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Application News

Software for Efficient Method Development

Automatic Optimization of Gradient Conditions by AI Algorithm and Seamless Method Transfer

-Consecutive Optimization at Different Column Oven Temperatures-

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User Benefits

- The AI algorithm of LabSolutions™ MD can automatically optimize gradient conditions to greatly reduce labor of LC method development.
- Gradient conditions are automatically optimized at different column oven temperatures consecutively.
- UHPLC methods efficiently developed in a short time can be automatically transferred to conventional HPLC methods while maintaining the separation pattern.

■ Introduction

In the typical LC method development, the process begins with “preparation” which includes mobile phase preparation, column installation, and creation of analysis schedules, then the analysis is started. After that, the acquired data is analyzed and “preparation” for the subsequent analysis is carried out, followed by starting the next analysis again. The method development progresses by repeating these processes, but in addition to the significant time required to repeatedly create analysis schedules, expertise in chromatography is necessary to explore optimal conditions based on data analysis. In other words, typical method development requires “human intervention”. Therefore, eliminating human involvement and automating such method development processes would be desirable to improve labor efficiency. This article introduces an approach for automatically optimizing gradient conditions at different column oven temperatures consecutively, identifying the combination of temperature and gradient conditions that meet the resolution criteria. Subsequently, a case study on the automatic adjustment of parameters for transferring an optimized UHPLC method to conventional HPLC is described.

■ Analytical Conditions and Target Compounds

The analytical conditions and target compounds are shown in Table 1. In this article, a mixture of seven compounds was used as the model sample. Resolution criteria was specified for the sample, and a combination of a temperature and gradient conditions that met the criteria were automatically searched using LabSolutions MD (A dedicated software for supporting method development : Technical Report C190-E309). Specifically, the automatic optimization of gradient conditions was consecutively applied at three different column oven temperatures (30, 40, and 50 °C).

Table 1 Analytical Conditions and Target Compounds

System : Nexera™ X3	
Sample : (1) Hydrocortisone, (2) Furosemide, (3) Ketoprofen, (4) Naproxen (5) Probenecid, (6) Diclofenac, (7) Indomethacin	
Mobile phase:	
Pump A : 0.1% formic acid in water	
Pump B : Acetonitrile	
Column : Shim-pack Scepter™ C18-120 *1 (100 mm × 3.0 mmI.D., 1.9 µm)	
Analytical conditions	
B Conc.	: 30% (0 min)→60% (X *2 min) →30% (X~X+5 min)
Column Temp.	: 30, 40, 50 °C
Flow rate	: 0.7 mL/min
Injection Vol.	: 5 µL
Detection	: 254 nm (SPD-M40, STD cell)
Criteria of automatic optimization of gradient conditions	
Minimal resolution	: 1.5

*1 : 227-31013-03 (Shimadzu GLC product number)
*2 : X = 16, 18, 20, 22, 24 (5 patterns)

■ Automatic Optimization of Gradient Conditions

Fig. 1 shows the workflow of automatic optimization of gradient conditions using LabSolutions MD. This software has a unique AI algorithm to automatically explore gradient conditions that satisfy resolution criteria by alternately repeating "improvement of gradient conditions by AI (condition search)" and "analysis under improved conditions (correction analysis)”. For the criteria, "resolution" and "elution time of the last peak" can be set. Automatic optimization can be performed by simply inputting flow rate and column oven temperature (Fig. 2), providing easy operation for anyone, regardless of experience in chromatography. In this article, automatic optimization of gradient conditions was applied at different column oven temperatures (30, 40, and 50 °C) consecutively to meet the criteria of minimal resolution of 1.5 (Fig. 2).

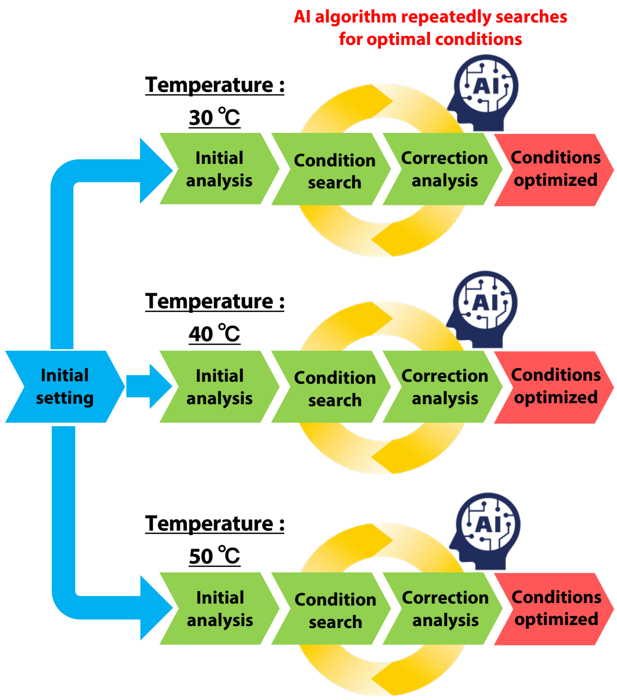

Fig. 1 Workflow for Automatic Optimization of Gradient Conditions by LabSolutions MD

Fig. 2 Setting for Automatic Optimization

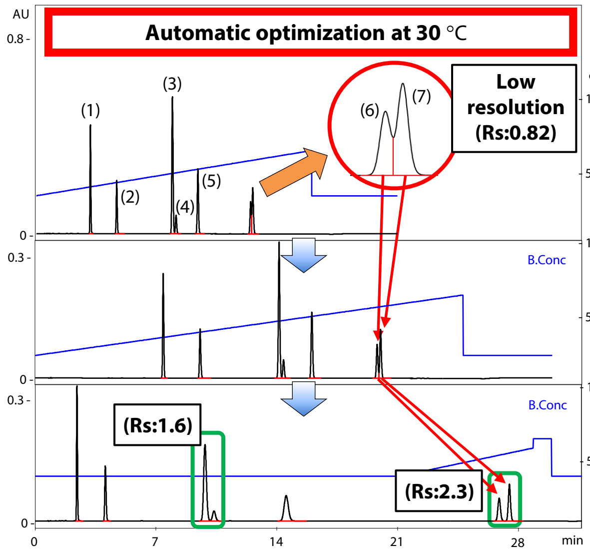

Fig. 3 Result of Automatic Optimization at 30 °C
* blue line shows gradient conditions

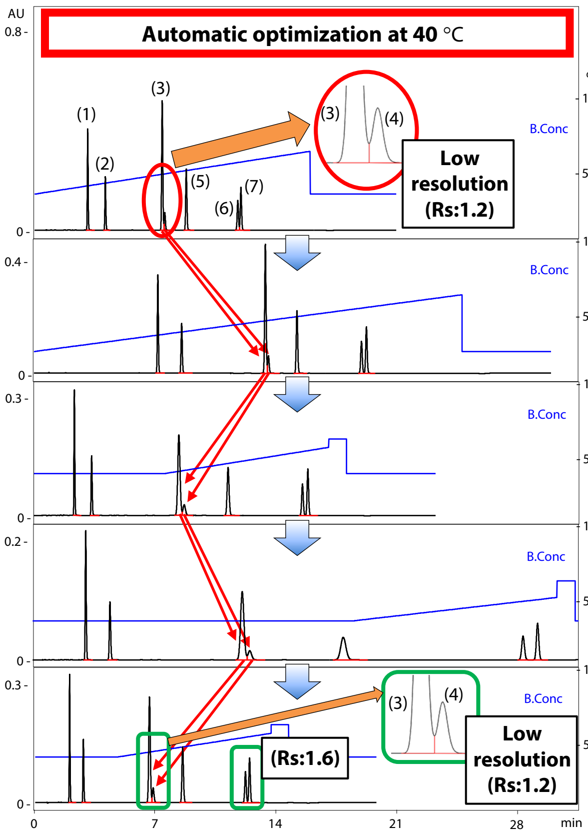

Fig. 4 Result of Automatic Optimization at 40 °C
* blue line shows gradient conditions

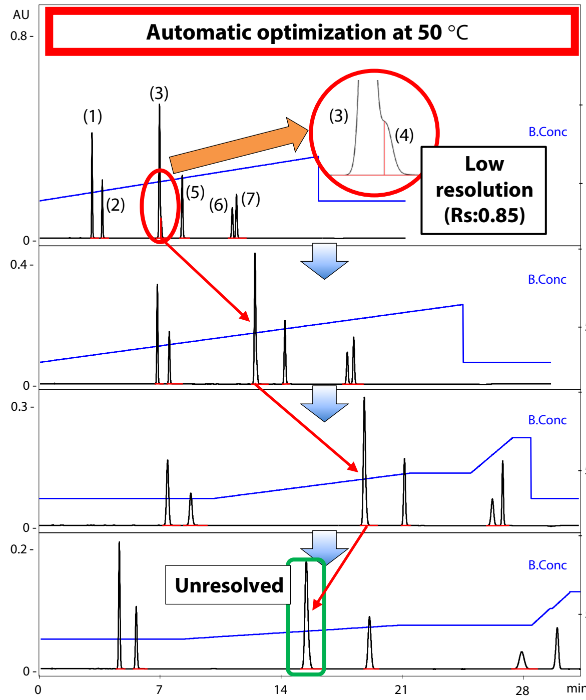

Fig. 5 Result of Automatic Optimization at 50 °C
* blue line shows gradient conditions

The results of automatic optimizations of gradient conditions at different column oven temperatures are shown in Fig. 3~5, respectively. Considering the minimal resolution of 1.5, peaks (6) and (7) were not well separated in the initial analysis when the column oven temperature was 30 °C (red circle in Fig. 3). Similarly, peaks (3) and (4) were not well separated at column oven temperatures of 40 °C and 50 °C (red circles in Fig. 4 and Fig. 5). Following the initial analysis, the gradient conditions that met the resolution criteria were automatically explored using “improvement of gradient conditions by AI” and “analysis under improved conditions”. At 30 °C, the optimized gradient conditions successfully met the resolution criteria (green box in Fig. 3). However, at 40 °C and 50 °C, even with automatic optimization, peaks (3) and (4) failed to meet the resolution criteria. These results indicate that the optimal column oven temperature is 30 °C, as it achieves the minimal resolution of 1.5 for all peaks. As demonstrated, the automatic optimization of gradient conditions can be applied consecutively to multiple column oven temperatures, enabling users to identify suitable conditions that meet the criteria, regardless of their experience in chromatography.

■ Seamless Method Transfer Support

Transferring analytical methods between systems with differing system volumes or column dimensions while maintaining consistent separation patterns necessitates appropriate adjustment of several LC parameters. Manual adjustment can be labor-intensive and prone to input errors during parameter calculation and system entry. LabSolutions MD addresses these challenges by automatically calculating the necessary LC parameters for method transfer and generating the corresponding method files, as illustrated in steps (1) to (4) of Fig. 6. For instance, a method optimized through automatic gradient optimization using Nexera X3 system with a 100 mm × 3.0 mm I.D., 1.9 µm column was successfully transferred to conventional HPLC conditions on Nexera lite system equipped with a 150 mm × 4.6 mm I.D., 5 µm column, as depicted in Fig. 6 (analytical conditions detailed in Table 2). By selecting the target system for method transfer (Fig. 6(1)), entering the column dimensions (Fig. 6(2)), and specifying the flow rate (Fig. 6(3)), LabSolutions MD automatically adjusts the parameters required for method transfer.

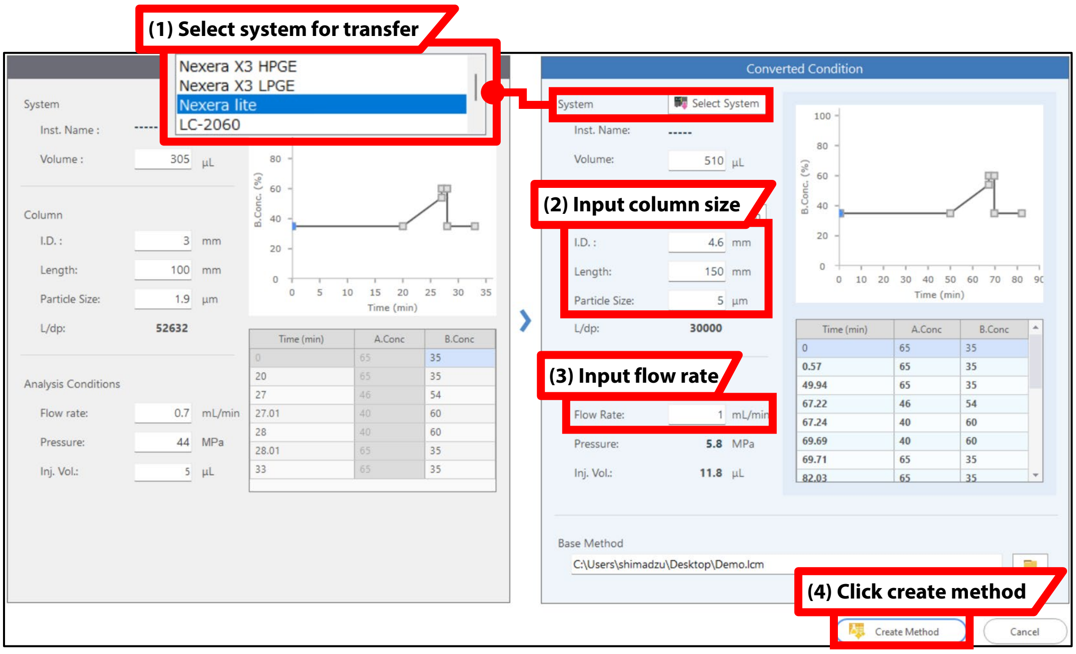

Fig. 6 Automatic Calculation of Parameters for Method Transfer by LabSolutions MD

Table 2 Analytical Conditions (Parameters Automatically Transferred by LabSolutions MD)

【Parameters before transfer】	
System	: Nexera X3
Column	: Shim-pack Scepter C18-120 (100 mm × 3.0 mmI.D., 1.9 µm)
Analytical conditions	
B Conc.	: 35% (0~20 min)→54% (27 min) →60% (27.01~28 min) →35% (28.01~33 min)
Flow rate	: 0.7 mL/min
Injection Vol.	: 5 µL
【Parameters after transfer】	
System	: Nexera lite
Column	: Shim-pack Scepter C18-120 *1 (150 mm × 4.6 mmI.D., 5 µm)
Analytical conditions	
B Conc.	: 35% (0~50 min)→54% (67 min) →60% (67.01~70 min) →35% (70.01~82 min)
Flow rate	: 1 mL/min
Injection Vol.	: 12 µL

*1 : 227-31020-05 (Shimadzu GLC product number)

■ Chromatogram Comparison Before and After Method Transfer

Fig. 7 presents a comparison of chromatograms before transfer (conditions are upper section of Table 2) and after transfer (conditions are lower section of Table 2 : parameters automatically calculated by LabSolutions MD). Although the chromatograms were acquired using different systems and columns, similar separation patterns were observed. Notably, even for the most challenging peaks, peaks (3) and (4), baseline separation was maintained before and after transfer. These results demonstrate that LabSolutions MD enables reliable method transfer while preserving separation patterns through automatic parameter adjustment.

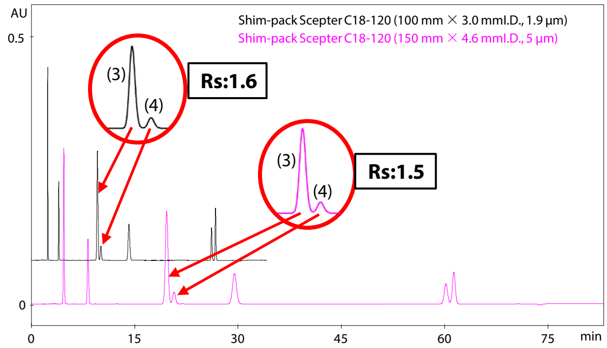

Fig. 7 Comparison of Chromatograms Before (Upper) and After (Lower) Method Transfer

■ Conclusion

Automatic optimization of gradient conditions using AI algorithm of LabSolutions MD was applied to a model sample (mixture of seven compounds of small molecule) at different column oven temperatures. As a result, the temperature and gradient conditions that met the resolution criteria were successfully explored. Furthermore, a case study in which an optimized method was transferred to conventional HPLC conditions while maintaining the separation pattern was also introduced, demonstrating how LabSolutions MD supports seamless method transfer by automatically adjusting the parameters across different systems and columns. In method development, human intervention, such as analysis batch creation and data analysis, is required to optimize gradient conditions. LabSolutions MD can provide significant labor savings in this area. For more information on LabSolutions MD, please refer to the Technical Report "Efficient Method Development Based on Analytical Quality by Design with LabSolutions MD Software (C190-E284)".

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