricultural product with the absorbent



Rack changer



Fig. 7 Comparison of Pretreatments for Pesticides

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