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Application News

Software for Efficient Method Development
Supercritical Fluid Chromatography System

Streamlining SFC Method Development Workflow Using LabSolutions™ MD

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User Benefits

- LabSolutions MD enhances the efficiency of the entire SFC method development workflow.
- Column screening can be automated using column switching valves, while mobile phase preparation and optimization of modifier composition can be automated using mobile phase switching valves.

■ Introduction

Supercritical Fluid Chromatography (SFC) is a separation technique that employs supercritical carbon dioxide as the primary mobile phase. Due to its high diffusion coefficient and low viscosity, supercritical carbon dioxide allows high flow-rate delivery at lower column backpressure compared to high-performance liquid chromatography (HPLC), enabling shorter analysis time. It is also particularly effective for the separation of structurally related compounds. However, supercritical carbon dioxide has a polarity similar to that of hexane and exhibits low elution strength on its own. Therefore, it is common practice to add organic solvents (modifiers) to the mobile phase to adjust its polarity. Methanol, acetonitrile, 2-propanol, and ethanol are commonly used as modifiers. By appropriate selection and combination of these modifiers, the interactions between the analytes and the column stationary phase can be controlled, allowing diverse separation patterns. Since column and modifier selection strongly influences retention behavior, optimal separation ideally requires evaluation across multiple columns and modifiers. However, such investigations are labor- and time-intensive. Additionally, gradient conditions, column oven temperature, and backpressure settings also contribute to the separation, and therefore these parameters must be optimized as well. In this article, efficient SFC method development using LabSolutions MD was demonstrated using a mixture of six small-molecule pharmaceuticals as a model sample.

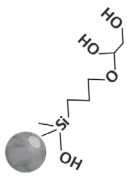
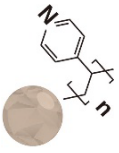
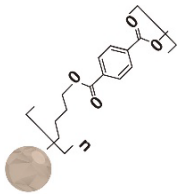
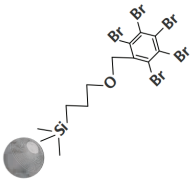
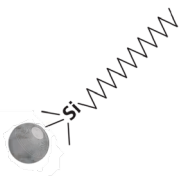
■ SFC Column Selection

Column screening is important for optimizing separation conditions because the same stationary phase often shows different retention behavior in SFC, and it is difficult to estimate SFC retention from existing HPLC data. Therefore, to employ a different type of stationary phase could be effective to change separation selectivity. The “six-column set”, a package of SFC columns (Shim-pack™ UC series) of different separation selectivities, is the best choice for the column screening. Column types and features are shown in Table 1.

■ Method Scouting System for SFC

Fig. 1 shows the flow diagram of the SFC method scouting system. By utilizing the column and mobile phase switching valves, six columns and seven modifiers can be switched automatically. Controlling this system with LabSolutions MD enables automated, comprehensive screening of columns and modifiers, resulting in a significant reduction of labor in method development.

Table 1 Features of Six-column Set

	Shim-pack UC-Diol Ⅱ	Shim-pack UC-Sil Ⅱ	Shim-pack UC-PolyVP
Chemistry			
Feature	• Normal phase separation • Suppressed non-specific interaction	• Excellent retention for basic compounds and recognition of steric structure	• Good peak shape without acidic or basic additive in mobile phase
	Shim-pack UC-PolyBT	**Shim-pack UC-PBr**	**Shim-pack UC-ODS**
Chemistry			
Feature	• Excellent recognition for aromatic compounds owing to π-π interaction	• Improved separation for shortly retained compounds in C18 column	• Reversed phase separation • Hydrophobic retention

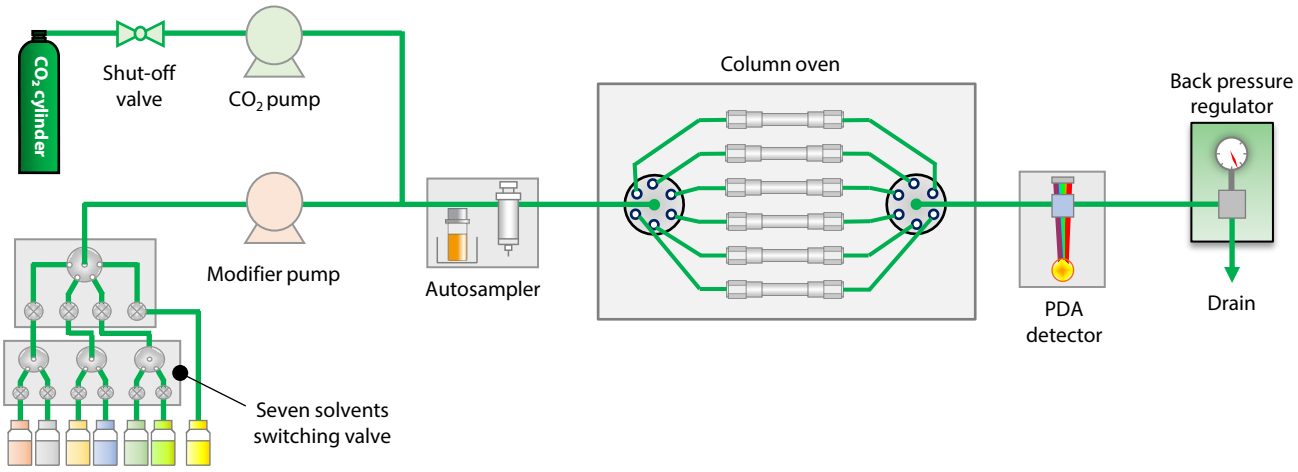


Fig. 1 Method Scouting System for SFC (Nexera™ UC)

■ Screening of Columns and Modifiers

Table 2 shows the screening conditions, and Table 3 lists the target compounds. Six candidate columns with different stationary phases (Table 1) were evaluated, along with three modifier compositions, consisting of 0%, 50%, and 100% acetonitrile in methanol. A comprehensive analysis schedule covering all 18 (6 × 3) combinations of columns and modifiers was generated to search for the optimal combination. LabSolutions MD enables error-free, automatic generation of analysis schedule under various conditions by combining different parameters such as columns and modifiers (steps (1)–(5) in Fig. 2). Column switching valves allow automatic selection of the desired column (step (2) in Fig. 2). Furthermore, mobile phase blending function automatically prepares the modifiers with the different compositions by simply clicking the desired modifier (step (1) in Fig. 2). This significantly reduces the amount of manual work and prevents human errors during preparation.

Table 2 Screening Conditions

System	: Nexera UC
Column 1	: Shim-pack UC-Poly VP *1
Column 2	: Shim-pack UC-Poly BT *2
Column 3	: Shim-pack UC-Diol II *3
Column 4	: Shim-pack UC-Sil II *4
Column 5	: Shim-pack UC-PBr *5
Column 6	: Shim-pack UC-ODS *6
	: (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	: CO_2
Pump B	
– Line A1	: Methanol
– Line A2	: Methanol
– Line B1	: Acetonitrile
– Line B2	: 100 mmol/L ammonium formate in methanol
– Line C1	: 100 mmol/L ammonium acetate in methanol
– Line C2	: 0.5% formic acid 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

*1 P/N：227-32507-03, *2 P/N：227-32501-03
*3 P/N：227-32606-29, *4 P/N：227-32607-29
*5 P/N：227-32602-09, *6 P/N：227-32608-24

Table 3 Target Compounds

	Name		Name
(A)	Antipyrine	(D)	Hydrocortisone
(B)	Probenecid	(E)	Clozapine
(C)	Naproxen	(F)	Indomethacin

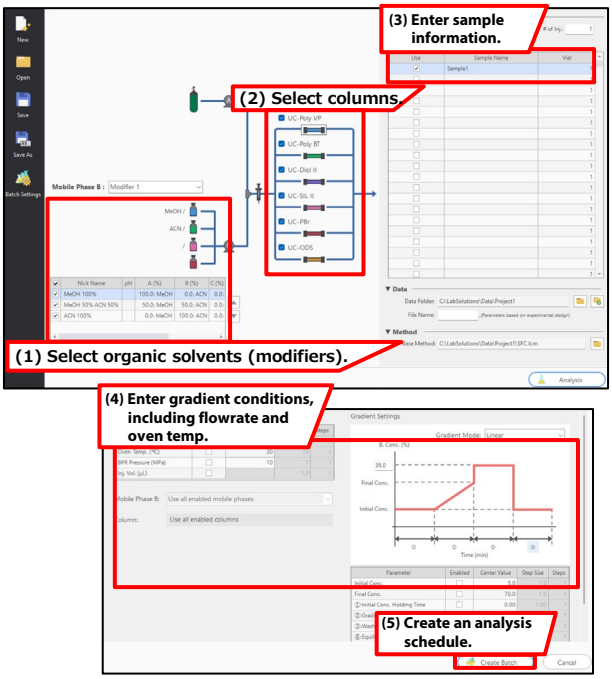


Fig. 2 Easy Creation of Analysis Schedule by LabSolutions MD

■ Results of Screening

The screening results for columns and modifiers are presented in Fig. 3–5. Changing the column or the modifier composition was found to significantly affect the retention and peak shape of each compound. Furthermore, under conditions using 100% acetonitrile without methanol as the modifier, peak intensity decreased or peaks were not detected for all columns.

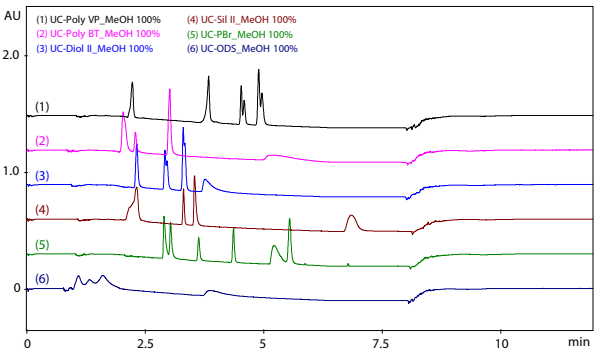


Fig. 3 Results of Column and Modifier Screening
(Modifier：Methanol 100%)

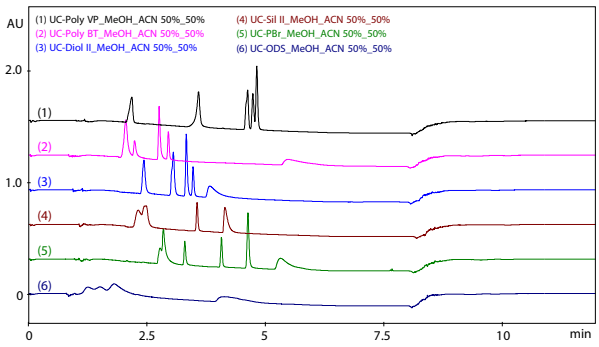


Fig. 4 Results of Column and Modifier Screening
(Modifier：Methanol / Acetonitrile = 1:1)

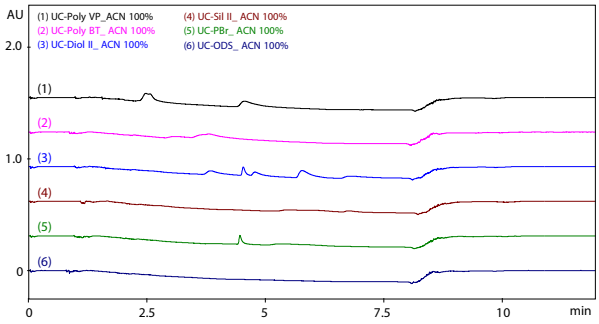


Fig. 5 Results of Column and Modifier Screening
(Modifier：Acetonitrile 100%)

■ 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. 6 shows Evaluation Value obtained through column and modifier screening and listed in the order from the highest to the lowest. Fig. 7 shows the chromatogram of the highest Evaluation Value.

Column Nick Name	MPB Nick Name	Evaluation Value
UC-PBr	MeOH 100%	107.428
UC-PBr	MeOH_ACN 50%_50%	105.380
UC-PolyVP	MeOH 100%	94.867
UC-Diol II	MeOH 100%	78.949
UC-PolyVP	MeOH_ACN 50%_50%	78.760
UC-Diol II	MeOH_ACN 50%_50%	76.209
UC-PolyBT	MeOH_ACN 50%_50%	70.629
UC-PolyBT	MeOH 100%	45.896
UC-SIL II	MeOH_ACN 50%_50%	43.175
UC-SIL II	MeOH 100%	36.768

Fig. 6 Ranking of Each Condition by Evaluation Value
(Top 10 Chromatograms Listed from the Highest to the Lowest)

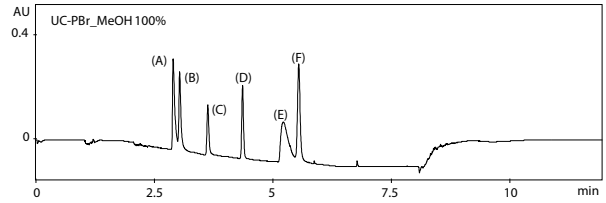


Fig. 7 Chromatogram with Highest Evaluation Value
(A) Antipyrine、(B) Probenecid、(C) Naproxen
(D) Hydrocortisone、(E) Clozapine、(F) Indomethacin

The condition with the highest Evaluation Value was a UC-PBr column with 100% methanol as the modifier. Moreover, the clozapine peak (Fig. 7 (E)) showed a tailing. Subsequently, an attempt was made to improve the separation and peak shape by adding additives to the modifier.

■ Optimization of Modifier Composition

Based on the condition with the highest Evaluation Value (column: UC-PBr, modifier: 100% methanol), the modifier compositions were optimized by adding 0–5% water to methanol and methanol solutions containing specified amounts of ammonium formate, ammonium acetate, or formic acid (See Table 4). The chromatograms obtained are shown in Fig. 8–11. Using methanol solutions with additives as modifiers was found to affect the retention times and peak shapes of each compound. The addition of ammonium formate or ammonium acetate sharpened the peak shape of clozapine (Peak E). Furthermore, increasing the water content tended to improve the resolution between clozapine (Peak E) and indomethacin (Peak F). Fig. 12 shows the results of applying Evaluation Value to the chromatograms obtained from the modifier composition study. The modifier composition providing the highest Evaluation Value was methanol containing ammonium formate (20 mmol/L) with 5% water (chromatogram (6) in Fig. 9). Based on this condition, the gradient conditions, column oven temperature, and backpressure settings were subsequently varied to further optimize the resolution.

Table 4 List of Conditions for Modifier Composition Evaluation

	Modifier composition	Water addition ratio (%)
1	Methanol	0
2		1
3		2
4		3
5		4
6		5
7	20 mmol/L ammonium formate in methanol	0
8		1
9		2
10		3
11		4
12		5
13	20 mmol/L ammonium acetate in methanol	0
14		1
15		2
16		3
17		4
18		5
19	0.1% formic acid in methanol	0
20		1
21		2
22		3
23		4
24		5

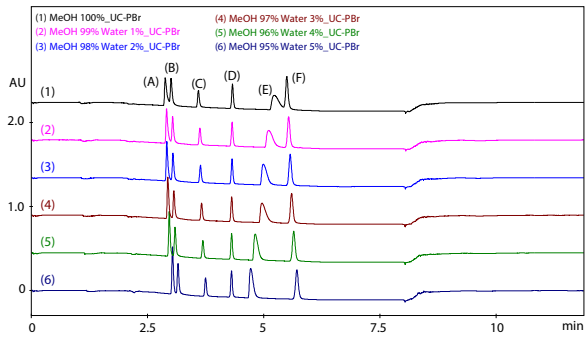


Fig. 8 Results of Optimization of Modifier Composition (Methanol)

(A) Antipyrine、(B) Probenecid、(C) Naproxen
(D) Hydrocortisone、(E) Clozapine、(F) Indomethacin

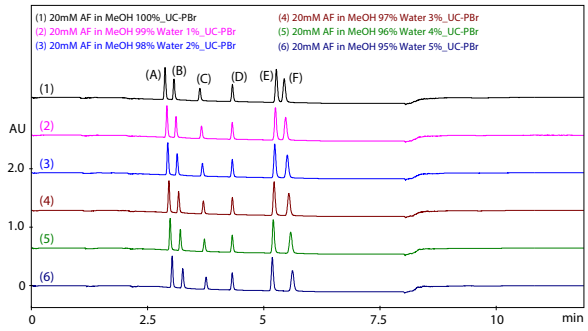


Fig. 9 Results of Optimization of Modifier Composition (20 mmol/L ammonium formate in methanol)

(A) Antipyrine、(B) Probenecid、(C) Naproxen
(D) Hydrocortisone、(E) Clozapine、(F) Indomethacin

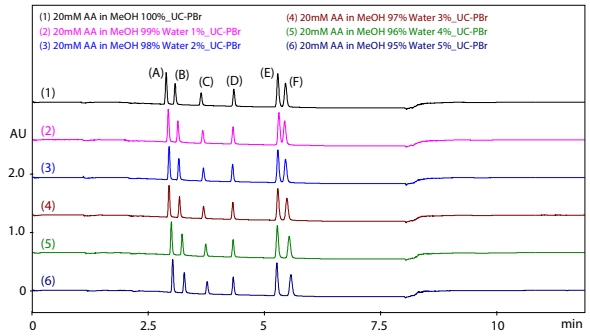


Fig. 10 Results of Optimization of Modifier Composition (20 mmol/L ammonium acetate in methanol)

(A) Antipyrine、(B) Probenecid、(C) Naproxen
(D) Hydrocortisone、(E) Clozapine、(F) Indomethacin

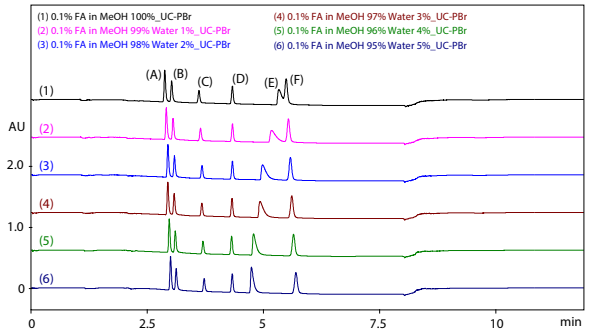


Fig. 11 Results of Optimization of Modifier Composition (0.1% formic acid in methanol)

(A) Antipyrine、(B) Probenecid、(C) Naproxen
(D) Hydrocortisone、(E) Clozapine、(F) Indomethacin

MPB Nick Name	Evaluation Value
20mM AF in MeOH 95% Water 5%	137.400
20mM AF in MeOH 96% Water 4%	134.104
20mM AA in MeOH 95% Water 5%	131.409
20mM AF in MeOH 97% Water 3%	129.774
20mM AA in MeOH 96% Water 4%	128.512
0.1% FA in MeOH 96% Water 4%	127.790
0.1% FA in MeOH 97% Water 3%	126.468
0.1% FA in MeOH 95% Water 5%	126.454
MeOH 96% Water 4%	126.281
20mM AF in MeOH 98% Water 2%	126.240

Fig. 12 Ranking of Each Condition by Evaluation Value (Top 10 Chromatograms Listed from the Highest to the Lowest)

■ Optimization of Gradient Conditions, Column Oven Temperature, and Backpressure

The gradient times of 5, 6, and 7 min (three conditions), column oven temperatures of 20, 30, and 40 ° C (three conditions), and backpressure settings of 10, 15, and 20 MPa (three conditions) were varied to examine their effects on separation under the optimized modifier composition (chromatogram in Fig. 9(6)). The chromatograms obtained are shown in Fig. 13–15. In addition, the resolutions of compounds (A) and (B) as well as (E) and (F), which exhibited relatively low resolution among all compounds, are also shown in the figures. Changes in each parameter affected the retention behavior of the compounds, thereby influencing the separation, indicating that optimization of these parameters is important. Furthermore, increasing the backpressure tended to shorten the retention times of all compounds (Fig. 15). This tendency is considered to result from increased CO_2 density at higher backpressure, which accelerates elution. Subsequently, the resolutions of the target compounds were visualized using a design space approach to explore optimal separation conditions that provide both high resolution and robustness.

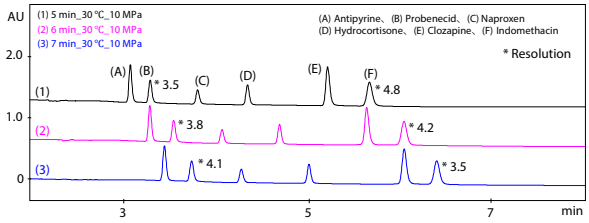


Fig. 13 Chromatograms with Different Gradient Times 5 min (1)、6 min (2)、7 min (3)

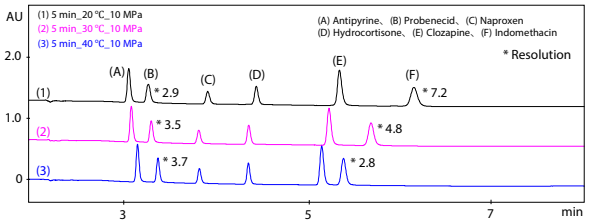


Fig. 14 Chromatograms with Different Column Oven Temperatures 20 °C (1)、30 °C (2)、40 °C (3)

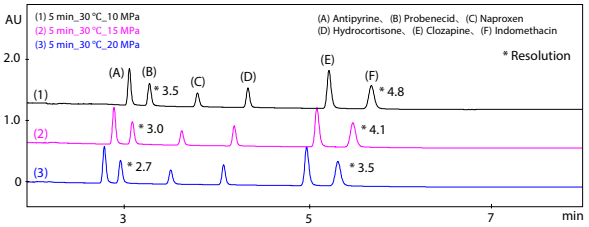


Fig. 15 Chromatograms with Different Backpressures 10 MPa (1)、15 MPa (2)、20 MPa (3)

■ Design Space Evaluation for Optimal Condition

LabSolutions MD can automatically search for optimal conditions that satisfy multiple criteria by overlaying design spaces. For instance, conditions meeting the criteria of resolutions of 3.5 or higher for both compound pairs (A, B) and (E, F) were explored using overlaid design spaces (Fig. 16). The green-framed region in Fig. 16 represents the area where the resolution of compounds (A) and (B) is below 3.5, while the orange-framed region represents the area where the resolution of compounds (E) and (F) is below 3.5. In the remaining region (black-hatched area), point A within the red circle was automatically identified as the optimal condition (gradient time: 6 min, column oven temperature: 30 °C, and backpressure: 10 MPa). Thus, conditions that meet a user-defined criteria for multiple peaks can be easily and rapidly identified by overlaying design spaces.

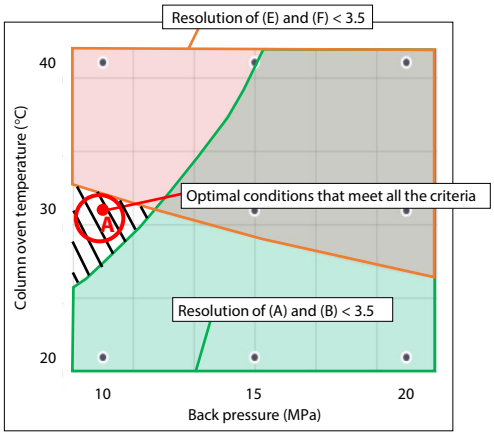


Fig. 16 Overlay of Design Spaces
(Gradient time : 6 min)

■ Chromatogram at Optimal Condition

Fig. 17 (upper) shows the chromatogram obtained under the optimal conditions (Point A) determined by the design space. The resolution between compounds (A) and (B) was 3.5 or higher, and that between compounds (E) and (F) was also 3.5 or higher, confirming that the specified criteria were satisfied. By comprehensively visualizing the resolutions within the design space, separation can be optimized without relying on the intuition or experience of chromatography. Fig. 17 (lower) shows the chromatogram obtained at an increased flow rate of 1.5 mL/min compared with 1.0 mL/min under the same optimized conditions. Due to the low viscosity and high diffusivity of supercritical carbon dioxide, high flow rates can be used without losing column efficiency, allowing for shorter analysis time.

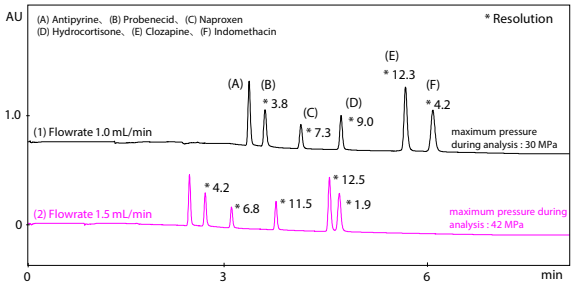


Fig. 17 Chromatograms at Optimal Condition
Flowrate 1.0 mL/min (upper), Flowrate 1.5 mL/min (lower)

■ Conclusion

A case study illustrating the streamlining of method development for SFC using LabSolutions MD is presented. Six columns and seven modifiers can be automatically switched using the column and mobile phase switching valves, which significantly reduces the labor required for method development. Furthermore, comprehensively visualizing resolutions within the design space enables searching for optimal separation conditions without relying on the intuition or experience of chromatographic experts. While this article focused on method development for SFC, the combined use of both SFC and LC techniques is expected to further enhance the efficiency of method development, since SFC and LC have different separation characteristics. For an example of an LC/SFC switching system applied to comprehensive method development for both techniques, please refer to Application News "01-01058."

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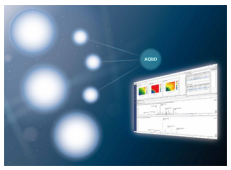

› Method Development System

Automatic Optimization of Gradient Conditions with···

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