

Improving Peak Shapes Affected by Sample Solvents in UHPLC Systems

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User Benefits

- ◆ Peaks broadened by sample solvents in UHPLC systems can be improved.
- ◆ Peak shapes can be improved using the “co-injection” automatic pretreatment function.

Introduction

Ultra high performance liquid chromatography (UHPLC) enables high speed and high separation analysis by using a UHPLC column packed with particles about 2 μm in size. UHPLC columns have an internal diameter that is narrower than general-purpose high performance liquid chromatography (HPLC) columns, which results in less diffusion of sample components within the column. However, because peak shapes are affected by diffusion that occurs throughout the system, including the column, using an HPLC system for analysis prevents the full benefit from UHPLC column performance capabilities to be obtained, due to system diffusion in areas other than the column. Therefore, a UHPLC system commonly employs tubing with an inner diameter of 0.1 mm or less.

On the other hand, the use of the UHPLC system which employs tubing with smaller inner diameter is prone to be affected with inadequate mixing of the sample solvent and mobile phase. Consequently, the peak shape may be broadened when using a sample solvent with a higher elution strength than the mobile phase.

That phenomenon can occur in cases (1) and (2) below.

- (1) When analysis is performed in a UHPLC system using HPLC analytical conditions.
- (2) When large volumes are injected and there is a large difference between the elution strength of the mobile phase and sample solvents.

This article introduces a case where peak shape was improved by using the automatic pretreatment function (co-injection) of autosamplers in Nexera series when peak broadening occurs in cases (1) and (2) above.

Key Considerations for Performing HPLC Analysis in a UHPLC System

If 10 μL of sample is injected using HPLC analytical condition in a UHPLC system, for example, the small volume in the tubing before reaching the column can result in the sample solvent not being fully diluted by the mobile phase when it reaches the column. Consequently, when using the sample solvent has a stronger elution strength than the mobile phase, its solvent effect can cause prominent sample band broadening that distorts peak shape (leading).

Using a standard configuration Shimadzu Nexera XR UHPLC system, analysis was performed using the HPLC analytical conditions indicated in Table 1. The resulting chromatogram is shown in Fig. 1. To minimize system diffusion, the Nexera XR features 0.1 mm I.D. tubing for sample flow lines. Due to the small tubing volume, the chromatograph shows peak leading caused by the solvent effect.

If an HPLC system is upgraded to a UHPLC system in order to increase analysis speed, for example, but the same HPLC analytical conditions are used directly in the UHPLC system, it can potentially cause the type of peak shape distortions shown. So, the sample solvent must be reconsidered.

Table 1 Analytical Conditions

System:	Nexera XR
Column:	Shim-pack™ VP-ODS*1 (150 mm × 4.6 mm I.D., 5 μm)
Mobile Phase:	Water/methanol = 70 : 30
Flowrate:	1.0 mL/min
Column Temp.:	40 °C
Injection Vol.:	10 μL
Detection:	272 nm (SPD-40V)
Sample:	Caffeine (sample solvent: methanol)
Co-Injected Reagent**2:	Water (15 μL each before and after the sample)

*1 P/N: S228-34937-91

**2 Sample diluent solvent for co-injection used in Fig. 4.

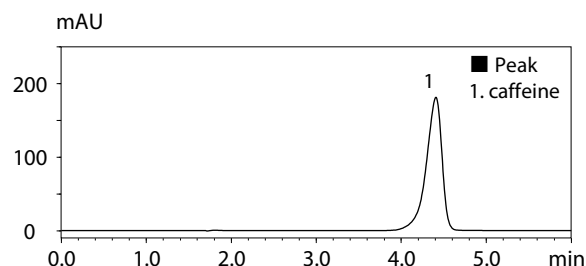


Fig. 1 Chromatogram Obtained with Standard Configuration Nexera XR System

Improving Peak Shape by Adding Tubing

The peak distortion shown in Fig. 1 was caused by small tubing volume and inadequate mixing between the sample solvent and the mobile phase. Therefore, the resulting sample band broadening can be reduced by adding additional tubing to the column inlet.

The chromatogram obtained with 600 mm × 0.3 mm I.D. tubing added to the column inlet of the standard Nexera XR system (configuration A) is shown in Fig. 2.

It shows how the additional tubing improved peak shape by inhibiting peak leading.

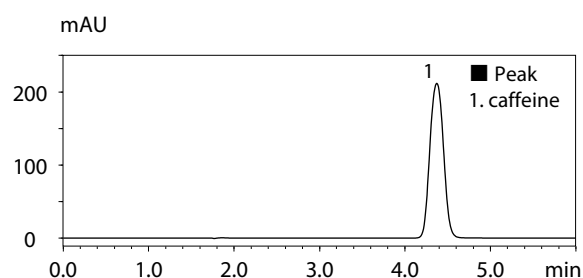


Fig. 2 Chromatogram Obtained with Nexera XR System Configuration A

However, increasing the tubing volume by adding tubing will increase system diffusion, which depending on the analytical conditions, in some cases can have the opposite effect of causing peak broadening and worse theoretical plate number.

Consequently, using a UHPLC system with additional tubing for UHPLC analysis requires particular care and in some cases might require disconnecting or reconnecting tubing for each analysis.

■ Improving Peak Shape Using the “Co-Injection” Automatic Pretreatment Function

Autosamplers in Nexera series are equipped as standard with a variety of automatic pretreatment functions such as the co-injection function. The co-injection function can be used to suction a solvent with low elution strength (co-injection reagent) from a specific vial and inject it together with the sample into the analytical column. The mixing action and wait time can also be specified for the process.

The co-injection function can provide the same effect as adding tubing, as described above. It also enables peak shape improvement by adjusting software settings, without disconnecting or reconnecting tubing.

The co-injection function was used for analysis with the settings in Table 1. Fig. 3 shows how the co-injection settings were configured. 15 µL of the co-injected reagent (water), sample, and 15 µL of the co-injected reagent (water) were successively suctioned, mixed within the needle, and then injected. The resulting chromatogram is shown in Fig. 4. It shows how co-injection improved peak shape.

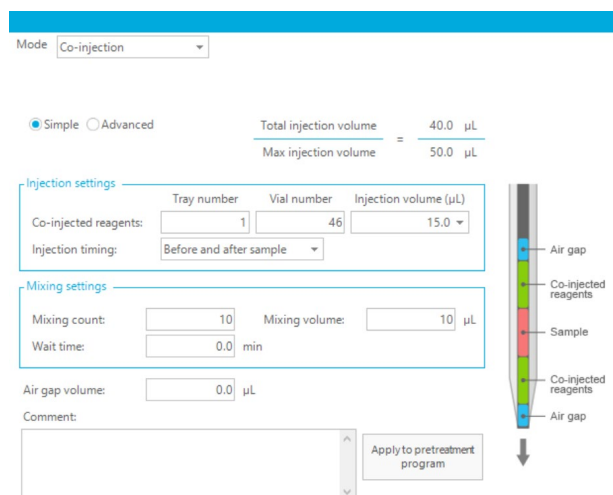


Fig. 3 Window for Specifying Co-Injection Settings (Simple) for Automatic Pretreatment Function

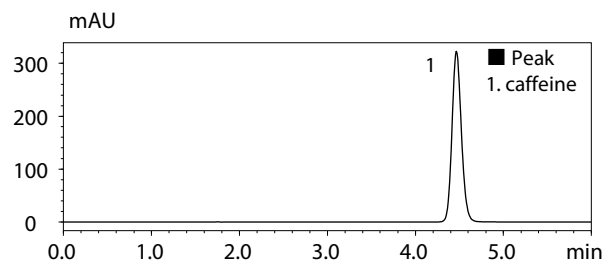


Fig. 4 Chromatogram with Water Co-Injected

The theoretical plate number for the respective peaks in Figs. 1, 2, and 4 are indicated in Table 2. Compared to the normal Nexera XR configuration, the theoretical plate number increased by a factor of about 1.3 with configuration A and by about a factor of 2.8 if co-injection is used. Even without changing the system configuration, simply using the co-injection function increased the theoretical plate number more than adding 600 mm × 0.3 mm I.D. tubing. These results demonstrate that peak shape can be improved by using co-injection of dilution solvent.

Table 2 Theoretical Plate Number with Additional Tubing or Co-Injection

	Theoretical plate number
Standard configuration	2737
Configuration A	3589
Using co-injection in standard configuration	7566

■ Sample Solvent Effects in UHPLC Analysis

UHPLC analysis often uses narrower internal diameter column than HPLC. Accordingly, the injection volume is smaller than for HPLC due to the smaller column cross-section. Therefore, even in UHPLC systems with a small tubing volume, the sample solvent is diluted by the mobile phase by the time it reaches the column.

However, in some cases, injection volumes are increased for analysis, such as for samples that contain low concentrations of target components.

If the injection volume is increased for UHPLC analysis and the sample solvent has a stronger elution strength than the mobile phase, peak shapes can be distorted because the sample can be affected by the sample solvent by the time it reaches the column.

In such cases also, using the “co-injection” automatic pretreatment function can improve peak shape.

■ Analytical Results from Co-Injecting a Dilution Solvent

Analysis was performed using a Nexera XR system with the conditions indicated in Table 3. The resulting chromatograms are shown in Fig. 5. Fig. 5 shows chromatograms obtained with and without co-injecting water. The results show that for normal analysis without co-injection, increasing the injection volume causes sample solvent effects that clearly cause peak distortion.

Table 4 indicates the theoretical plate number determined from each chromatogram in Fig. 5. Assuming theoretical plates are 100 % for a 1 µL injection, a graph of the theoretical plate number for each injection volume is shown in Fig. 6. For normal analysis, the theoretical plates decrease to 63 % for 2 µL and decrease significantly to 5 % for 10 µL. On the other hand, if water is co-injected as the dilution solvent, the decrease of the theoretical plate number is improved compared to those of normal analysis with the value being 70 % for 5 µL and 29 % for 10 µL. In addition, the peak shape is maintained enough to allow quantitation.

Table 3 Analytical Conditions

System:	Nexera XR
Column:	Shim-pack XR-ODS II*1 (75 mm × 3.0 mm I.D., 2.2 µm)
Mobile Phase:	Water/methanol = 70 : 30
Flowrate:	1.0 mL/min
Column Temp.:	40 °C
Injection Vol.:	1, 2, 5, and 10 µL
Detection:	272 nm (SPD-40V)
Sample:	Caffeine (sample solvent: methanol)
Co-Injected Reagent:	Water (15 µL each before and after the sample)

*1 P/N: S228-41624-91

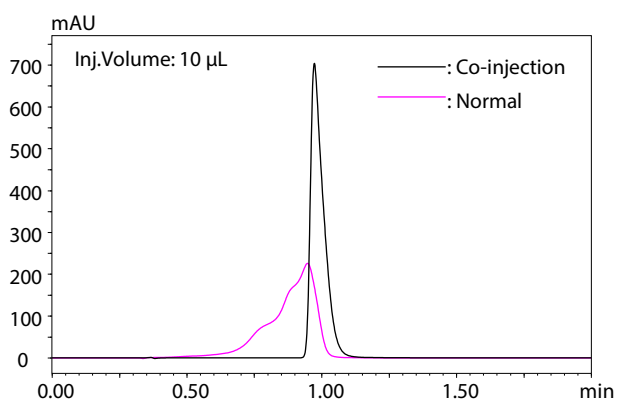
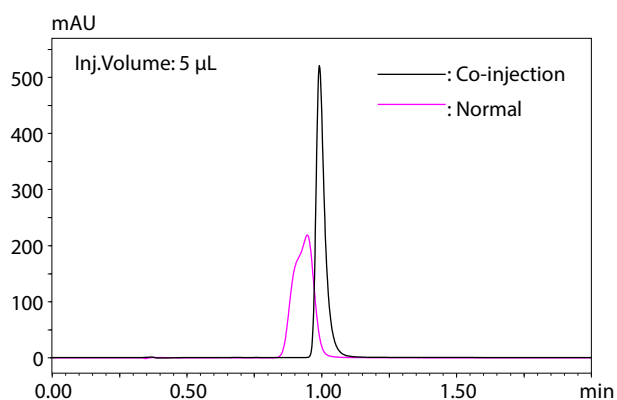
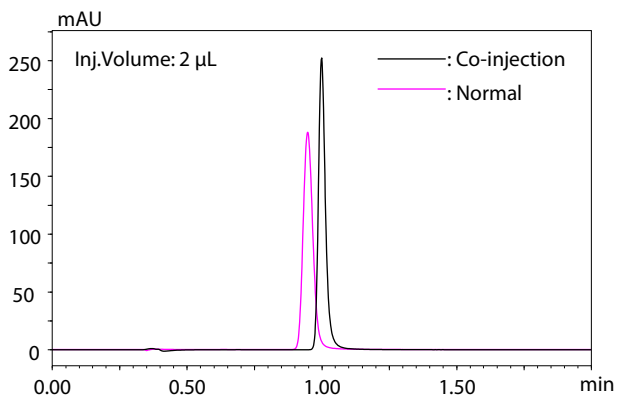
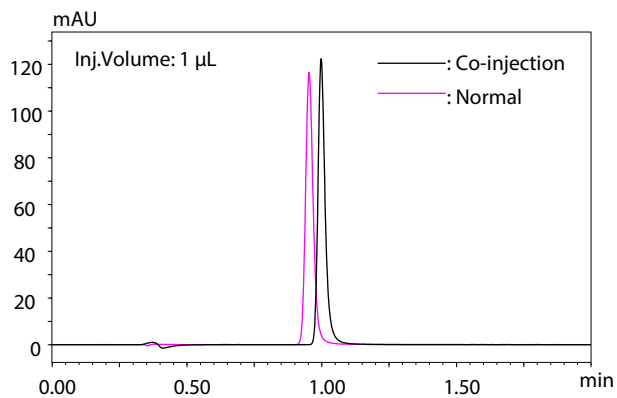


Fig. 5 Comparison of Chromatograms at Respective Injection Volumes with and without Water Co-Injection

Table 4 Theoretical Plate Number for Respective Injection Volumes with and without Co-Injection

volume \ mode	Co-injection mode	Normal injection
1 µL	6746	5226
2 µL	6964	3277
5 µL	4708	766
10 µL	1928	265

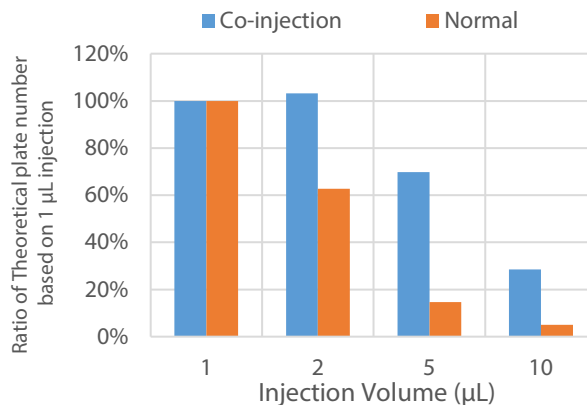


Fig. 6 Percent Change in Theoretical Plates Assuming 100 % Theoretical Plates for a 1 µL Injection

Conclusion

This article describes how if peak broadening occurs due to sample solvent effects, the “co-injection” automatic pretreatment function included with Nexera XR systems can be used to improve peak shape using only software settings and without making any special changes to the system itself.

Because the co-injection function can prevent sample solvent effects, Nexera XR with its function as standard can be used for analysis with a wide range of analytical condition settings.

Note that the “co-injection” automatic pretreatment function is included in not only the Nexera XR system but also all other Shimadzu Nexera series autosamplers.

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