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Technical Report

Supercritical Fluid Chromatography

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Abstract:

Advances in column technology have led to a renewed interest in supercritical fluid chromatography, which uses a supercritical fluid as its mobile phase. Compared to liquid, supercritical fluids have low viscosities and high diffusivities. In this report, starting from the basic principles of supercritical fluid chromatography, we introduce examples of high-speed, high-resolution analysis and chiral separation.

Keywords: supercritical fluid chromatography, SFC

1. Supercritical Fluid

A supercritical fluid is a state of substance wherein the temperature and pressure are both above its critical point (Fig. 1). Supercritical fluids can dissolve substances better than gases and are more diffusive and have lower viscosities than liquids (Table 1). Although various substances have particular critical points, the especially low critical point of carbon dioxide (critical temperature: 31.1°C, critical pressure: 7.38 MPa) makes it easy to handle. As it is non-flammable, inert, low-cost, and non-toxic, it has been widely used in industrial processes, such as for decaffeination of coffee beans and extraction of hops extract and flavor compounds (Fig. 2). Supercritical fluids are also used in analytical fields, including as the main mobile phase in supercritical fluid chromatography (SFC) and the main extracting solvent in supercritical fluid extraction (SFE).

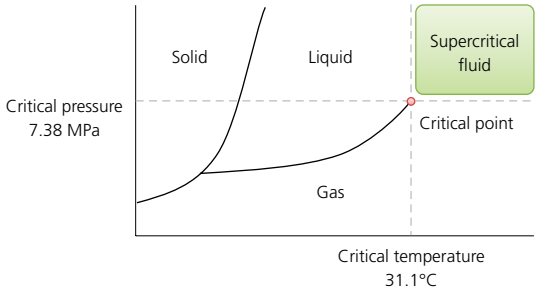


Fig. 1 Phase Diagram for Carbon Dioxide

Table 1 Properties of Supercritical Fluids

	Diffusion coefficient (cm^2/s)	Density (g/cm^3)	Viscosity ($g/cm \cdot s$)
Liquid	10^{-6}	1	10^{-2}
Supercritical fluid	10^{-3}	0.2 to 0.8	10^{-3}
Gas	10^{-1}	10^{-3}	10^{-4}

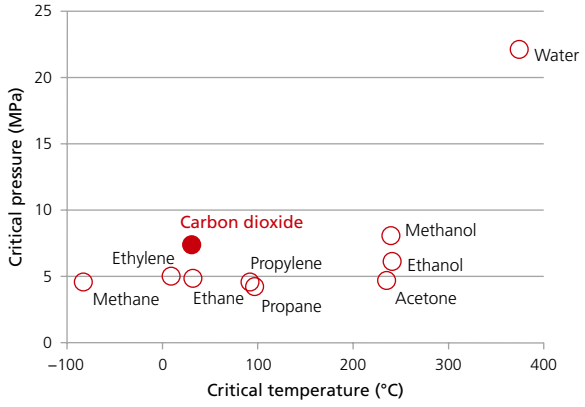


Fig. 2 Critical Points of Various Substances

2. Supercritical Fluid Chromatography

SFC is a separation technique that uses a supercritical fluid as its main mobile phase (often supercritical carbon dioxide). Because of the properties of supercritical fluids, which include low viscosities and high diffusivities, SFC can be performed at a lower column back pressure than conventional high-performance liquid chromatography (HPLC). Additionally, a high-speed analysis can be performed at high flow rates and a high-resolution analysis can be performed by using a longer column. Also, recent advances in SFC systems and in the packed columns made for SFC allow analyses to be performed with HPLC-like operation.

Although supercritical carbon dioxide has a similar hydrophobicity as hexane, this property alone is often insufficient for the elution of target compounds from a column. These target compounds can be eluted by adding an organic solvent, called a modifier, to modify the polarity of the mobile phase. Organic solvents that are compatible with carbon dioxide, such as methanol, ethanol, isopropyl alcohol, and acetonitrile, are used as modifiers. Organic solvents with an added acid (e.g., formic acid or acetic acid), salt (e.g., ammonium formate or ammonium acetate), or base (e.g., diethylamine) are also used as modifiers for the analysis of highly polar compounds.

1 Analytical & Measuring Instruments Division

3. Nexera UC

The Nexera UC platform can accommodate a wide variety of analyses and pretreatments and includes an (1) SFC system, (2) online SFE-SFC system, and (3) offline SFE system. A major difference between a Nexera UC system and a conventional HPLC system is the addition of a back pressure regulator to prevent mobile phase vaporization inside the column and the pump that delivers the carbon dioxide. The Nexera UC platform is based around the Nexera ultra high-performance liquid chromatograph, with each Nexera UC system configured by adding a newly developed carbon dioxide delivery unit (LC-30ADSF), a back pressure regulator unit that allows high-precision pressure control (SFC-30A), and an extraction unit used for SFE (SFE-30A) (Fig. 3). The autosamplers and other units designed for liquid chromatography can be used in the Nexera UC system.

(1) SFC system

SFC systems include an SFC-UV system that uses a UV (or PDA) detector, a UFMS system (SFC-MS) that uses a mass spectrometer (MS) that is suitable for high-speed analyses by SFC, and a chiral screening system that automatically switches between multiple columns and modifiers to examine the analytical conditions.

(2) Online SFE-SFC system

Online SFE-SFC systems combine SFE and SFC online to automatically perform all steps from target compounds extraction from solid samples to analysis.

(3) SFE pretreatment system

Offline SFE pretreatment systems are specifically designed to extract target compounds from solid samples.

The characteristic properties of the supercritical fluid used in the Nexera UC SFC systems, which include high diffusivity and low viscosity, allow for low column pressures even at high flow rates, enabling high-speed analyses while maintaining column efficiency. Because of these factors, the Nexera UC SFC systems can shorten analysis times to between one third and one fifth of the time required for HPLC analysis using the same size column. (Fig. 5).

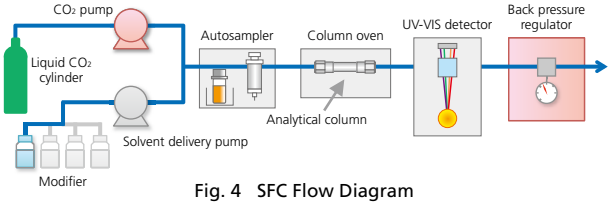
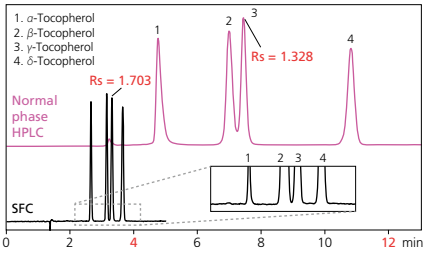


Fig. 4 SFC Flow Diagram



SFC conditions

Column	:	Shim-pack UC-SIL (4.6 mm I.D. × 250 mm L. 5 µm)
Modifier	:	MeOH
Modifier conc.	:	5 %
Flow rate	:	3.5 mL/min
Temperature	:	40°C
Detection	:	UV 290 nm
Back pressure	:	10 MPa

Normal phase HPLC conditions

Column	:	Shim-pack HRC-SIL (4.6 mm I.D. × 250 mm L. 5 µm)
Mobile phase	:	HEX/IPA 99/1 (V/V)
Flow rate	:	1 mL/min
Temperature	:	40°C
Detection	:	UV 290 nm

Fig. 5 Comparison between HPLC and SFC

SFC-UV System

SFC-MS System

Fig. 3 Nexera UC Systems

By using the same column packing material for separation in the Nexera UC systems as that used in normal phase HPLC analysis (e.g., silica gel), normal phase HPLC analyses can be easily transferred to SFC analyses while improving the resolution and increasing the analysis speed, as shown in Fig. 5. Transferring analyses from normal phase HPLC to SFC can also substantially reduce the volume of organic solvents consumed per analysis, as shown in Fig. 6, which also reduces analysis costs. SFC is an environment- and user-friendly technique as it reduces consumption of toxic organic solvents.

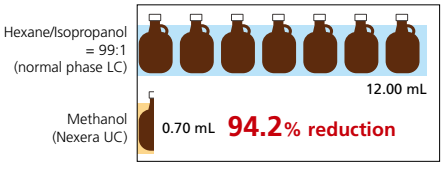


Fig. 6 Comparison between HPLC and SFC of Organic Solvent Consumption

When using a mass spectrometer for SFC, equipment used for LC/MS can be used as is. In SFC, a make-up solution is added after column separation to promote ionization. Conventional SFC systems used pressure regulators that had a large internal volume. This required the flow path of the column eluate to be split before entering the mass spectrometer to suppress the effect of extra-column dispersion (Fig. 7(a)). The Nexera UC systems use a proprietary low-internal volume design for their back pressure regulator (patent pending). This allows the flow path to enter the back pressure regulator and mass spectrometer in series, so all the column eluate enters the mass spectrometer (Fig. 7(b)). Increasing the volume of eluate introduced to the mass spectrometer in this way enables higher sensitivity analysis, and precludes the effects of split ratio variation, etc., resulting in highly reproducible SFC/MS analysis (Fig. 8, Table 2).

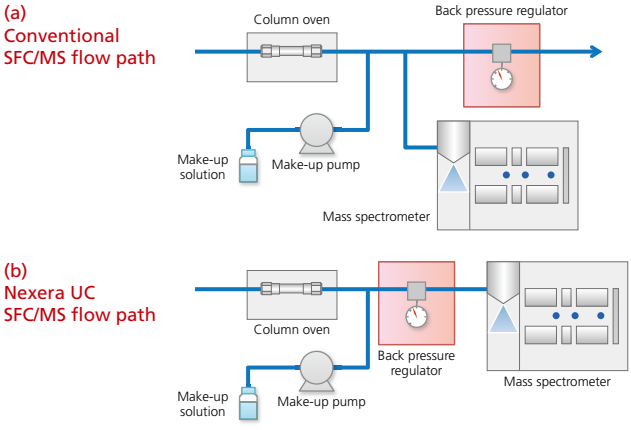
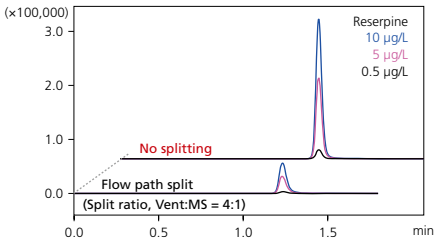


Fig. 7 SFC-MS Flow Path



Column : Shim-pack UC-Diol 4.6 mm I.D. × 150 mm L.
Modifier : MeOH
Modifier conc. : 30 %
Flow rate : 3.0 mL/min
Temperature : 40°C
Back pressure : 10 MPa
Injection volume : 1 µL
Sample : Reserpine (0.5, 5, 10 µg/L)
Detection : TQ mass spectrometer ESI(+) *m/z* 609.3 > 195.0

Fig. 8 Sensitivity With and Without Flow Path Splitting

Table 2 MS Reproducibility With and Without Flow Path Splitting

	Injection volume (µL)	Retention time		Area		Height	
		Ave.	%RSD	Ave.	%RSD	Ave.	%RSD
Flow path split	0.1	0.359	0.64	6,583	18.83	2,361	17.29
	1	0.356	0.25	81,467	4.26	26,656	3.88
	2	0.355	0.32	156,831	2.18	49,721	3.28
No splitting	0.1	0.356	0.09	16,264	6.18	7,673	6.17
	1	0.353	0.05	155,170	2.43	71,971	2.23
	2	0.35	0.07	325,739	1.16	142,350	1.19

Column : Shim-pack UC-Diol 4.6 mm I.D. × 150 mm L. 5 µm
Modifier : MeOH with 0.1 % w/v ammonium formate
Modifier conc. : 30 %
Flow rate : 2.0 mL/min
Temperature : 40°C
Back pressure : 10 MPa
Injection volume : 1 µL
Detection : TQ mass spectrometer ESI(–) *m/z* 351.20 > 271.20 (prostaglandin 100 µg/L)

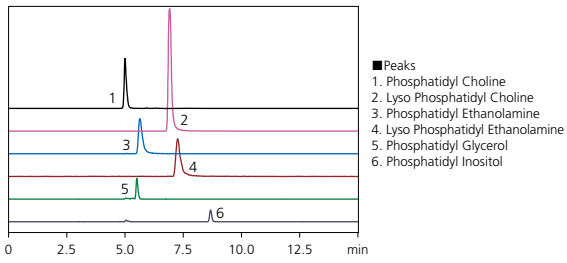
4. Shim-pack UCX Series Columns for SFC

Because of the high diffusivity of the mobile phase used in SFC, the separation behavior substantially changes based on the column stationary phase and modifiers used. The Shim-pack UCX series columns are designed for SFC and encompass eight different stationary phases, as shown in Table 3. This allows the columns to accommodate the separation of a wide variety of compounds.

Table 3 Shim-pack UCX Series Columns

	Functional group	Pore size	Surface area	Carbon content
Shim-pack UC-RP	Octadecyl group + polar functional group	10 nm	—	9%
Shim-pack UC-GIS II	Octadecyl group		450 m^2/g	11%
Shim-pack UC-Diol	Diol group			20%
Shim-pack UC-Sil	—			—
Shim-pack UC-Amide	Carbamoyl group			18%
Shim-pack UC-NH2	Aminopropyl group			8%
Shim-pack UC-Phenyl	Phenethyl group			9.5%
Shim-pack UC-CN	Cyanopropyl group			14%

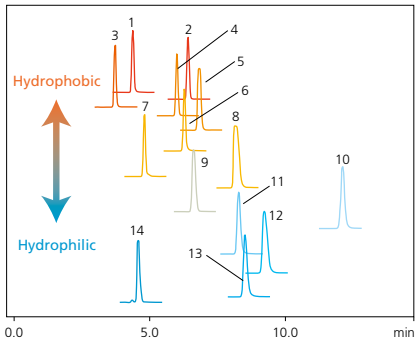
Fig. 9 shows an example analysis of phospholipids using the Shim-pack UCX-Diol column. This column allows separation of phospholipids by class, as with normal phase LC. Phospholipids can also be separated by molecular species using the same modifier conditions paired with a different column, such as the Shim-pack UCX-GIS II, which has an octadecyl group stationary phase. Using different stationary phases but the same mobile phase, SFC can be used to recreate the retention behaviors observed with normal phase and reverse phase HPLC, providing a variety of other separation behaviors. This is of substantial benefit for the analysis of complex samples.



Column : Shim-pack UC-Diol 4.6 mm I.D. × 150 mm L. 5 µm
Modifier : MeOH with 0.1 % w/v ammonium formate
Modifier conc. : 10 % (0 min) → 40 % (10–15 min)
Flow rate : 3.0 mL/min
Temperature : 40°C
Make-up solution : MeOH with 0.1 % w/v ammonium formate
Make-up flow rate : 0.1 mL/min
Back pressure : 10 MPa
Detection : TQ mass spectrometer (ESI)

Fig. 9 Phospholipid Analysis

Fig. 10 shows an example analysis of pesticides of a wide range of polarities—from hydrophobic to hydrophilic—using the Shim-pack UCX-RP column. The Shim-pack UCX-RP column is unique in having a stationary phase that combines octadecyl and polar functional groups. This stationary phase is able to retain a wide range of compounds, including both hydrophobic and hydrophilic compounds. This column allows the simultaneous analysis of pesticides that were previously difficult to analyze without changing the analytical conditions, thereby providing improved analytical efficiency.



No.	Compound	log *P*
1	Carbofuran	7.4
2	Ethofenprox	6.9
3	Fenpropathrin	6.0
4	Pyriproxyfen	5.0
5	Pyraclostrobin	4.0
6	Linuron	3.0
7	Aminocarb	1.9
8	Ethoxysulfuron	1.0
9	Halosulfuron methyl	0.0
10	Bentazone	–0.5
11	Chlorsulfuron	–1.0
12	Rimsulfuron	–1.5
13	Nicosulfuron	–1.8
14	Vamidothion	–4.2

Column : Shim-pack UC-RP 4.6 mm I.D. × 150 mm L. 5 µm
Modifier : MeOH with 0.1 % w/v ammonium formate
Modifier conc. : 0 % (0 min) → 10 % (11 min) → 30 % (14 min) → 40 % (14.01–17 min)
Flow rate : 3.0 mL/min
Temperature : 40°C
Make-up solution : MeOH with 0.1 % w/v ammonium formate
Make-up flow rate : 0.1 mL/min
Back pressure : 15 MPa
Detection : TQ mass spectrometer (ESI)

Fig. 10 Pesticide Analysis

5. Chiral Separation

In the field of pharmaceuticals, research is underway in the area of drug discovery using chiral columns for rapid chiral separation. Finding the appropriate combination of analytical column and mobile phase for a given analyte from the wide variety of chiral columns available requires a substantial amount of time and labor. Therefore, there is a demand to improve the speed of condition scouting for chiral separations.

The speed and labor required for scouting chiral compound separation conditions can be improved by combining Shimadzu's Nexera UC chiral screening system and the wide range of polysaccharide derivative CHIRALPAK and CHIRALCEL series chiral columns (Daicel Corporation).

The Nexera UC chiral screening system includes an SFC system, solvent switching valves, and column switching valves and is able to acquire comprehensive data by automatic and continuous screening of the modifier conditions on a maximum of 12 columns. Its mobile phase blending function can also mix up to four different solvents to user-defined ratios for analysis under a variety of separation conditions, which significantly simplifies the workflow of condition scouting for chiral compounds.

Also, Method Scouting Solution for Nexera UC is software that presents a graphical user interface environment developed to support the process of separation condition scouting for chiral compounds (Fig. 11). This software provides database management for analytical columns, mobile phases, and modifiers, which improves management efficiency and can reduce the number of operating errors that arise with multiple operators. The software provides powerful support for work related to chiral compound analysis, including work such as the calculation of required modifier and sample volumes, column washing, changeover of enclosed liquids at the end of analysis to prevent column degradation, and estimation of analysis completion times.

Here, we present chiral separation screening results for omeprazole obtained from all 36 possible combinations of the 12 chiral columns (Daicel Corporation) and three modifier conditions (Fig. 12, 13).

The Nexera UC chiral screening system utilizes SFC to select the mobile phase and optimize the separation conditions in a short time period, which improves R&D efficiency during the drug discovery stage of pharmaceutical production.

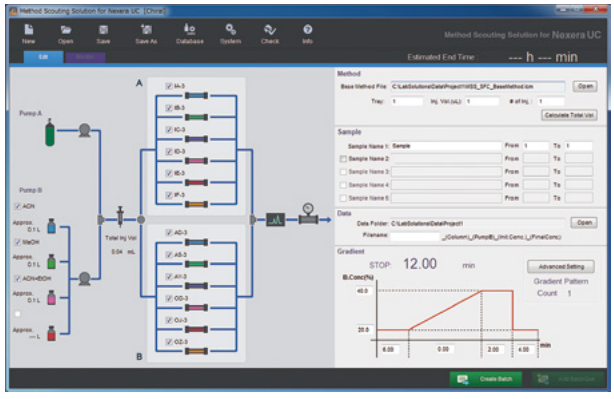


Fig. 11 Configuration Window of Method Scouting Solution Software

Column
(1) CHIRALPAK IA-3/SFC
(2) CHIRALPAK IB-3/SFC
(3) CHIRALPAK IC-3/SFC
(4) CHIRALPAK ID-3/SFC
(5) CHIRALPAK IE-3/SFC
(6) CHIRALPAK IF-3/SFC
(7) CHIRALPAK AD-3/SFC
(8) CHIRALPAK AS-3/SFC
(9) CHIRALPAK AY-3/SFC
(10) CHIRALCEL OD-3/SFC
(11) CHIRALCEL OJ-3/SFC
(12) CHIRALCEL OZ-3/SFC

3.0 mm I.D. × 100 mm L. 3 µm

×

Modifier
(1) MeOH
(2) EtOH
(3) Acetonitrile/EtOH 75/25 (V/V)

Modifier conc	:	20 %
Flow rate	:	3.0 mL/min
Temperature	:	40°C
Back pressure	:	10 MPa
Injection volume	:	1 µL
Detection	:	UV (300 nm)

Fig. 12 Screening Conditions

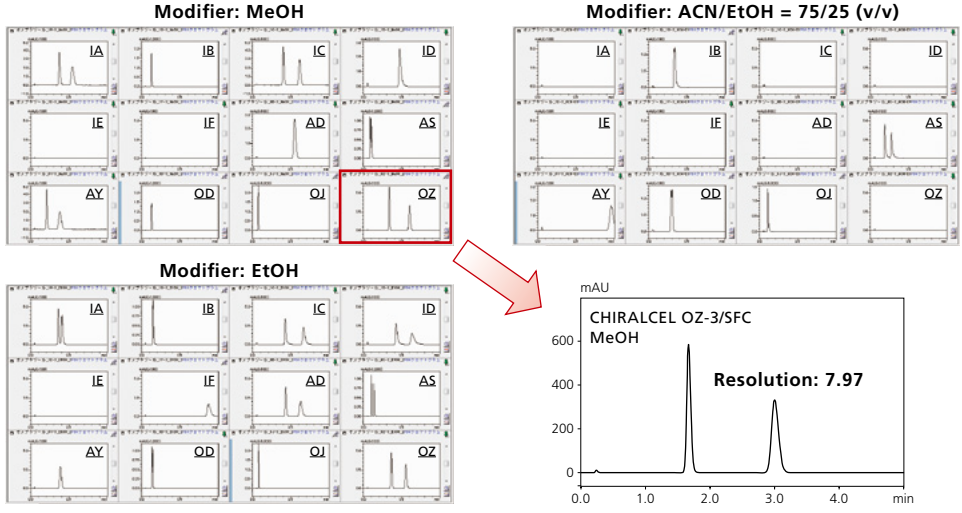


Fig. 13 Screening Results

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