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Supercritical Fluid Chromatography System / Ultra High Performance Liquid Chromatograph

Application News

Diversification of Separation Selectivity Using Supercritical Fluid Chromatography

-Efficient Search for Optimal Separation Conditions by Combining LC and SFC-

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User Benefits

- Because LC and SFC have different separation characteristics, SFC has the potential to separate compounds that are difficult to resolve by LC.
- Both LC and SFC analyses can be performed on a single system.
- By utilizing mobile phase and column switching valves, LabSolutions™ MD enables automated screening of mobile phases and columns.

■ Introduction

Supercritical Fluid Chromatography (SFC) is a separation technique that employs supercritical carbon dioxide as the primary mobile phase. Owing to its high diffusion coefficient and low viscosity, supercritical carbon dioxide allows high flow-rate delivery at lower column backpressure compared to Liquid Chromatography (LC), enabling shorter analysis time. It also provides excellent separation performance for structurally related compounds. However, since supercritical carbon dioxide has a polarity similar to that of hexane, it often lacks sufficient elution strength on its own. Therefore, organic solvents (modifiers) such as methanol, acetonitrile, 2-propanol, and ethanol are commonly added to the mobile phase to adjust polarity. By appropriately selecting and combining these modifiers, the interaction between analytes and the stationary phase can be finely tuned, resulting in diverse separation patterns. Because SFC and LC exhibit different retention behaviors, compounds that are difficult to separate by LC may be effectively separated by SFC. Accordingly, combining both techniques in method development can improve the efficiency of searching for optimal separation conditions. On the other hand, both column and mobile phase selection have a strong influence on retention behavior. To obtain optimal separation, it is ideal to examine multiple types of columns and mobile phases. However, this process is highly labor- and time-intensive. This study demonstrates efficient optimization of separation conditions using Nexera™ UC/s (LC/SFC switching system), which allows LC and SFC analyses to be performed on a single instrument, in combination with the LabSolutions MD, a dedicated software for supporting method development. Column and mobile phase screening were conducted using a mixed sample containing six small-molecule pharmaceutical compounds as a model sample.

■ Features of LC/SFC Switching System

Nexera UC/s enables both LC and SFC analyses on a single system (flow diagram: Fig. 1). The system is configured by adding a supercritical CO_2 delivery pump and backpressure regulator unit to a standard LC system. By switching the ON/OFF status of the delivery pump and backpressure, both LC and SFC analyses can be carried out. The organic solvent delivery pumps, autosampler, column oven, and detector can be shared between LC and SFC, enabling space-saving and cost-effective operation. Existing LC systems can also be upgraded. In combination with a PDA (photodiode array) detector and the single quadrupole mass spectrometer LCMS-2050, the system allows obtained mass data to be used for identification of known compounds and estimation of unknown impurities. When controlled by LabSolutions MD, up to 12 columns and 7 types of organic solvents (modifiers) can be automatically switched, enabling comprehensive screening for both LC and SFC and significantly reducing labor in method development.

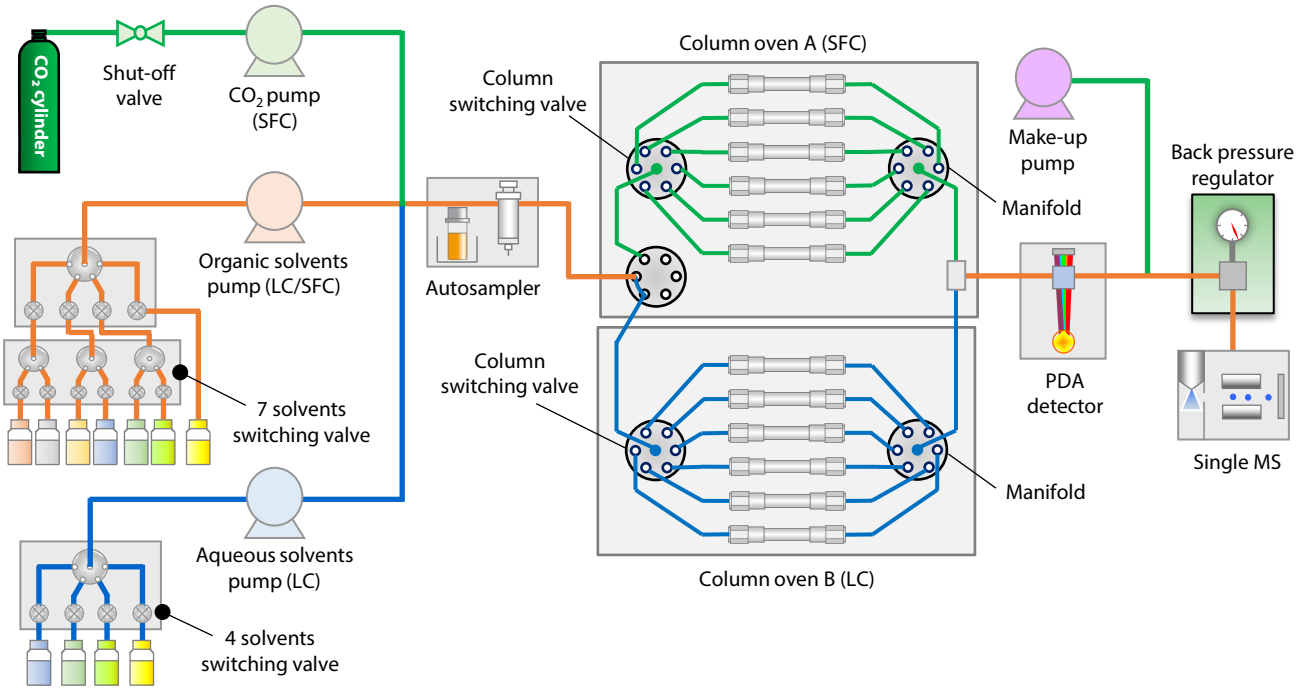

Fig. 1 LC/SFC Switching System (Nexera UC/s)
(Green: SFC flow path, Blue: LC flow path, Orange: Common flow path for LC and SFC)

■ Column and Mobile Phase Screening

A case study of screening to find the optimal combination of columns and mobile phases for SFC analysis is presented (screening conditions: Table 1; target compounds: Table 2). Three columns with different stationary phases were evaluated. Three modifier compositions (0%, 50%, and 100% acetonitrile in methanol) were tested. A comprehensive analysis schedule covering all 9 (3×3) combinations of columns and modifiers was generated to identify the optimal combination. LabSolutions MD enables error-free automatic generation of analysis schedules combining columns, mobile phases, and other parameters (steps (1)–(5) in Fig. 2). Column switching valves allow automatic selection of the desired column (step (2) in Fig. 2). Modifier composition changes are automatically prepared using the mobile phase blending function by simply selecting the desired composition (step (1) in Fig. 2). This reduces manual preparation work and prevents errors. LC and SFC switching is performed automatically by executing the analysis schedule generated in LabSolutions MD. The specified switching method (Fig. 3) automatically replaces the flow path, allowing consecutive analyses without manual intervention or influence from the previous analysis.

Table 1 Screening Conditions

System	: Nexera UC/s (SFC)
Column 1	: Shim-pack™ UC-Poly BT *1
Column 2	: Shim-pack UC-PBr *2
Column 3	: Shim-pack UC-ODS *3
	(150 mm × 3.0 mm I.D., 3.0 µm)
Column Temp.	: 30 °C
Injection volume	: 5 µL (100 mg/L)
Sample solvent	: Methanol
Mobile phases	
Pump A (CO2)	: CO2
Pump B (Organic)	
– Line A	: Methanol
– Line B	: Acetonitrile
– Line C	: 100 mmol/L ammonium formate in methanol
– Line D	: Water
Flow rate	: 1 mL/min
Time program (%B)	: 5% (0 min) →70% (5 - 7 min)→5% (7.01-12 min)
Detection	: 254 nm (SPD-M40、high-pressure flow cell)
BPR pressure	: 10 MPa
BPR Temp.	: 50 °C
System	: LCMS-2050
Ionization	: ESI/APCI (DUIS™)、positive and negative mode
Mode	: SCAN (*m/z* 100-1000)
Nebulizing gas	: 2.0 L/min (N_2)
Drying gas	: 5.0 L/min (N_2)
Heating gas	: 7.0 L/min (N_2)
DL temp.	: 200 °C
Desolvation temp.	: 450 °C
Interface Voltage	: +3.0 kV / -2.0 kV
Make-up pump	
Mobile phase	: Methanol
Flow rate	: 0.1 mL/min

*1 P/N：227-32501-03, *2 P/N：227-32602-09
*3 P/N：227-32608-24

Table 2 Target Compounds

	Name	Monoisotopic mass *
(A)	Antipyrine	188
(B)	Probenecid	285
(C)	Naproxen	230
(D)	Hydrocortisone	362
(E)	Clozapine	326
(F)	Indomethacin	357

* rounded to the nearest unit mass

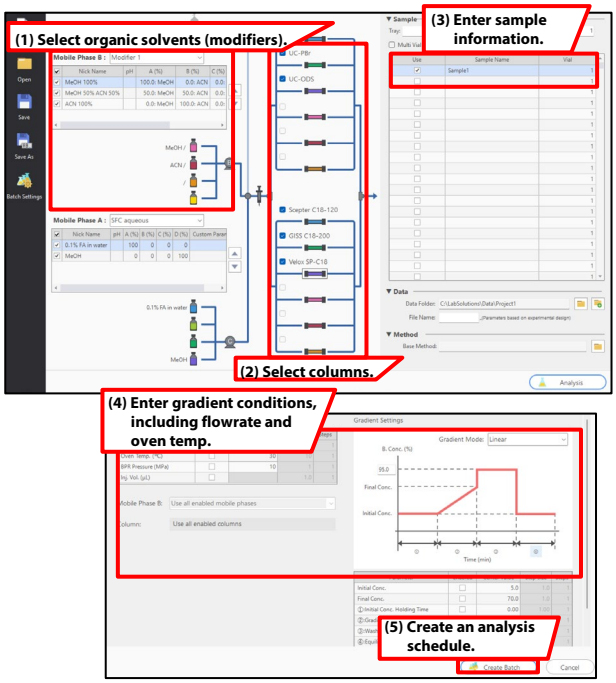

Fig. 2 Easy Creation of Analysis Schedule by LabSolutions MD

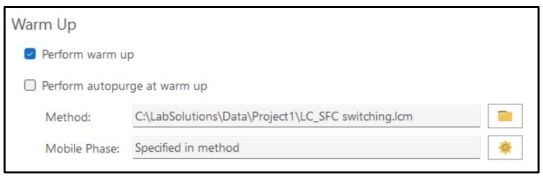

Fig. 3 Method Selection Screen for LC/SFC Switching

■ Results of Screening

The screening results are shown in Fig. 4. Changing columns or modifier compositions significantly affected retention and peak shapes of all compounds.

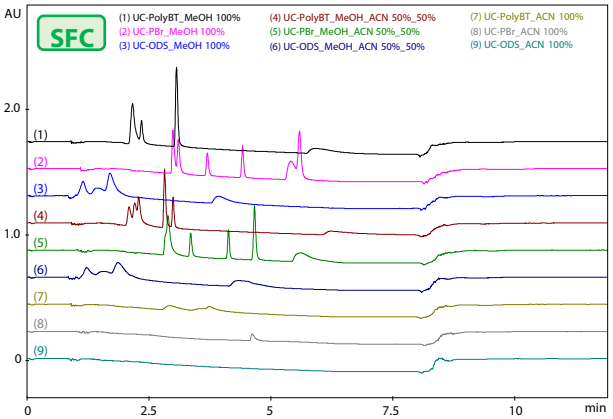

Fig. 4 Results of Column and Mobile Phase Screening

■ Quickly Find Optimal Condition

Because screening generates as many chromatograms as the number of analysis schedule, they must be evaluated to determine which one is the optimal. Checking all chromatograms manually is time-consuming. LabSolutions MD can quickly and easily find optimal condition using equation (Eq. 1) below to quantitatively evaluate the separation.

$$(\text{Evaluation Value}) = P \times (Rs_1 + Rs_2 + \ldots + Rs_{P-1}) \qquad \text{(Eq. 1)}$$

Evaluation Value is calculated as the number of peaks detected (P) multiplied by the sum of resolution factor (Rs) for all peaks. Fig. 5 shows Evaluation Value obtained through column and mobile phase screening and listed in the order from the highest to the lowest. Fig. 6 shows the chromatogram of the highest Evaluation Value.

SFC

Column Nick Name	MPB Nick Name	Evaluation Value
UC-PBr	MeOH 100%	102.253
UC-PolyBT	MeOH_ACN 50%_50%	82.573
UC-PBr	MeOH_ACN 50%_50%	80.245
UC-PolyBT	MeOH 100%	45.530
UC-ODS	MeOH 100%	26.823
UC-ODS	MeOH_ACN 50%_50%	6.968
UC-PolyBT	ACN 100%	2.818
UC-PBr	ACN 100%	0.000
UC-ODS	ACN 100%	0.000

Fig. 5 Ranking of Each Condition by Evaluation Value

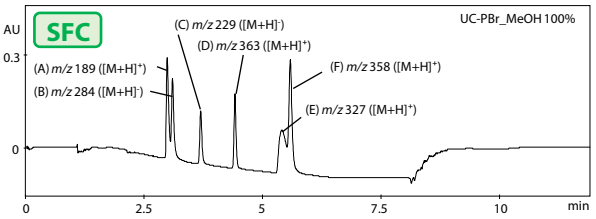

Fig. 6 Chromatogram with Highest Evaluation Value
(A) Antipyrine、(B) Probenecid、(C) Naproxen
(D) Hydrocortisone、(E) Clozapine、(F) Indomethacin

The optimal condition was a UC-PBr column with 100% methanol as the modifier. The clozapine peak (Fig. 6E) exhibited slight tailing. Subsequently, additives were added to the modifier in an attempt to improve separation and peak shape.

■ Improvement of Separation and Peak Shape Using Additives

Based on the condition with the highest Evaluation Value (UC-PBr, 100% methanol), ammonium formate (20 mmol/L) was added to methanol, and water was further added at 0%, 2%, and 4% to evaluate the effect on separation of each compound. The resulting chromatograms are shown in Fig. 7. Adding ammonium formate sharpened the clozapine peak (Fig. 7(2)). Increasing water content improved separation between clozapine (E) and indomethacin (F), with the best separation observed at 4% water (Fig. 7(4)).

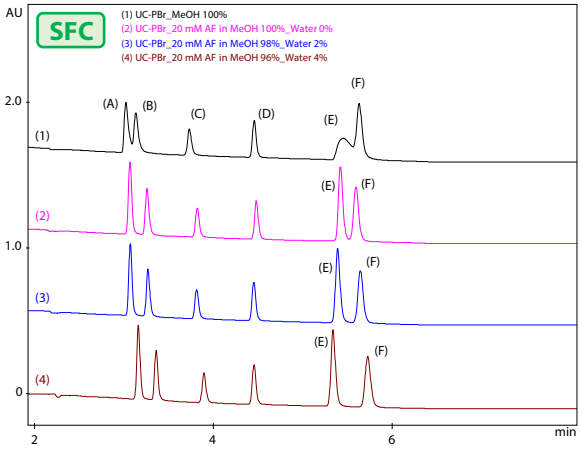

Fig. 7 Chromatograms with Varying Modifier Compositions
(A) Antipyrine、(B) Probenecid、(C) Naproxen
(D) Hydrocortisone、(E) Clozapine、(F) Indomethacin

Fig. 8 compares SFC chromatograms after modifier optimization (Upper) and LC chromatograms using water-based (0.1% formic acid) and methanol-based mobile phases (Lower). Mass data obtained by LCMS-2050 indicated that elution orders of compounds differ significantly between LC and SFC. Mass detection enables identification even when elution orders differ. SFC with ammonium formate and water in the modifier achieved good separation and peak shapes for all compounds. The results suggest that compounds difficult to separate by LC have the potential to be separated by SFC.

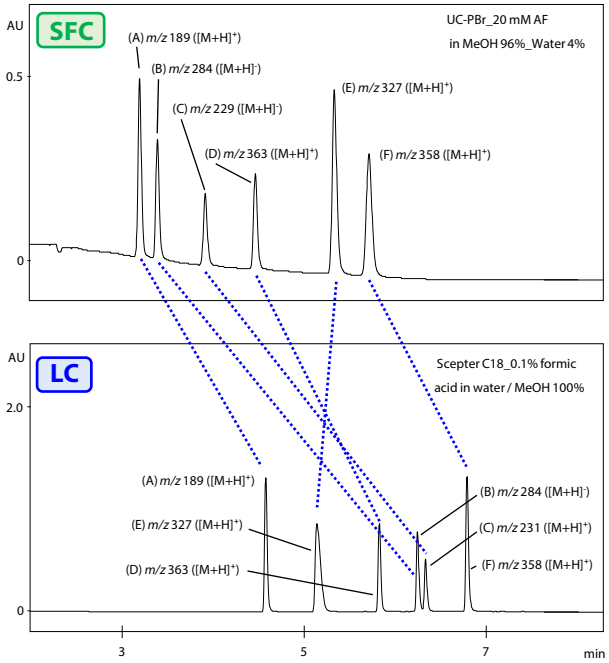

Fig. 8 Comparison of Chromatograms After Modifier Composition Optimization
SFC (Upper), LC (Lower)
(A) Antipyrine、(B) Probenecid、(C) Naproxen
(D) Hydrocortisone、(E) Clozapine、(F) Indomethacin

■ Conclusion

Because SFC and LC have different separation characteristics, combining both techniques in method development can improve the efficiency of finding optimal separation conditions. Nexera UC/s allows automated switching between LC and SFC analyses on a single system. Using the LCMS-2050, mass-based identification of known compounds and estimation of unknown impurities is possible. Combining this with LabSolutions MD enables automated switching of up to 12 columns and 7 modifiers, allowing comprehensive condition screening for both LC and SFC and significant labor savings. This article demonstrated the use of column and mobile phase screening for efficient method development using both LC and SFC. In SFC, gradient conditions, column oven temperature, and backpressure settings also contribute to separation selectivity, and optimization of these parameters is important. For details on a complete workflow for efficient SFC method development, please refer to Application News "01-01057."

01-01058-EN First Edition: Nov. 2025

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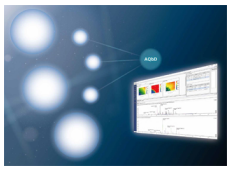

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