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Application News

Supercritical Fluid Chromatography System
Software for Efficient Method Development

Seamless Purification Workflow Enabled by Supercritical Fluid Chromatography

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User Benefits

- SFC enables high flow rate operation at lower column back pressure than LC, resulting in shorter analysis time and improved throughput in preparative purification.
- Because the supercritical carbon dioxide rapidly evaporates during fraction collection, the labor required for post-purification drying and powderization can be greatly reduced.
- Optimization of separation conditions can be streamlined by LabSolutions™ MD.

■ Introduction

Supercritical Fluid Chromatography (SFC) is a separation technique that uses supercritical carbon dioxide as the primary mobile phase. Owing to its high diffusion coefficient and low viscosity, it enables high flow-rate delivery at lower column backpressure than liquid chromatography (LC), allowing shorter analysis time. Preparative LC is widely used in pharmaceuticals, food, and chemical industries for purifying target compounds, and SFC can also be applied to preparative purification. In preparative LC, post-treatment such as solvent evaporation and concentration is typically required after fraction collection. In contrast, in preparative SFC the supercritical carbon dioxide used as the mobile phase readily evaporates under ambient conditions, significantly reducing the effort required for concentration processes. To recover target compounds with high purity, separation conditions that adequately resolve them from other components and impurities must be established. Because preparative conditions consume large amounts of sample and mobile phase, separation conditions are typically optimized at the analytical scale before scale-up for fractionation. This article presents an example of applying SFC to a seamless preparative purification workflow (Fig. 1). In addition, LabSolutions MD, a dedicated software for supporting method development, is used for efficient optimization of separation conditions.

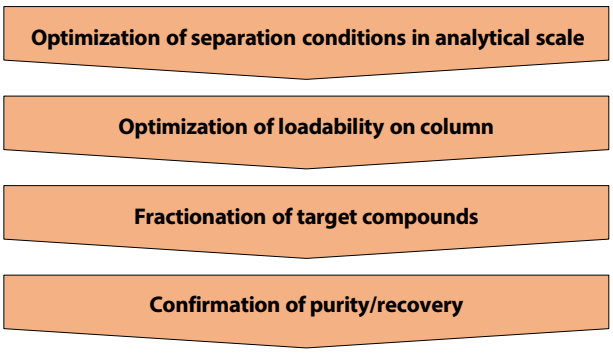

Fig. 1 Workflow of Preparative Purification

■ Overview of the Analytical and Preparative SFC System

The flow path of the analytical and preparative SFC systems is shown in Fig. 2. The analytical system (upper part of Fig. 2) is used for the optimization of separation conditions at the analytical scale, evaluation of sample loading, and confirmation of purity and recovery. The preparative system (lower part of Fig. 2) is used for the fractionation of target compounds. By combining the single quadrupole mass spectrometer LCMS-2050, mass information of the target compound can be obtained during the optimization of separation conditions. In addition, MS signals can be used as triggers during fractionation. To achieve both fractionation and MS detection, the flow path is split and a portion of the mobile phase is introduced into MS using a make-up pump. In this study, a seamless preparative purification workflow was demonstrated using the systems with a five-component mixture of small-molecule pharmaceuticals (target compound for fractionation: Naproxen) as a model sample.

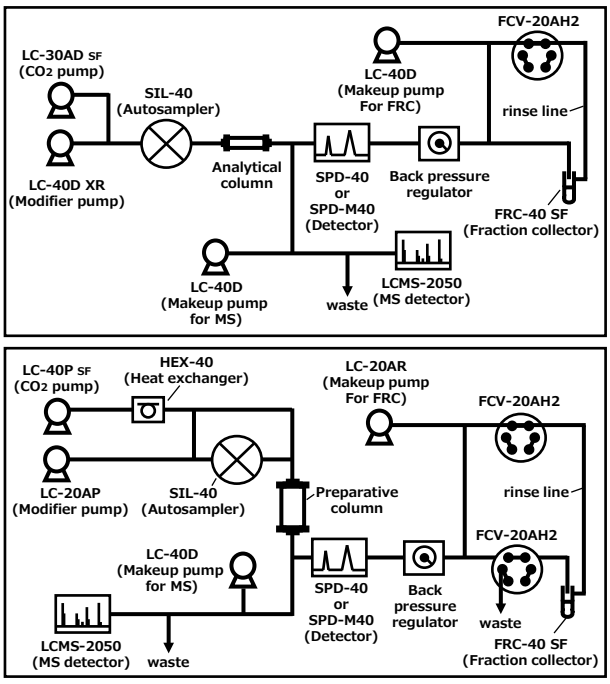

Fig. 2 Analytical SFC System "Nexera UC" (Upper)
Preparative SFC System "Nexera UC Prep" (Lower)

■ Optimization of Separation Conditions in Analytical Scale

The separation conditions for the target compound (Naproxen) were optimized using the analytical system. UV chromatogram before optimization (analytical conditions: Table 1) is shown in Fig. 3, where Disopyramide elutes close to Naproxen. Increasing the sample load may further deteriorate the separation and reduce the purity of the recovered fraction. Therefore, improvement of the separation is essential. Separation was optimized by varying the gradient conditions in nine profiles (three levels each for the initial concentration and gradient slope). LabSolutions MD, which automatically generates analysis schedules with systematically varied LC parameters, was used to streamline this investigation (Fig. 4). Because supercritical carbon dioxide enables high flow-rate delivery at low column backpressure, the flow rate was set to 2.5 mL/min for the column used in this study (4.6 mm i.d.), reducing the analysis time to about 10 minutes per run. The chromatograms are shown in Fig. 5.Considering the analysis time, the condition that improved the resolution between Naproxen and Disopyramide (initial concentration: 15%; gradient slope: 10 min, Fig. 5(2)) was selected as the base condition for evaluating sample loading. During separation optimization, mass information can also be obtained using LCMS-2050, which can be used to confirm synthesized compounds and estimate unknown impurities.

* In SFC analysis, in addition to gradient conditions, parameters such as modifier composition and column oven temperature also affect separation selectivity. For further information on efficient separation optimization, refer to Application News No. 01-01057.

Table 1 Analytical Conditions

Mobile Phases	
Pump A	: CO_2
Pump B	: 20 mmol/L ammonium formate in methanol
Column	: Shim-pack UC-PBr (250 mm × 4.6 mm I.D., 5 µm)*1
Sample	: (A) Antipyrine, (B) Probenecid, (C) Naproxen, (D) Disopyramide, (E) Betamethasone
Sample concentration	: 500 mg/L (C), 125 mg/L (D), 50 mg/L (A, B, E)
Sample solvent	: Methanol
Injection volume	: 10 µL
Flowrate (MS makeup)	: 2 mL/min (Methanol)
Flowrate (FRC makeup)	: 0.8 mL/min (Methanol)
SFC Conditions	
Time program	: B Conc. 15%(0 min)→50%(10 min) →15%(10-13 min)
Column Temp.	: 25 ˚C
Flowrate	: 2.5 mL/min
Sample loop size	: 50 µL
Detection (UV)	: 254 nm (SPD-40, high-pressure flow cell)
BPR pressure	: 10 MPa
BPR Temp.	: 50 ˚C
MS conditions	
Ionization	: ESI/APCI (DUIS), positive and negative
Mode	: SCAN (*m/z* 100-500)
Nebulizing gas flow	: 2.0 L/min (N_2)
Drying gas flow	: 5.0 L/min (N_2)
Heating gas flow	: 7.0 L/min (N_2)
DL Temp.	: 200 ˚C
Desolvation Temp.	: 100 ˚C
Interface voltage	: 3.0/-2.0 kV (positive/negative)

*1 P/N : 227-32602-02

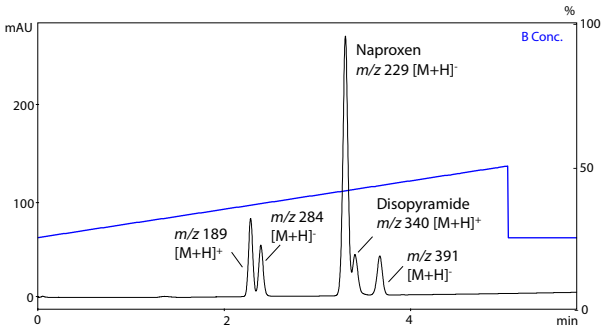

Fig. 3 UV Chromatogram Before Optimizing the Separation

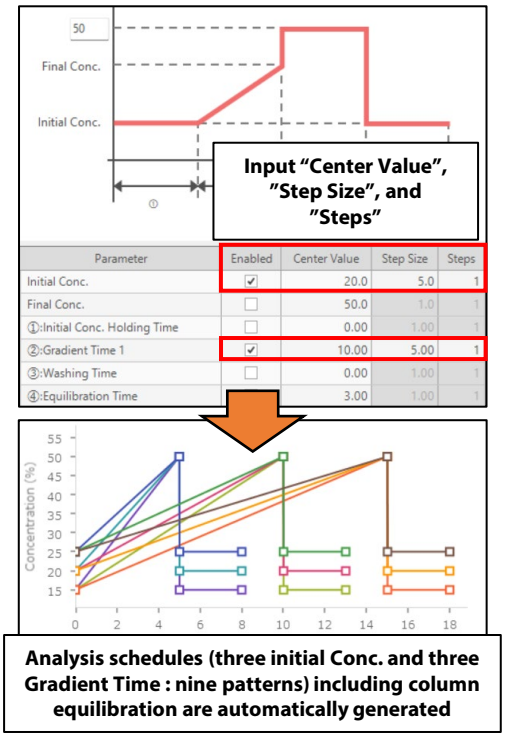

Fig. 4 Automatic Generation of Analysis Schedules by LabSolutions MD

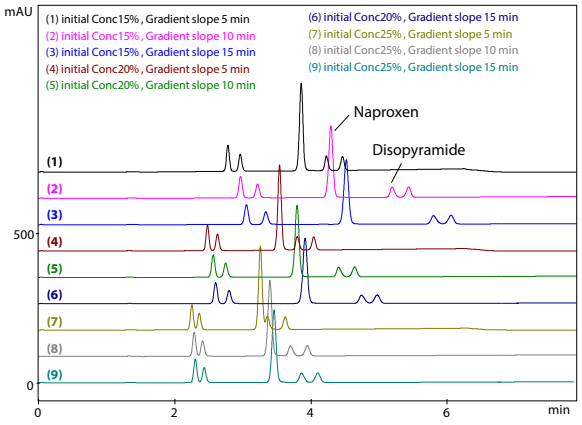

Fig. 5 Results of Optimizing Separation Conditions by LabSolutions MD

■ Optimization of Loadability on Column

Fig. 6 shows the results of loadability evaluation conducted at injection volumes of 5, 10, 15, 20, and 25 µL using Naproxen (5000 mg/L) under the optimized conditions at the analytical scale (Fig. 5(2)). Even at the maximum injection volume of 25 µL, the separation between Naproxen and Disopyramide remained sufficient. Therefore, scaling-up was performed using an injection volume of 25 µL, followed by preparative fractionation.

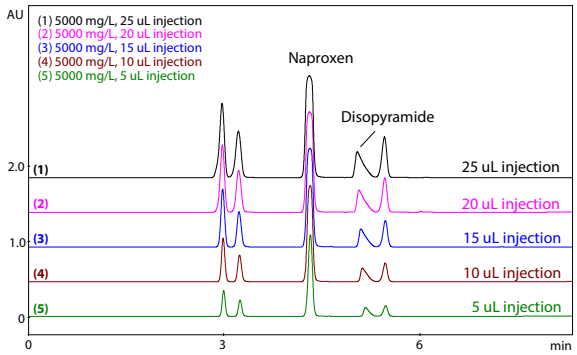

Fig. 6 Results of Evaluation of Sample Loading Capacity

■ Fractionation of Target Compounds

Naproxen was fractionated using a UV trigger. The preparative conditions are presented in Table 2 (only the parameters differing from those in Table 1 are listed). Based on the cross-sectional area ratio (approximately 20-fold) between the preparative column (20 mm I.D.) and the analytical column (4.6 mm I.D.), the flow rate was scaled up to 50 mL/min (with a constant linear velocity before and after scaling-up), and the injection volume was increased to 500 µL. UV chromatogram obtained after scaling-up is shown in Fig. 7 (the blue area represents the fractionation area). A separation profile similar to that obtained at the analytical scale was observed, allowing Naproxen to be fractionated while maintaining sufficient separation from Disopyramide. In addition, because the supercritical carbon dioxide in the mobile phase evaporates during fractionation, the collected sample can be recovered in a concentrated state. When scaling up from analytical to preparative analysis, various parameters must be calculated and transferred to create a preparative method file. However, this process is often labor-intensive and prone to transcription errors. LabSolutions MD automatically calculates the required parameters for method transfer and generates a preparative method file reflecting these parameters (Steps (1)–(4) in Fig. 8). By simply selecting the target system (Fig. 8(1)) and entering the column size (Fig. 8(2)) and flow rate (Fig. 8(3)), a preparative method file can be automatically generated, significantly reducing manual operations.

Table 2 Preparative Conditions

Column	: Shim-pack UC-PBr (250 mm × 20 mm I.D., 5 µm)*1
Sample Concentration	: 5000 mg/L (C), 1250 mg/L (D), 500 mg/L (A, B, E)
Injection Volume	: 500 µL
SFC Conditions	
Flowrate	: 50 mL/min
Flowrate (FRC makeup)	: 5 mL/min (Methanol)
Heat exchanger Temp.	: 25 °C
Sample loop size	: 2 mL
Syringe size	: 2.5 mL
Detection (PDA)	: 254 nm (SPD-M40, high-pressure flow cell)

*1 P/N : 227-32602-04

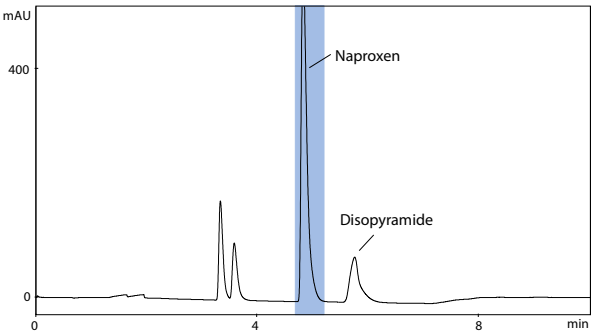

Fig. 7 UV-triggered Preparative Chromatogram
*Fractionation area is colored blue.

■ Confirmation of Purity/Recovery

Fig. 9 shows the chromatogram obtained when the fractionated Naproxen was re-injected into the analytical system, together with the chromatogram of a mixed standard solution prepared at the same concentration as the fractionated Naproxen (used as a reference for recovery calculation). The purity and recovery of the fractionated Naproxen are summarized in Table 3. Favorable results were obtained for both purity and recovery. Although a high purity (Table 3) was achieved in this study using UV-triggered fractionation, MS-triggered fractionation can be effective for recovering target compounds with higher purity when impurities elute close to the target compound. For further information on MS-triggered fractionation, refer to Application News No. 01-00651.

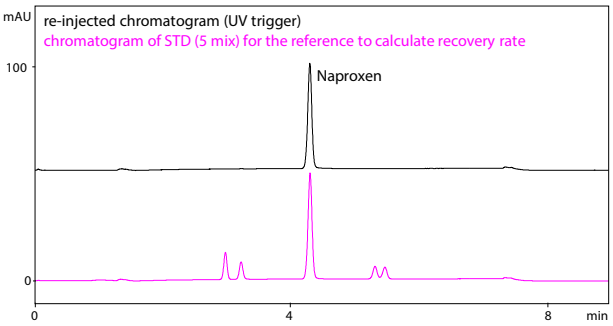

Fig. 9 Re-injected Chromatogram (Upper)
Chromatogram of STD (Lower)

Table 3 Purity and Recovery Rate of Fractionated Naproxen

	Purity (Area %)	Recovery Rate (%)
Naproxen	100	99.4

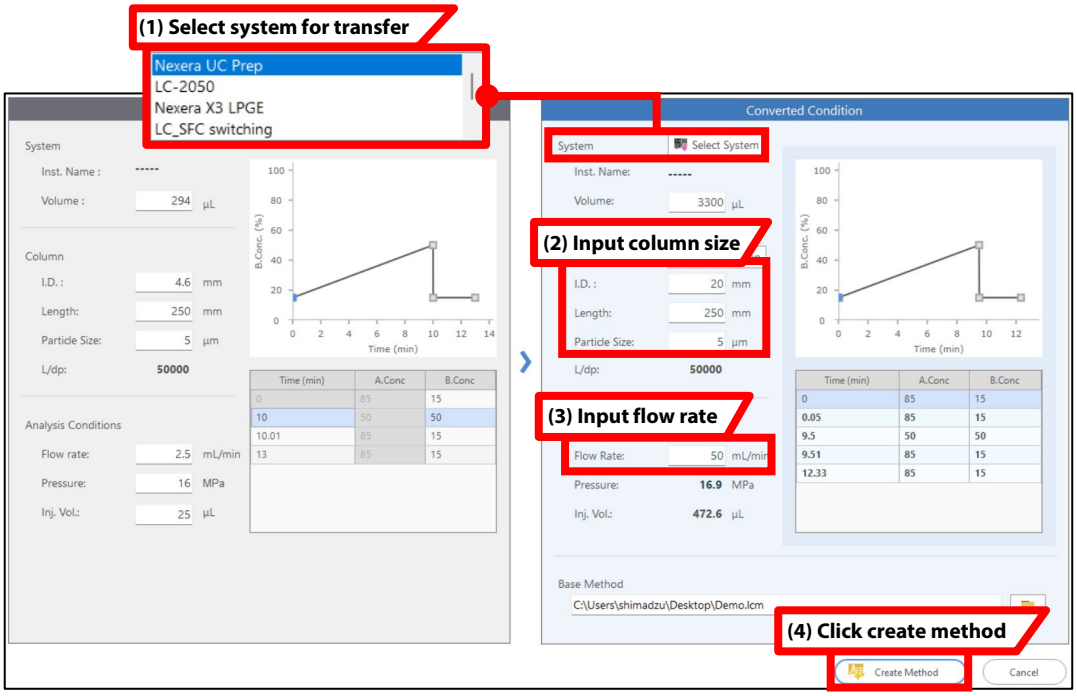

Fig. 8 Automatic Generation of Preparative Method File by LabSolutions MD

■ Fractionation at the Analytical Scale

Although not included in the preparative purification workflow shown in Fig. 1, fractionation at the analytical scale can also be performed using the analytical SFC system. This approach is useful when only a small amount of fraction (e.g., several milligrams) is required for purposes such as confirmation of synthesized compounds. Analytical-scale fractionation can be achieved by connecting a fraction collector (FRC-40 SF) to the analytical SFC system (flow path diagram: Fig. 2, upper). In analytical-scale fractionation, target compounds can be collected directly into 1.5 mL vials or 96-well plates. For example, when fractionated into a 1.5 mL vial, the collected sample can be directly used for purity analysis, eliminating the need for transferring the sample from the collection container. During SFC fractionation, rapid volume expansion of carbon dioxide as it transitions from the supercritical state to the gas phase can cause splashing of the collected fraction, which may reduce recovery. By using the gas–liquid separator LotusStream™ (Fig. 10), carbon dioxide is vented outward while the collected liquid flows down the column and drips vertically, enabling high recovery while suppressing sample scattering and carryover. As a reference, Fig. 11 shows the result of MS-triggered fractionation of Naproxen under the optimized analytical-scale conditions (Fig. 5(2)). Fig. 12 shows the chromatogram obtained when the fractionated Naproxen was re-injected, together with the chromatogram of a mixed standard solution prepared at the same concentration as the fractionated Naproxen (used as a reference for recovery calculation). The purity and recovery of the fractionated Naproxen are summarized in Table 4. Favorable results were obtained for both purity and recovery, even in analytical-scale fractionation.

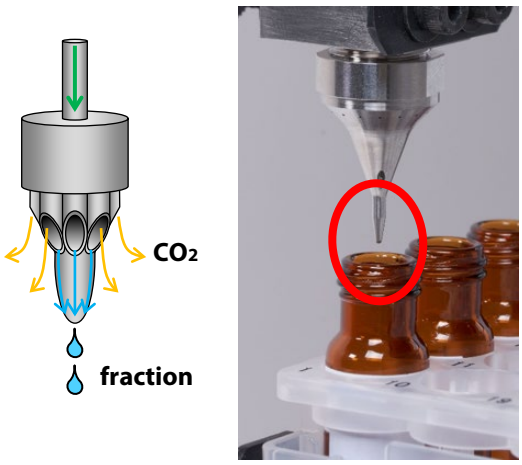

Fig. 10 Structure of Gas–Liquid Separator LotusStream (Left)
Appearance During Fractionation (Right)

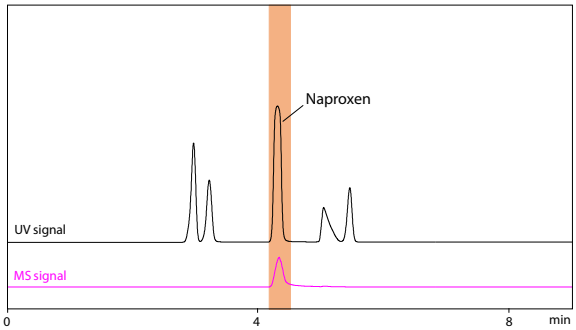

Fig. 11 Preparative Chromatogram Obtained by MS-trigger
*The orange area indicates the fractionation area.

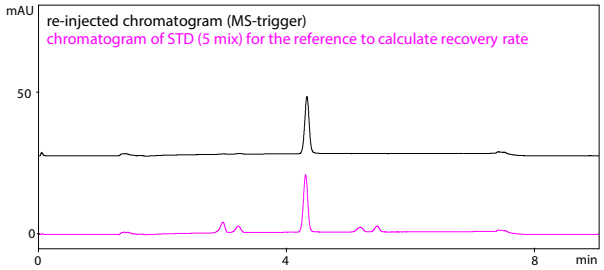

Fig. 12 Re-Injected Chromatogram for Purity/Recovery Confirmation (Upper)
Chromatogram of the Mixed Standard Solution (Lower)

Table 4 Purity and Recovery Rate of Fractionated Naproxen

	Purity (Area %)	Recovery Rate (%)
Naproxen	100	101.1

■ Conclusion

A seamless preparative purification workflow using SFC was demonstrated with a five-component mixture of small-molecule pharmaceuticals as a model sample. Compared with LC, SFC enables high flow-rate delivery at lower column backpressure, allowing shorter analysis time. As a result, throughput in preparative purification can be improved. In LC, post-treatment processes such as drying and powderization are typically required after fractionation. In contrast, in SFC the supercritical carbon dioxide used as the mobile phase evaporates during fractionation, significantly reducing the labor required for post-fractionation processing. Furthermore, the automatic analysis schedule generation function of LabSolutions MD streamlines the optimization of separation conditions, and the mass information obtained using LCMS-2050 can be utilized as qualitative information for confirmation of synthesized compounds. These results demonstrate that the use of SFC can improve the efficiency of the overall preparative purification workflow. Although this article presented an example of applying SFC to preparative purification, an example of a preparative purification workflow using LC can be found in Application News No. 01-00937.

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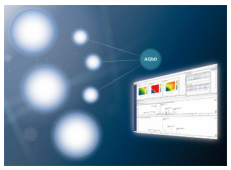

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