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High Performance Liquid Chromatograph
Software for Efficient Method Development

Application News

Achieving Sharp Peaks and High Sensitivity with Nexera™ X4

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User Benefits

- The low-dispersion design of Nexera X4 suppresses extra-column band broadening within the system, enabling the column to deliver its maximum separation performance. This also results in sharper peak shapes and improved detection limits for impurity analysis.
- Using LabSolutions™ MD enables a more efficient workflow for developing and optimizing separation conditions.

■ Introduction

In high-performance liquid chromatography, minimizing sample band broadening both inside and outside the column is essential to achieving high separation performance. For example, impurity analysis often requires the separation of structurally similar components, demanding high separation performance and high sensitivity. Nexera X4 (Fig. 1) is a next-generation UHPLC system that inherits the advanced technologies of Shimadzu's Nexera series and delivers top-class analytical performance. Its industry-leading*1 low-dispersion design provides exceptionally sharp peaks and outstanding separation capability. In addition, Nexflow technology achieves low dispersion without changing the inner diameter of the flow lines, thereby minimizing system-derived band broadening while reducing the risk of tube blockage. This allows UHPLC columns to perform at their full potential and enables highly efficient separations. All high-pressure flow lines that samples pass through employ end-surface-sealed connections, minimizing dead volume–induced peak broadening. Tool-free fittings (Fig. 2) are also used to enhance usability. This article introduces an example in which impurities were separated with high resolution and high sensitivity using Nexera X4. It also describes how separation conditions were efficiently optimized by combining the system with LabSolutions MD, a dedicated software for supporting method development.

*1 : ECBB value: 7.0 µL

Fig. 1 Nexera™ X4

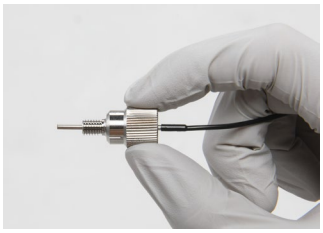

Fig. 2 Tool-Free Fittings

■ Analytical Conditions and Target Compounds

The analytical conditions and target compounds are summarized in Table 1. In this study, hydrocortisone, a small-molecule pharmaceutical compound, was used as the model sample.

Table 1 Analytical Conditions and Target Compounds

System : Nexera X4	
Sample : Hydrocortisone	
Mobile phase	
Pump A : 0.1% formic acid in water	
Pump B : Acetonitrile	
Column : Shim-pack Scepter™ C18-120 *1 (50 mm × 2.1 mm I.D., 1.9 µm)	
Analytical conditions	
B Conc.	: X *2 %(0 min)→95%(Y *3 ~Y+0.5 min) →X%(Y+0.5 ~ Y+1.7 min)
Column Temp.	: 30, 40, 50 °C
Flow rate	: 0.5 mL/min
Mixer	: Micro mixer
Sample loop Vol.	: 15 µL
Injection Vol.	: 1 µL
Detection	: 254 nm (SPD-M40 X4, STD cell)

*1 : P/N 227-31012-03
*2 : X (Initial Conc.)= 5, 10
*3 : Y (Gradient time)= 1.0, 1.2, 1.4

■ High-Resolution, High-Sensitivity Impurity Analysis with Nexera X4

Fig. 3 compares chromatograms obtained using Nexera X4 and a typical UHPLC system under the analytical conditions summarized in Table 1 (initial concentration: 5%, gradient time: 1 min, column oven temperature: 30 ˚C). Compared with the typical UHPLC system, Nexera X4 provides higher resolution between impurity peaks (indicated by red boxes in Fig. 3) and greater peak heights. Table 2 summarizes the resolution and peak height for each impurity on both systems. Nexera X4 achieved approximately 1.5-fold higher peak heights than the typical UHPLC system, demonstrating improved separation and enhanced sensitivity. Because further improvements in separation between impurities were still desirable, LabSolutions MD was used to streamline the search for optimal separation conditions.

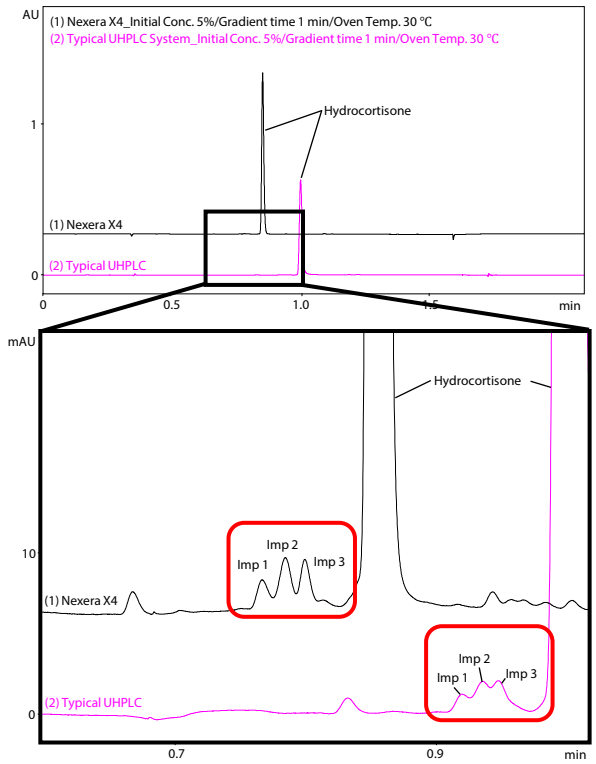

Fig. 3 Comparison of Chromatograms for Impurity Analysis
(1) Nexera X4, (2) Typical UHPLC System
(Baseline compensation by blank data applied for typical UHPLC.)

Table 2 Comparison of Resolution and Peak Height for Each Impurity

	System	Resolution	Peak height
Imp 1	**Nexera X4**	-	**1716**
	Typical UHPLC	-	1107
Imp 2	**Nexera X4**	**1.13**	**2937**
	Typical UHPLC	0.89	1894
Imp 3	**Nexera X4**	**1.00**	**2686**
	Typical UHPLC	0.56	1929

■ Enhancing the Efficiency of Separation Optimization Using LabSolutions MD

LabSolutions MD can automatically generate analysis schedules while avoiding human error based on comprehensive combinations of user-defined parameters (Steps (1)–(4) in Fig. 4). This enables anyone to perform separation optimization easily and quickly. For example, when varying the gradient time (red box in Fig. 4), an entered center value of 1.2 min is adjusted by one step above and below the center at 0.2-min increments, resulting in automatically generated schedules of 1.0, 1.2, and 1.4 min. In this study, a total of 18 analytical conditions (2 × 3 × 3) were automatically generated by combining two initial concentrations (5% and 10%), three gradient times (1.0, 1.2, and 1.4 min), and three column oven temperatures (30, 40, and 50 ° C). These conditions were then used to comprehensively evaluate the separation.

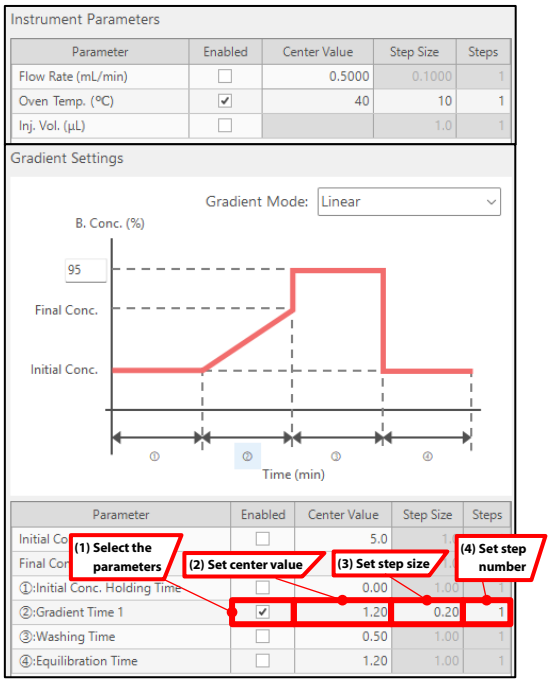

Fig. 4 Analysis Schedule Creation Screen

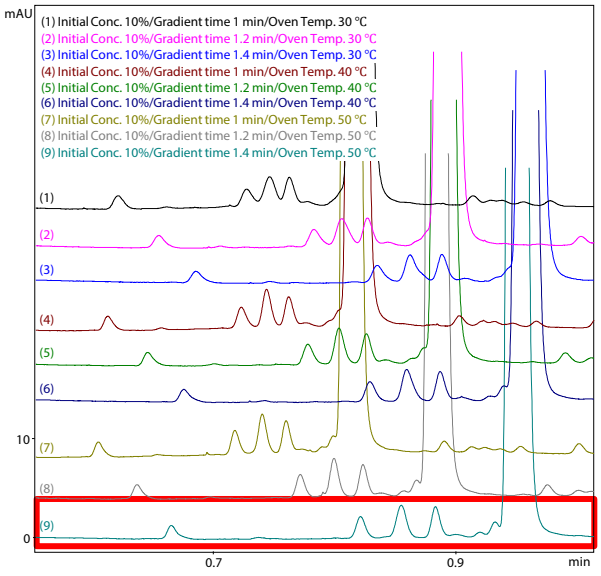

Fig. 5 Chromatograms Obtained During Separation Optimization

■ Rapid Identification of Optimal Condition

Representative chromatograms obtained during the separation optimization are shown in Fig. 5. In the process of optimizing separation conditions, varying multiple parameters results in a large number of chromatograms, making it a time-consuming task to determine which conditions provide the desired separation. This process typically requires both substantial effort and a certain level of chromatographic expertise. LabSolutions MD enables users to quickly and easily identify optimal conditions by quantitatively evaluating the separation performance for each condition using the equation shown below (Eq. 1), eliminating the need to rely on intuition or experience.

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

Evaluation Value is calculated as the number of peaks detected (P) multiplied by the sum of the resolution factors (Rs). Fig. 6 shows the Evaluation Value obtained from the investigation of initial concentration, gradient time, and column oven temperature, ranked in descending order. The highest Evaluation Value was obtained under the condition with an initial concentration of 10%, a gradient time of 1.4 minutes, and a column oven temperature of 50 ˚C, corresponding to chromatogram (9) in Fig. 5. A comparison of chromatograms before and after optimization is shown in Fig. 7, demonstrating that comprehensive condition screening with LabSolutions MD greatly streamlined the workflow and significantly improved the separation of each impurity.

Initial Conc. (%)	Gradient Time (min)	Oven Temp. (°C)	Evaluation Value
10	1.4	50	36.005
5	1.4	50	35.429
10	1.2	50	34.978
10	1.4	40	33.803
5	1.2	50	32.873
5	1.4	40	32.803
10	1.2	40	31.391
10	1	50	30.794
10	1.4	30	30.644
5	1.2	40	30.384

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

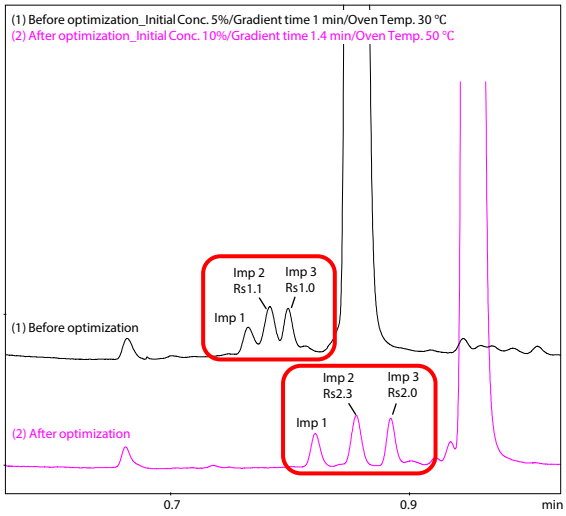

Fig. 7 Comparison of Chromatograms Before and After Optimization by Nexera X4
(1) Before Optimization, (2) After Optimization

■ Conclusion

High-resolution and high-sensitivity impurity analysis was demonstrated using Nexera X4. The system's low-dispersion design minimizes extra-column band broadening, enabling excellent separation performance even for structurally related impurities. Suppression of sample band spreading results in sharper peak shapes, contributing to improved detection limits. In addition, when combined with the method development support software LabSolutions MD, the efficiency of exploring optimal separation conditions is further enhanced.

01-01175-EN First Edition: Jun. 2026

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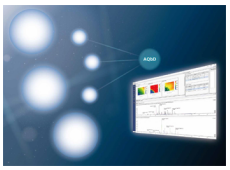

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