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Application News

Software for Efficient Method Development
Ultra High Performance Liquid Chromatograph

Automatic Optimization of Sample Solvent Composition for Achieving Sharp Peak Shapes

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User Benefits

- The automatic pretreatment function integrated into the autosampler enables efficient optimization of sample solvent composition, which can be automatically adjusted during analysis.
- The desired composition of sample solvent can be easily specified via the LabSolutions™ MD interface.

■ Introduction

In LC analysis, the composition of the sample solvent is critical for achieving proper peak shape. If the sample solvent is a stronger eluting solvent than the mobile phase, sample band condensation at the column inlet may be insufficient, leading to sample band broadening. For instance, increasing the organic solvent ratio in the sample solvent to dissolve low-polar compounds may deteriorate the peak shapes of early-eluting compounds in reversed-phase chromatography. Therefore, determining the optimal ratio of organic solvent in the sample solvent is essential. However, manually preparing multiple solvent compositions is extremely time-consuming. The automatic pretreatment function of the autosamplers (Nexera™ series), in combination with the method development support software LabSolutions MD, enables consecutive analyses using different sample solvent compositions without manual intervention. This allows evaluation of the effect of sample solvent composition on peak shape while significantly reducing the labor involved in optimization. In this study, we demonstrate the automated determination of the optimal sample solvent composition using metoclopramide, a small-molecule drug, as a model compound.

■ Automatic Pretreatment Function

The automatic pretreatment function integrated into the autosampler enables drawing and ejecting specified volumes of reagents and solvents from any vial, as well as mixing them within the needle. As an example, Fig. 1 illustrates the procedure for automatically adjusting the water-to-methanol ratio in the sample solvent using this function. The autosamplers (Nexera series) supports up to three rinsing solvents via the multi-rinse function, and in this procedure, methanol and water were supplied from these rinsing lines. By modifying the "methanol eject volume" (2) and “water eject volume” (3) in Fig. 1, sample solvents with desired methanol-to-water ratios can be automatically prepared. In addition, adjusting the “sample drawing volume” (1) allows for not only changing the sample solvent composition but also diluting the sample solution to a specified concentration.

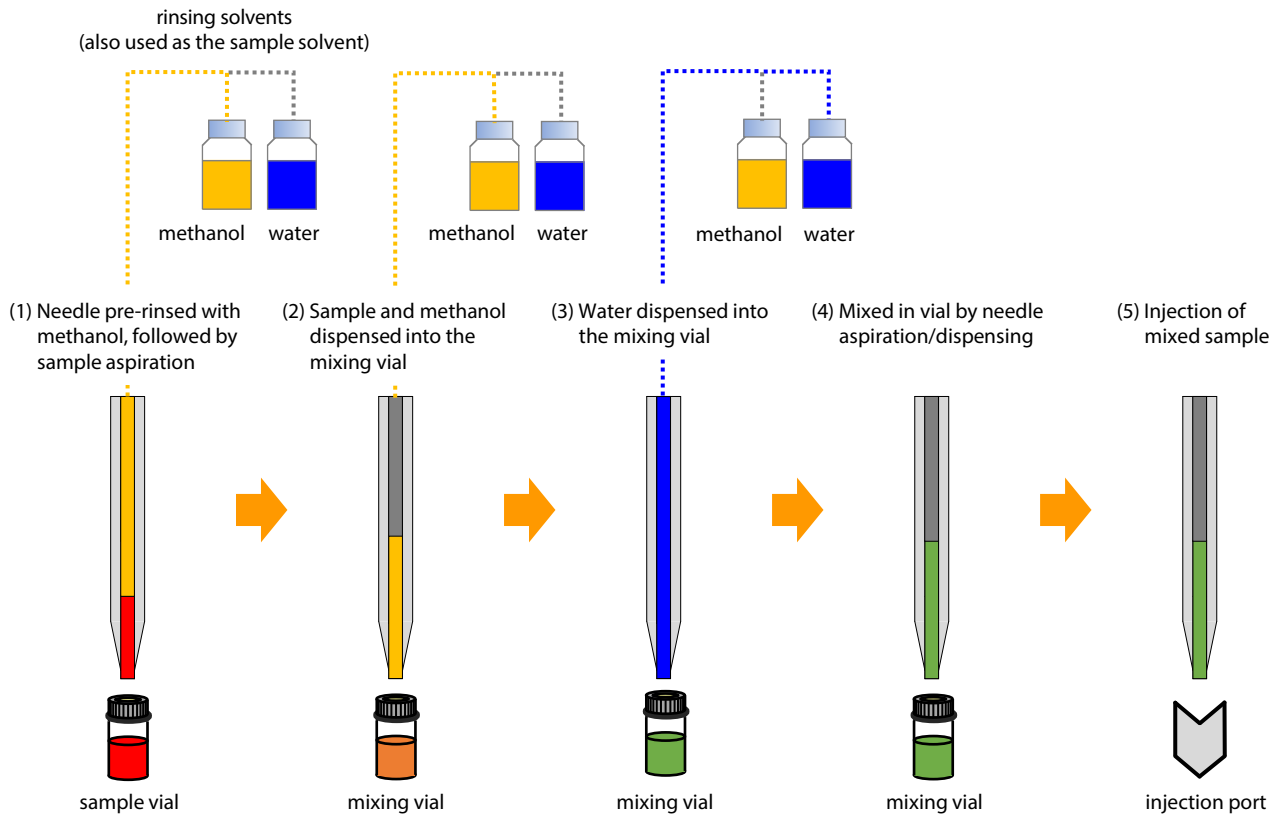

Fig. 1 Automatic Preparation of Sample Solvent Using the Pretreatment Function

■ Analytical Conditions and Target Compounds

A 1000 mg/L solution of metoclopramide, a small-molecule drug, was prepared in 100% methanol as a model sample. The water content in the sample solvent was then varied from 0% to 90% in 10% increments (A 10-fold dilution was applied during automatic sample solvent preparation), and its effect on peak shape was evaluated under the analytical conditions shown in Table 1. By simply specifying the desired water content on the LabSolutions MD interface (red frame in Fig. 2), the autosampler's automatic pretreatment function adjusts the sample solvent composition during consecutive analyses. This eliminates the need for manual preparation of multiple solvent mixtures, significantly reducing the workload involved in optimizing sample solvent composition for ideal peak shapes.

Table 1 Analytical Conditions and Target Compounds

System	: Nexera X3
Sample	: Metoclopramide
Sample solvents	: Methanol / Water = 100 - X : X*1
Column	: Shim-pack Scepter™ C18-120*2 (100 mm × 3.0 mm I.D., 1.9 µm)
Mobile phases	
Pump A	: 0.1% formic acid in water
Pump B	: Methanol
Temperature	: 40 °C
Injection volume	: 5 µL (100 mg/L)
Flow rate	: 0.6 mL/min
B Conc. (Isocratic)	: 50%
Detection	: 254 nm (SPD-M40, STD cell)

*1 : X = 0, 10, 20, 30, 40, 50, 60, 70, 80, 90
*2 P/N : 227-31013-03

		Pretreatment Program	
Sample Name	Vial	Water in sample solvent (%)	Dilution factor
Metoclopramide_0	1	0	10
Metoclopramide_10	1	10	10
Metoclopramide_20	1	20	10
Metoclopramide_30	1	30	10
Metoclopramide_40	1	40	10
Metoclopramide_50	1	50	10
Metoclopramide_60	1	60	10
Metoclopramide_70	1	70	10
Metoclopramide_80	1	80	10
Metoclopramide_90	1	90	10

Fig. 2 Setting Screen of Water Ratio in Sample Solvent (LabSolutions MD)

■ Effect of Sample Solvent Composition on Peak Shape

Fig. 3 (left) shows the chromatograms obtained when the water content in the sample solvent was varied using the automatic pretreatment function. For comparison, Fig. 3 (right) presents chromatograms obtained by manually varying the water content. Fig. 4 illustrates the variation in peak height as a function of water content in the sample solvent. As shown in Fig. 3, at low water contents (0–30%), peak broadening was observed due to the stronger elution strength of the sample solvent, resulting in poor peak shapes. In contrast, as the water content increased, peak shape improved and peak height gradually recovered. Nearly constant peak heights were achieved at water contents of 50% or higher. These results suggest that increasing the water content in the sample solvent reduces its elution strength relative to the methanol-rich mobile phase (50% methanol), leading to improved peak shapes. As shown in Fig. 4, peak height plateaued at water contents of 50% or higher, indicating that a water content of at least 50% is suitable for this target compound.

It was observed that increasing the water content above 50% led to a modest improvement in peak shape. However, higher water content tends to reduce the solubility of the sample. Although good peak shapes may still be achieved, using 100% water as the sample solvent may lead to poor accuracy and precision due to limited solubility. Sample solubility also depends on co-existing compounds, but based on the results obtained with standard solutions, a water content of approximately 50–70% appears optimal for balancing peak shape and solubility. The selection of an appropriate sample solvent is critically important, as it significantly affects both chromatographic behavior and quantitative reliability. Furthermore, as shown in Fig. 4, the variation in peak height (red line) obtained using the automatic pretreatment function closely matches that obtained with manual preparation (blue line), with no significant difference observed. This confirms that the automatic pretreatment function performs equivalently to manual preparation.

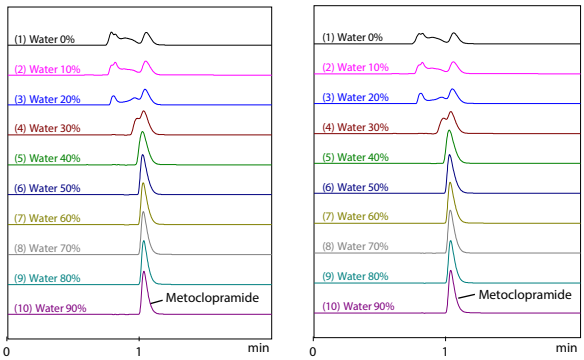

Fig. 3 Chromatograms Obtained with Different Water/Methanol Ratios in the Sample Solvent
Left: Prepared by Automatic Pretreatment Function, Right: Prepared Manually.
(Peak heights normalized to a maximum value of 1.)

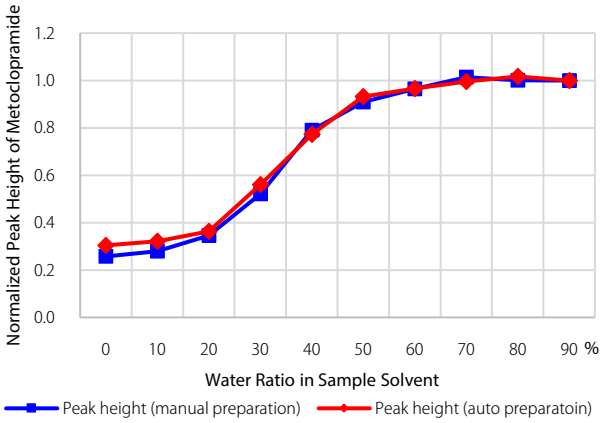

Fig. 4 Relationship Between Water Content in the Sample Solvent and Peak Height.
(Peak heights normalized to a maximum value of 1.)

■ Conclusion

Optimizing the composition of the sample solvent is essential for achieving appropriate peak shapes. However, manual preparation of multiple solvent compositions is time-consuming. The automatic pretreatment function of the autosampler, in combination with LabSolutions MD, enables consecutive analyses with automatic variation of the sample solvent composition. This significantly reduces the effort required for optimization. Since the optimal sample solvent composition depends on both the physicochemical properties of the analyte and the mobile phase conditions, optimization is necessary for each sample. These tasks, however, can be greatly reduced by utilizing the automatic pretreatment function.

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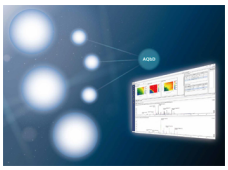

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