

# Application News

High Performance Liquid Chromatograph

## 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.

### 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.



Fig. 1 Nexera™ X4

\*1 : ECBB value: 7.0 µL

### 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 a 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.	: 5%(0 min)→95%(1~1.5 min) →5%(1.5~2.7 min)
Column Temp.	: 30 °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



Fig. 2 Tool-Free Fittings

### 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. Compared with the typical UHPLC system, Nexera X4 provides higher resolution between impurity peaks (indicated by red circles 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.

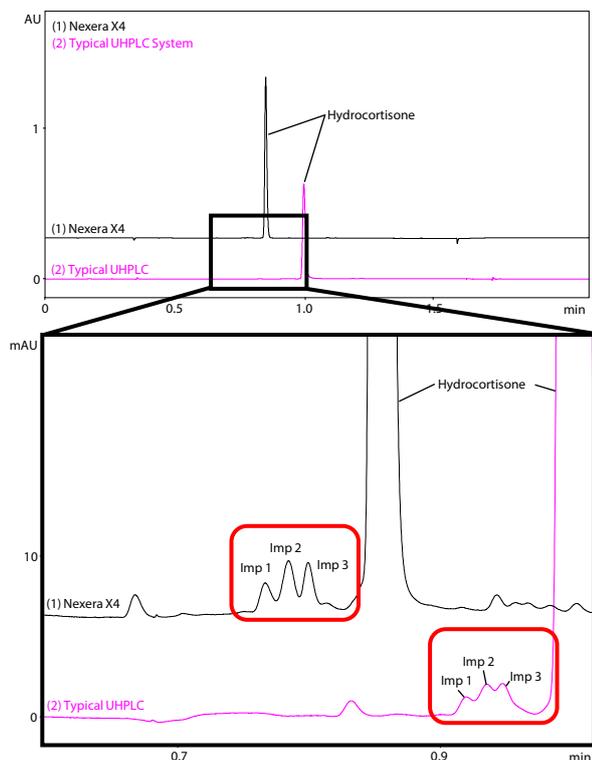


Fig. 3 Comparison of Chromatograms for Impurity Analysis  
(1) Nexera X4, (2) Typical UHPLC System  
(baseline compensation by blank data applied for typical UHPLC)

Table 2 Comparison of Resolution and Peak Height for Each Impurity

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

## ■ 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.

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