

# Application News

## No. L522

### High Performance Liquid Chromatography

## Peak Shape Improvement Using the Auto-Pretreatment Function (Co-injection) of i-Series Plus Integrated Liquid Chromatograph

Ultra-high performance liquid chromatography (UHPLC) requires a system with even lower dispersion than that of conventional high performance liquid chromatography (HPLC). In addition, the UHPLC system commonly employs tubing with an inner diameter of 0.1 mm or less. This is because the internal diameter of columns used for UHPLC systems is small with 2.0 to 3.0 mm being the most popular and therefore more prone to be affected by extra-column dispersion compared to HPLC.

However, smaller internal diameters in tubing mean that mixing of the sample and mobile phase within tubing becomes difficult. If the sample solvent is organic and has a higher concentration than that of the mobile phase, the solvent may affect the sample dispersion at the column and result in broad peaks. The small I.D. of the column emphasizes this phenomenon.

This article introduces a case where peak shape was improved by using the automatic pretreatment function (co-injection) of the i-Series Plus model Nexera™-i MT to automatically mix the sample together with the dilution solvent and then perform analysis.

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### Auto-pretreatment Function

i-Series Plus models have an automatic pretreatment function for the autosampler with three modes ("Dilution", "Reagent", and "Co-Injection") available with templates. The mode used in this article is "Co-Injection (Simple)". The "Co-Injection (Simple)" mode is capable of aspirating solvent for co-injection from a specific vial and injecting the solvent together with the sample into the analysis column.

This mode allows the mixing and standing functions additionally. Fig. 1 and Fig. 2 show the setting screen and resulting operational procedure respectively.

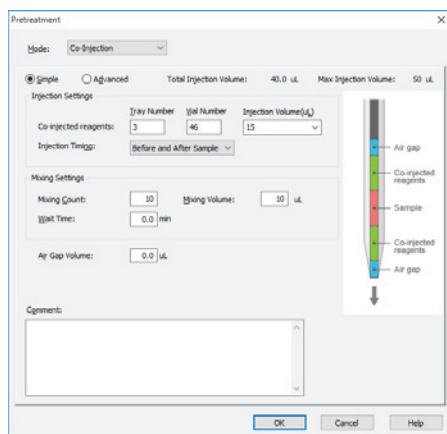


Fig. 1 Co-injection (Simple) Settings Screen of the Automatic Pretreatment Function

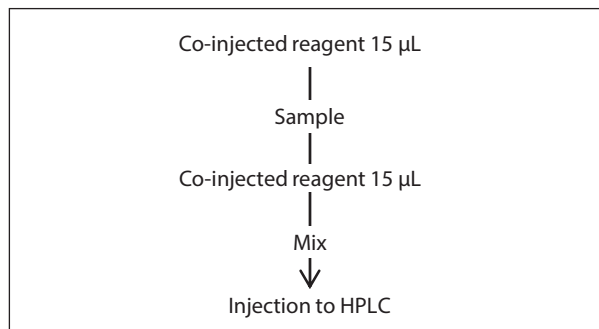


Fig. 2 Co-injection (Simple) Process

### Results from Co-injecting Dilution Solvent

Table 1 lists the analytical conditions and Fig. 3 shows the 1 µL injection -based normalized number of theoretical plate for each injection volume. The co-injection procedure was shown in Fig. 2. Fig. 4 shows the chromatograms obtained with and without co-injection of dilution solvent. For standard analysis without co-injection, it is clear that the peak shape worsens as the injection volume increases due to the effects of the sample solvent. As for number of theoretical plate, the decreased number of theoretical plate values of 84 % and 13 % at 2 µL and 10 µL injection volume compared to that of 1 µL injection was observed. On the other hand, when water was co-injected as the dilution solvent, the drop was improved compared to those of standard analysis with the value being 89.9 % for 5 µL and 61.3 % for 10 µL. In addition, the peak shape was maintained enough to allow quantitation.

Table 1 Analytical Conditions

Column	: Shim-pack™ XR-ODSII 75 mmL. × 3.0 mmI.D., 2.2 µm
Mobile phase	: Water/Methanol = 7/3 (v/v)
Flow rate	: 1.0 mL/min
Column temp.	: 40 °C
Injection volume	: 1, 2, 5, 10 µL
Detection	: UV 272 nm
Sample	: Caffeine (Water/Methanol = 4/6)
Co-injected reagent	: Water

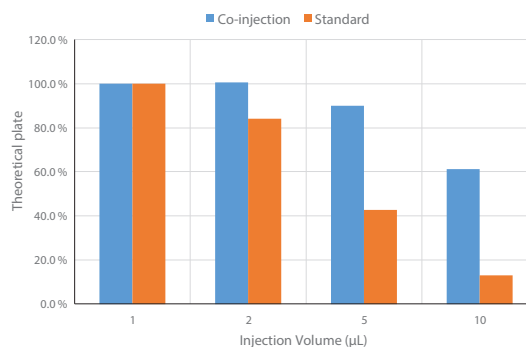


Fig. 3 Theoretical Plates for Each Injection Volume (with 1 µL being 100 %)

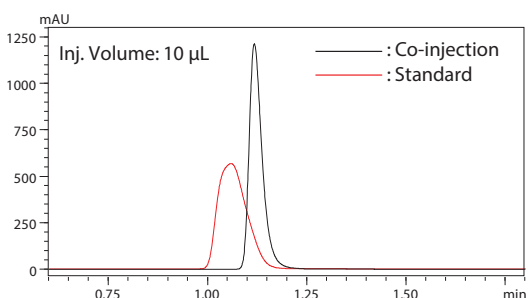
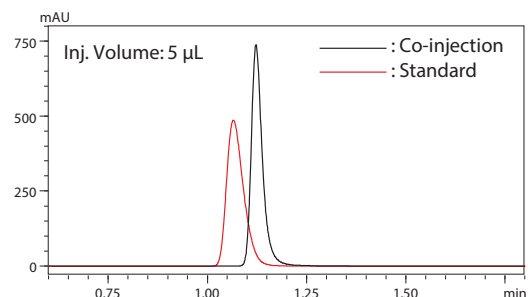
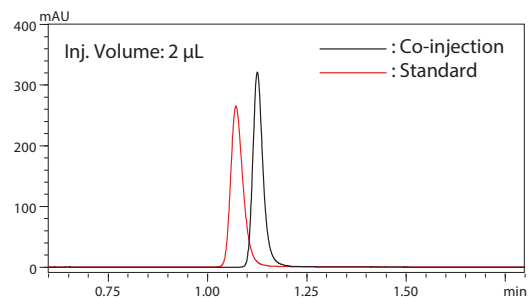
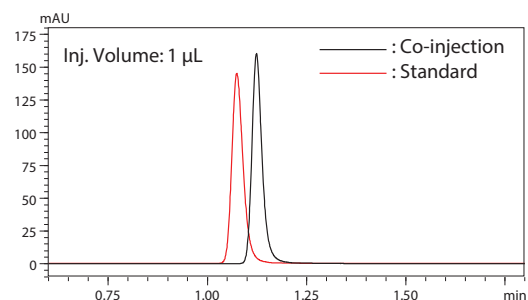


Fig. 4 Peak Shape Comparison When Diluent is Co-injected

#### Effects of Co-injection on Ion Pair Analysis

In ion pair analysis as well, deteriorated peak shape or split peaks can be seen due to inadequate mixing of the sample and the mobile phase containing the ion pair reagent caused by small I.D. of the tubing. In some cases the peak shape can be improved by co-injecting a solvent containing the ion pair reagent. Table 2 lists the analytical conditions and Fig. 5 shows the co-injection settings. Fig. 6 compares the chromatograms obtained with and without co-injection.

Table 2 Analytical Conditions

Column	: Shim-pack™ XR-ODSII 75 mmL. × 3.0 mmI.D., 2.2 µm
Mobile phase	: Dissolve 3.4 g of monobasic potassium phosphate and 1.7 g of sodium lauryl sulfate in 1000 mL of a mixture of water and acetonitrile (1:1)
Flow rate	: 0.8 mL/min
Column temp.	: 40 °C
Injection volume	: 15 µL
Detection	: UV 345 nm
Sample	: Berberine chloride (water)
Co-injected reagent	: Mobile phase

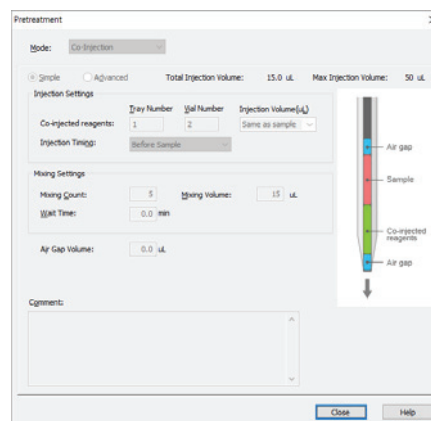


Fig. 5 Settings for Co-injecting a Mobile Phase Containing an Ion Pair Reagent

As shown by the chromatograms in Fig. 6, in ordinary analysis, both peaks 1 and 2 are split. This is most likely because the sample compounds and the mobile phase containing the ion pair reagent were not mixed adequately before the column, therefore resulting in partial ion pair formation.

On the other hand, co-injection of the mobile phase containing the ion pair reagent enabled adequate mixing of the sample and the mobile phase within the needle and analysis resulted in a proper peak shape.

As described, co-injection, which is a feature of the automatic pretreatment function, can be utilized for (U) HPLC analysis with easy parameter settings of the solvent used for dilution or reaction on a single screen.

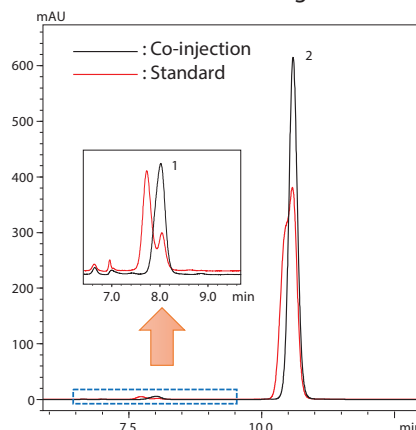


Fig. 6 Peak Shape Comparison When a Mobile Phase Containing an Ion Pair Reagent is Co-injected

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