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Technical Report

Improving Peak Shape Using the Automatic Pretreatment Function (Co-Injection) in the SIL-40 Series Autosamplers

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Abstract:

If a sample solvent selected in accordance with the solubility of a compound has a higher elution strength than the mobile phase, this can have a negative impact on the chromatogram. For example, if the ratio of organic solvent in a sample solvent is heightened in order to dissolve a compound with low polarity, this can have a detrimental effect on peak shape for quickly eluted compounds in reverse-phase chromatography, and even compromise the reliability of quantitative results. This article illustrates the use of the co-injection function, one aspect of the automatic pretreatment functionality included as standard in autosamplers in the Nexera™ series (SIL-40 series), to improve peak shape by injecting a solvent with a weak elution strength simultaneously with the sample in order to reduce the effect of the sample solvent. The co-injection function can be specified easily via the LabSolutions workstation software.

Keywords: Automated pretreatment functionality, co-injection

1. Co-Injection Function

In addition to regular injection procedures, autosamplers in the Nexera series (SIL-40 series) are equipped as standard with a variety of automatic pretreatment functions for diluting samples or adding reagents for example. One such function, the co-injection function, can be used to take a specified volume of reagent or solvent from a specified vial, and inject it together with the sample, or to mix it with the sample inside the needle.

Using this function, a specified volume of reagent (or solvent) from a specified vial can be injected together with the sample (Fig. 1).

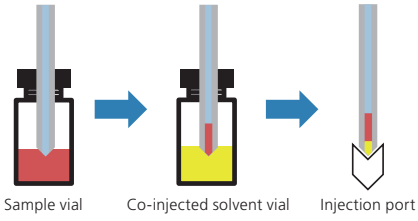

Fig. 1 Illustration of the Injection Process

2. Settings for the Co-Injection Function

Settings for co-injection and other standard automatic pretreatment functions can be configured easily using a LabSolutions template, and saved in a method file (Fig. 2).

The applicable parameter settings are indicated below.

(1) Tray and vial number for the reagent to be co-injected
(2) Injection volume of reagent to be co-injected
(3) Injection timing (before, after, or before and after the sample)
(4) Mixing count and volume (number of agitation cycles within the needle, and the volume)
(5) Wait time (wait time after mixing before injection)
(6) Air gap volume inserted before and after aspirating the sample and the co-injected reagent

It is also possible to successively aspirate user-defined volumes from multiple vials for co-injection, as shown in Fig. 3.

In addition to co-injection, programmed pretreatment actions not specified in a template can also be changed and executed.

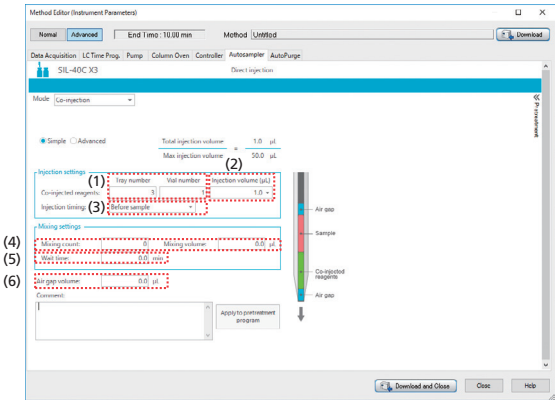

Fig. 2 Settings for Co-Injection Function

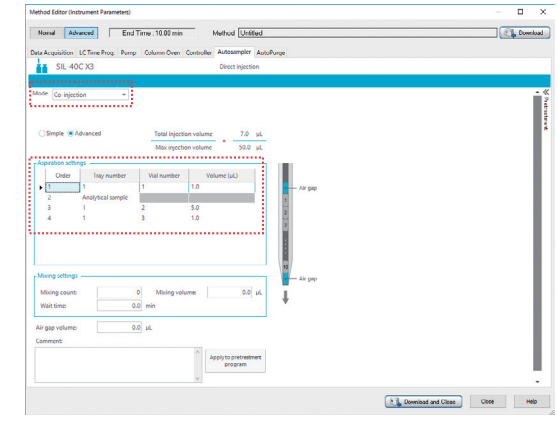

Fig. 3 Settings for Co-Injection Function (Advanced)

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3. Example of the Use of Co-Injection to Improve Peak Shape

The simultaneous analysis of lipid mediators and related substances is mainly performed in the context of biomarker discovery and in disease research fields. Because lipid analysis involves the use of sample solvents with a high organic solvent ratio, peak shapes for weakly retained components can become distorted during reverse-phase chromatography.

In this example, the co-injection function was used to improve the peak shapes for a mixed standard solution prepared with 100 % methanol as the solvent.

The effect of co-injecting water using the analytical conditions indicated in Table 1 is shown in Fig. 4. Co-injection improved both the peak shape and the tailing factor (S). It was particularly effective for components with fast retention times.

Table 1 Analytical Conditions

Column	: C8 column (2.1 mm I.D. × 150 mm L, 2.6 µm)
Mobile Phase A	: 0.1 % aqueous formic acid solution
Mobile Phase B	: Acetonitrile
Rinse Solution	: Acetonitrile
Other Conditions	: Compatibility with Shimadzu LC/MS/MS method package for lipid mediators
Sample	: Standard sample of lipid mediators and related compounds (mixture of 196 components in methanol solution)
Co-Injected Solvent	: Water (5, 10, and 15 µL)

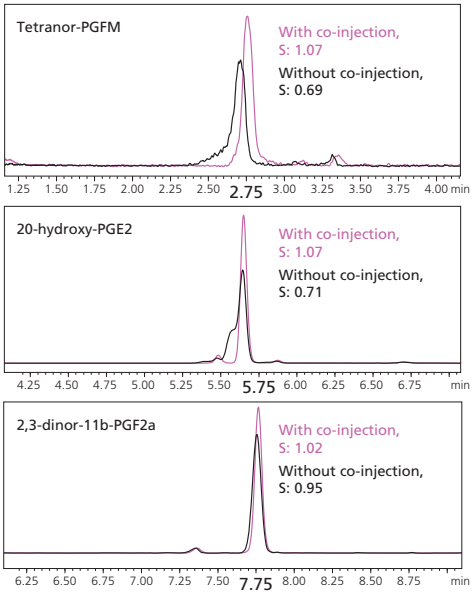

Fig. 4 MRM Chromatograms for Various Lipids
(with either 15 µL or no co-injection)
(The tailing factor S was calculated by the USP method.)

Fig. 5 shows the relationship between the quantity of co-injected solvent (water) and the improvement in peak shape. The greater the volume of co-injected water, the greater the improvement in the leading slope for both of the compound peaks, and in the resolution (Rs) for both components.

Further improvements in peak shape and resolution can be expected from optimizing the type and volume of co-injected solvent.

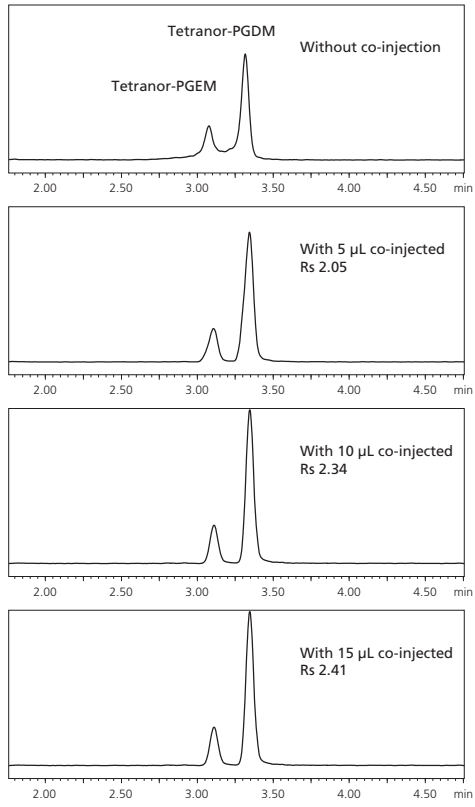

Fig. 5 Comparison of Resolution between Lipid Isomers
(with 5 ,10 ,15 µL or no co-injection)
(Resolution Rs was calculated by the USP method.)

4. Summary

- Autosamplers in the SIL-40 series are equipped as standard with a function for automatically pretreating samples inside the autosampler, including co-injection and dilution.
- For reverse-phase chromatography with samples that have a high organic solvent ratio, the use of the co-injection function to simultaneously inject a solvent with the sample can improve the shape of peaks with a short elution time, and improve the reliability of quantitative analysis results.

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