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Technical Report

Improving Separation and Method Development Efficiency Using the Nexera UC/s UHPLC/SFC Switching System

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Abstract:

Pharmaceuticals, foods, environmental testing, and many other fields require a wide variety of separation methods, such as for separating chiral compounds and structural isomers. Due to the difference in separation selectivity between ultra high performance liquid chromatography (UHPLC) and supercritical fluid chromatography (SFC) using supercritical carbon dioxide and organic solvents, SFC has been highly anticipated in recent years as a new separation method. By combining both UHPLC and SFC, the Nexera UC/s UHPLC/SFC switching system enables both UHPLC and SFC analysis using a single system. Since SFC has different separation characteristics than UHPLC, it can improve separation between isomers. By using two separation methods, UHPLC and SFC, for screening during the method development process, the system can result in configuring superior analytical conditions more quickly.

Keywords: Supercritical fluid chromatography (SFC), ultra high performance liquid chromatography (UHPLC), and switching

1. Supercritical Fluid Chromatography

Supercritical fluid chromatography (SFC) is a separation technique that uses a supercritical fluid as the primary mobile phase. Because supercritical fluids have lower viscosity and higher diffusivity, they result in lower column back pressure than separation by UHPLC. That means they can be used for high speed analysis and high separation analysis at high flowrates.

Typically, the supercritical fluid used for SFC is supercritical carbon dioxide. The polarity of supercritical carbon dioxide is known to be similar to hexane. However, because it is difficult to elute target components from the column using supercritical carbon dioxide alone, an organic solvent is added to the mobile phase as a modifier. That changes the polarity of the mobile phase, so that the target components can be eluted from the column.

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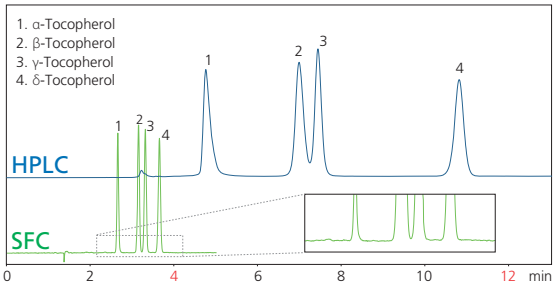
2. Differences Between HPLC and SFC Separation Characteristics

SFC column efficiency does not decrease as much as HPLC, even at high flowrates. Therefore, SFC enables high speed analysis and shorter analysis times, but can result in a different elution order and different separation selectivity. SFC can improve separation of even the compounds that are difficult to separate by HPLC.

(1) Shorter Analysis Time

Due to the low viscosity and high diffusivity of supercritical carbon dioxide, SFC column back pressure is low even at high flow rates, which enables analysis speed to be increased without losing column efficiency. As shown in Fig. 1, using a column packed with the same particle size as HPLC, SFC can achieve three to five times shorter analysis times than HPLC, without sacrificing separation.



Normal Phase HPLC Conditions
Column : Shim-pack HRC-SIL (4.6 mm I.D. × 250 mm long, 5 µm)
Mobile Phase : HEX/IPA = 99/1 (V/V)
Flowrate : 1 mL/min
Temperature : 40 °C
Detection : UV 290 nm

SFC Conditions
Column : Shim-pack UC-SIL (4.6 mm I.D. × 250 mm long, 5 µm)
Modifier : MeOH
Modifier ratio : 5 %
Flowrate : 3.5 mL/min
Temperature : 40 °C
Detection : UV 290 nm
Back pressure : 10 MPa

Fig. 1 Comparison of HPLC and SFC

(2) Elution Order

HPLC and SFC have different elution characteristics. Fig. 2 shows HPLC and SFC Chromatograms for three types of drug components. An ODS column was used for both HPLC and SFC, but the elution order was different. Components with high retention by HPLC are eluted quickly by SFC, which results in clear peaks that enable highly sensitive detection. SFC is also useful for changing the elution order overlapping contaminant components.

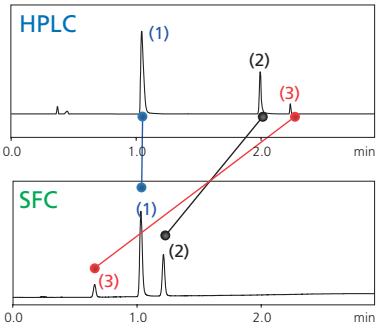


Fig. 2 Separation of Three Types of Drug Components (Upper: HPLC and Lower: SFC)

1 Analytical & Measuring Instruments Division

(3) Improved Separation Selectivity

Since SFC has different separation patterns than HPLC, SFC can improve separation between isomers. Fig. 3 shows comparison of HPLC and SFC separation using structural isomers. Triterpenes oleanic acid and ursolic acid were inadequately separated by HPLC, but fully separated by SFC. Consequently, the differences in separation selectivity for HPLC and SFC can be used effectively to improve the separation of isomers and contaminant components that were difficult to separate.

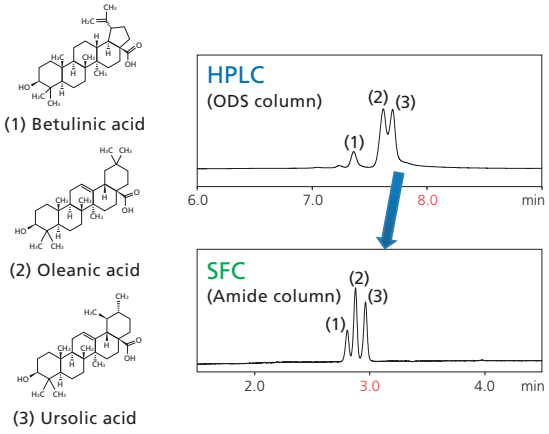


Fig. 3 Separation of Structural Isomers

3. UHPLC/SFC Switching System

When considering separation conditions, using both UHPLC and SFC can help determine even better separation conditions. Therefore, the Nexera UC/s UHPLC/SFC switching system provides the ability to use both UHPLC and SFC analysis modes in a single system. Fig. 4 shows a flow diagram for this system. The system was configured by adding a supercritical carbon dioxide delivery unit and back pressure regulator unit to a standard UHPLC system. Both UHPLC and SFC analysis modes can be used by switching (control mode ON or OFF) of delivery units and switching the back pressure regulator pressure.

Sharing the solvent delivery unit (for pumping organic solvents), autosampler, column oven, and detector for both SFC and UHPLC analysis minimizes space requirements and equipment cost and improves the equipment utilization rate. In addition, an existing UHPLC system can be upgraded to this system. In addition, by using the mobile phase solvent switching valve in combination with the column switching valve, mobile phase conditions can be changed automatically and continuously for up to twelve columns to enable a wide variety of condition that improve method development efficiency.

4. Evaluating Separation of Enantiomer Using Two Types of Separation Methods

Synthesizing new drugs in pharmaceutical fields requires the efficient separation of enantiomer. The optimization of column and mobile phase requires extremely large amounts of time and effort. Therefore, a more efficient method scouting process is needed.

The following describes an example of using the UHPLC/SFC switching system to increase the speed of method scouting. The Nexera UC/s UHPLC/SFC switching system was used to automatically optimize the separation conditions for two chiral compounds (omeprazole and warfarin). A total of 36 combinations of six chiral columns (CHIRALPAK® series) and the three mobile phases shown in Tables 1 and 2 (18 combinations each for UHPLC and SFC) were measured automatically.

Method scouting results for omeprazole are shown in Fig. 5. The two chiral compounds can be separated using several of the UHPLC or SFC condition. A visual comparison of chromatograms was prepared easily using the Quant Browser function in LabSolutions, as shown in Fig. 5.

Table 1 UHPLC Analytical Conditions for Chiral Compounds

No.	Mobile phase (Upper: A and Lower: B)	Others
1	Hexane Ethanol	B Conc. (%) : 20% (Isocratic) Flow Rate : 2 mL/min Column Temperature : 40 °C Inj. Vol. : 1 µL Detection : PDA@220 nm Step GE 0 - 6 min 20% Analysis 6 – 8 min 40% Column washing 8 – 12 min 20% Equilibration
2	Hexane Isopropyl alcohol	
3	Methyl tertiary butyl ether Ethanol	

Table 2 SFC Analytical Conditions for Chiral Compounds

No.	Modifier	Others
1	Methanol	Modifier Conc. (%) : 20% (Isocratic) Flow Rate : 3 mL/min Column Temperature : 40 °C Inj. Vol. : 1 µL BPR Press : 10 MPa Detection : PDA@220 nm Step GE 0 - 5 min 20% Analysis 5 – 7 min 40% Column washing 7 – 10 min 20% Equilibration
2	Ethanol	
3	Acetonitrile / Ethanol = 75 / 25 (v/v)	

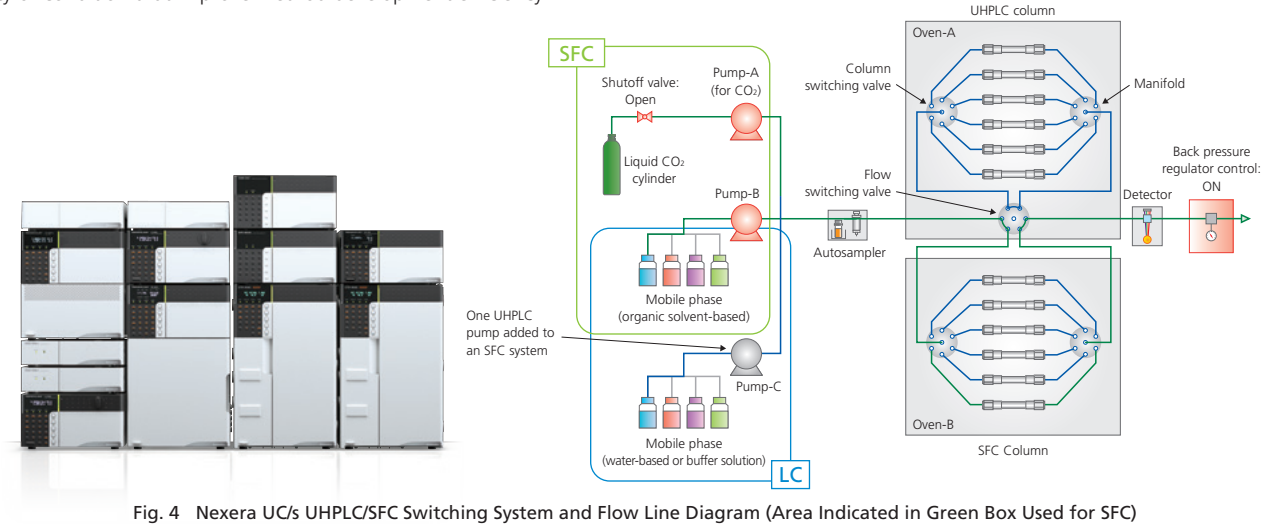


Fig. 4 Nexera UC/s UHPLC/SFC Switching System and Flow Line Diagram (Area Indicated in Green Box Used for SFC)

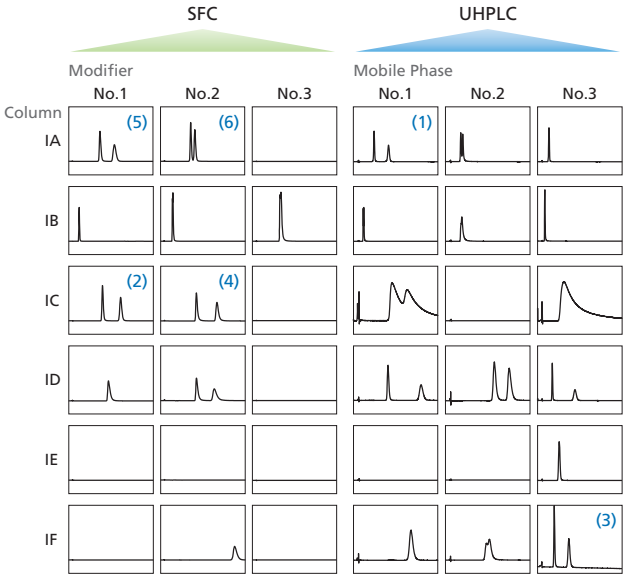


Fig. 5 Chromatograms of Omeprazole Using 36 Types of Analytical Conditions
(The chromatogram rank numbers from Table 3 are indicated in the upper right corner of corresponding chromatograms.)

In addition to a visual comparison of chromatograms, separation can also be evaluated based on resolution or other parameters. Table 3 shows the results from using Multi-Data Report software to evaluate separation. The software automatically ranks all the chromatograms with resolution greater than a given criteria (in this case, 1.5). Evaluating the separation conditions using Multi-Data Report determined that the UHPLC conditions indicated in Table 3 resulted in the best separation for omeprazole. (The chromatogram obtained using the optimal conditions is shown in Fig. 6.) Similarly, it indicated that SFC conditions obtained the best results for warfarin (Fig. 7).

This demonstrates that superior separation conditions can be determined efficiently by using both UHPLC and SFC for method scouting, even for the complicated process of evaluating separation conditions for chiral analysis.

Table 3 Evaluation of Separation Conditions for Omeprazole Using Multi-Data Report

Ranking	(1)	(2)	(3)	(4)	(5)	(6)
Analytical Condition	UHPLC	SFC	UHPLC	SFC	SFC	SFC
	IA	IC	IF	IC	IA	IA
	Mobile Phase No.1	Modifier No.1	Mobile Phase No.3	Modifier No.2	Modifier No.1	Modifier No.2
Resolution	6.24	5.60	5.55	5.40	3.43	1.59
Selectivity	1.92	1.65	2.22	1.69	1.57	1.17
Symmetry Factor (Peak 1)	1.06	1.39	1.20	1.90	1.46	1.32
Symmetry Factor (Peak 2)	1.05	1.27	1.19	1.64	1.31	1.28
Retention Factor (Peak 1)	3.18	4.71	2.42	5.05	4.30	4.10
Retention Factor (Peak 2)	6.11	7.76	5.38	8.51	6.75	4.80
Area % (Peak 1)	51.04	50.06	50.66	50.06	50.15	49.46
Area % (Peak 2)	48.96	49.94	49.34	49.94	49.85	50.54
Detected Peaks	2	2	2	2	2	2

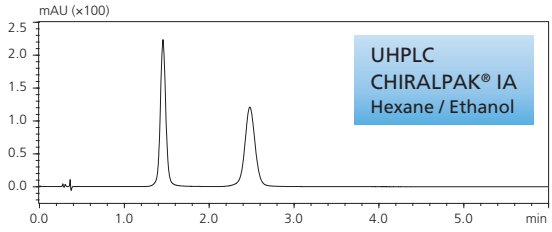


Fig. 6 Chromatogram of Omeprazole Using Optimal Conditions

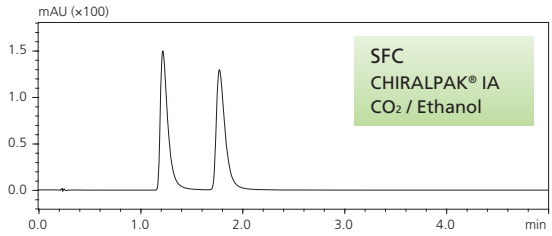


Fig. 7 Chromatogram of Warfarin Using Optimal Conditions

5. Dedicated Software That Supports UHPLC/SFC Switching

The Nexera UC/s UHPLC/SFC switching system enables both UHPLC and SFC analysis without reconnecting flow lines. Furthermore, this system also includes the functions for performing the following automatically for continuous analysis involving both UHPLC and SFC analysis modes.

1) Purge mobile phase for preceding analysis mode from flow lines.

2) Switch over flow lines, wash the column with the mobile phase for the next analysis mode, and equilibrate the column.

Method Scouting Solution, which is dedicated software that supports switching between UHPLC and SFC modes, can be used to automatically create and execute a batch table for action required for switching modes, such as purging the mobile phase used for the preceding mode and washing lines with the mobile phase required for the next mode.

Consequently, there is no need to perform complicated operations, such as creating methods for each combination of column and mobile phase. By simply specifying the (1) mobile phases, (2) columns, (3) vials, (4) base method used for analysis, and (5) gradient conditions (initial concentration, final concentration, and gradient), the software can automatically generate a batch table for use in method scouting. That ensures that even first-time users can smoothly use automatic switching between UHPLC and SFC.

In addition, the columns and mobile phases used can be managed in a database to improve management efficiency and minimize operating errors when multiple operators are involved.

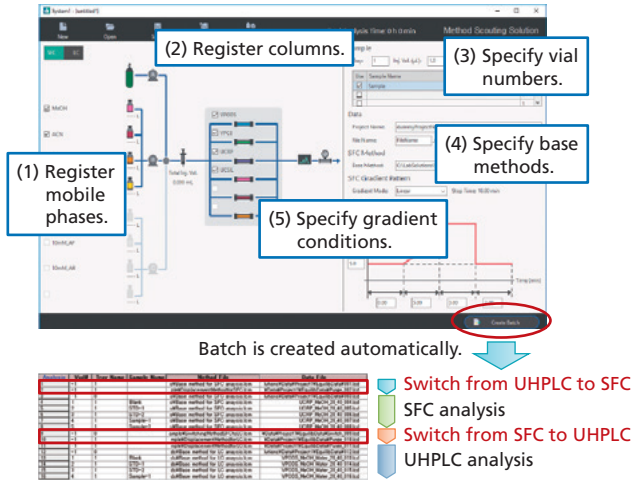


Fig. 8 Method Scouting Solution

6. Principle of Switching Between UHPLC and SFC Modes

6-1. Operating Principle

Fig. 9 shows the actions involved in switching from UHPLC to SFC analysis using the Nexera UC/s UHPLC/SFC switching system. The system can be switched between UHPLC and SFC modes in about ten minutes. The following describes the processes mentioned in section 5 above, including purging the mobile phase used for the preceding analysis mode, washing the column with the mobile phase for the next analysis mode, and equilibrating the mobile phase.

(1) Switching from UHPLC to SFC

The operating processes for switching from the UHPLC analysis mode are as follows.

1) Stop UHPLC analysis and then stop pumping the mobile phase.
2) Using the specified flow line purging method, purge the mobile phase used for UHPLC analysis by pumping only organic solvent through the flow lines. To ensure the mobile phase for UHPLC is thoroughly purged, including any water, buffer solutions, precipitated salts, or other substances, use methanol or other organic solvent that is compatible with both UHPLC and SFC to purge the lines.
3) Start supplying the supercritical carbon dioxide and controlling back pressure and also switch over the flow line switching valve at the same time. Pump the mobile phase through the column used for SFC analysis to purge and equilibrate the column. Also inject methanol to rinse out the sample loop.

(2) Switching from SFC to UHPLC

A similar process is used to switch flow line from SFC to the UHPLC mode. To purge the supercritical carbon dioxide remaining in the lines when switching from the SFC analysis mode, use the specified flow line purging method to pump only organic solvent through the lines. Use an organic solvent that is compatible with UHPLC, such as methanol. After purging the lines, switch over the switching valve to switch flow lines. Then pump the mobile phase through the column used for UHPLC analysis to purge and equilibrate the column.

Different solvent delivery unit and back pressure regulator valve settings are specified in methods for UHPLC and SFC analysis modes. Of the three solvent delivery units, set the concentration setting to zero percent for the unit not used for UHPLC and SFC analysis modes, respectively. Specify settings so that back pressure is controlled and supercritical carbon dioxide is supplied during SFC analysis, but not during UHPLC analysis.

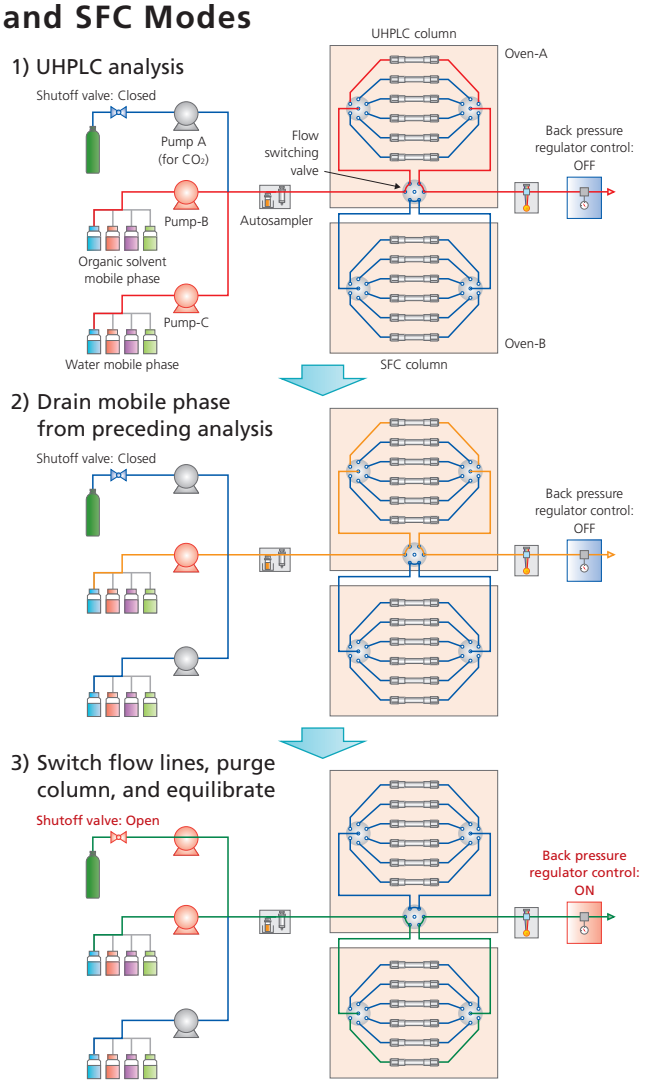


Fig. 9 Actions for Switching from UHPLC to SFC Analysis

6-2. Reproducibility for Continuous Switching

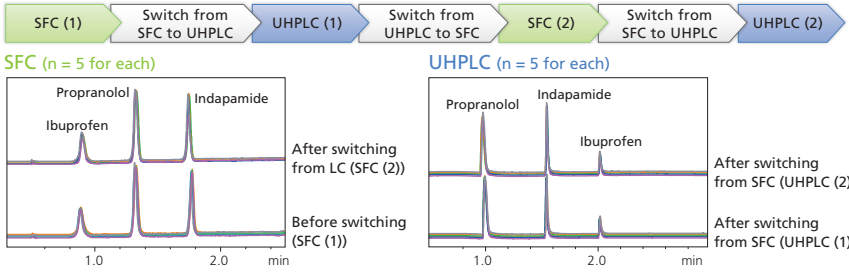


Fig. 10 Chromatograms for Three Drug Components Obtained by Switching Between UHPLC and SFC Analysis Modes

Using the switching system, three drug components were analyzed by continuous switching between UHPLC and SFC analysis three times. The resulting chromatograms are shown in Fig. 10. The chromatograms show that reliable results were obtained, with no effects from switching flow lines, even if mobile phases and separation characteristics are significantly different.

Note: CHIRALPAK® is a registered trademark of Daicel Corporation.

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