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Application News

Software for Efficient Method Development
Ultra High Performance Liquid Chromatograph

Automatic Optimization of Separation Conditions by AI Algorithm
-Optimization under Isocratic Elution-

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User Benefits

- The AI algorithm of LabSolutions™ MD can automatically optimize separation conditions, greatly reducing the workload involved in LC method development.
- By optimizing the separation under isocratic elution, method transfer between different systems becomes easier.
- Automatic optimization under isocratic elution can efficiently convert existing gradient methods to isocratic ones.

■ 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 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. In this article, a case study is presented in which a mixture of low-molecular-weight compounds was used as a model sample, and conditions meeting the resolution criteria were automatically explored using the dedicated software for supporting method development "LabSolutions MD". For gradient elution, method transfer between different systems while maintaining the separation pattern requires appropriate adjustment of various LC parameters, and achieving an exact match of the separation pattern before and after transfer can be challenging. In this article, optimization of separation conditions under isocratic elution was carried out to facilitate smoother method transfer.

■ Analytical Conditions and Target Compounds

Analytical conditions and target compounds are listed in Table 1. In this study, a mixture of five low-molecular-weight compounds was used as a model sample, prepared in two patterns with different combinations of compounds. Resolution and final peak elution time criteria were set for these samples, and separation conditions meeting these criteria were automatically explored using LabSolutions MD.

Table 1 Analytical Conditions and Target Compounds

System : Nexera™ X3	
Sample 1 : (A) Antipyrine, (B) Benzoic acid, (C) Salicylic acid, (D) Hydrocortisone, (E) Furosemide	
Sample 2 : (A) Antipyrine, (B) Benzoic acid, (C) Furosemide, (D) Naproxen, (E) Probenecid	
Mobile phase	
Pump A : 0.1% formic acid in water	
Pump B : Acetonitrile	
Column : Shim-pack Scepter™ C18-120 *1(100 mm × 3.0 mm I.D., 1.9 µm)	
Analytical conditions	
B Conc.	: 5%(0 min)→80%(X~X+1*2 min) →5%(X+1~X+5 min)
Column Temp.	: 40 °C
Flow rate	: 0.7 mL/min
Injection Vol.	: 5 µL
Detection	: 254 nm (SPD-M40, STD cell)
Criteria of automatic optimization	
Minimal resolution	: 1.5
Time of last eluting peak	: < 5 min
Gradient mode	: Isocratic

*1 P/N : 227-31013-03
*2 X = 2, 3, 4, 5, 6 (for sample 1), X = 3, 4, 5, 6, 7 (for sample 2)

■ Automatic Optimization of Separation Conditions

Fig. 1 shows the workflow for automatic optimization of separation conditions using LabSolutions MD. This software is equipped with a unique AI algorithm that automatically explores conditions satisfying the set criteria by iteratively performing "improvement of separation conditions by AI (condition search)" and "analysis under the improved conditions (correction analysis)." The criteria can be set for the resolution of any peak and the elution time of the last peak. In this study, the minimum resolution was set to 1.5 and the elution time of the last peak to less than 5 minutes, and separation conditions meeting these criteria were automatically explored, taking into account the reduction of analysis time. Optimization was performed under isocratic elution (Fig. 2).

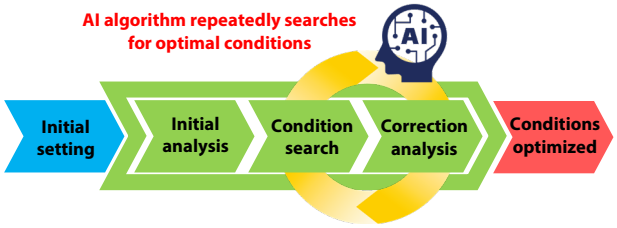

Fig. 1 Workflow for Automatic Optimization of Separation Conditions by LabSolutions MD

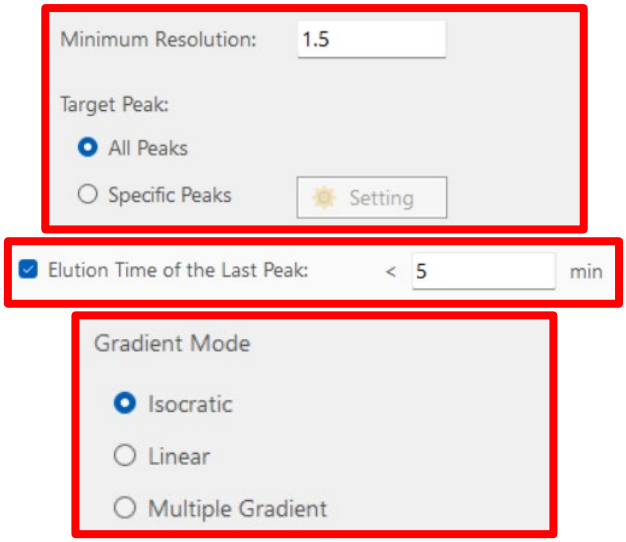

Fig. 2 Setting for Automatic Optimization

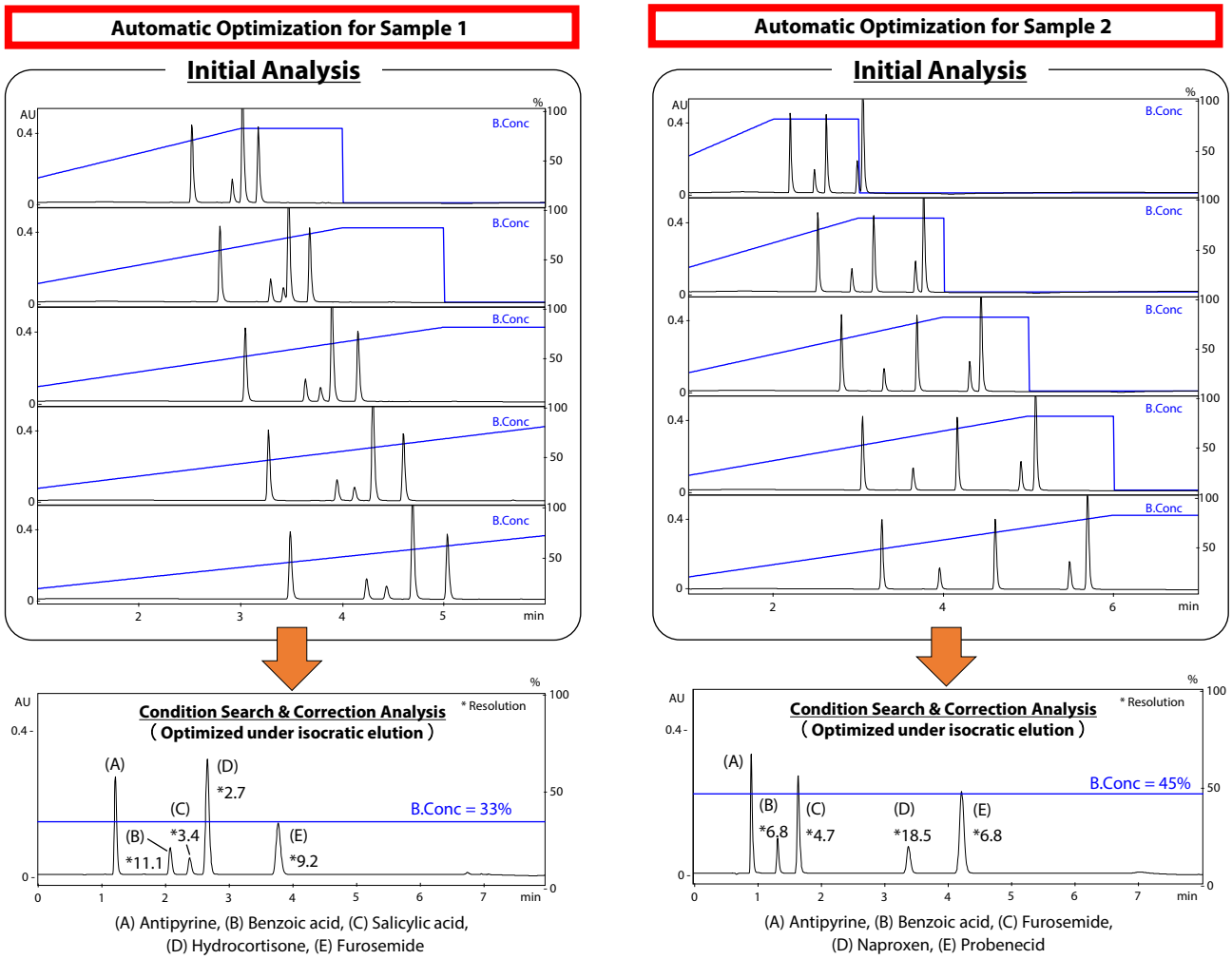

Fig. 3 Results of Automatic Optimization for Sample 1 (left) and Sample 2 (right)
(The blue line indicates the gradient conditions)

The results of automatic optimization of separation conditions are shown in Fig. 3. After initial analyses were performed using five gradient conditions (Table 1), the AI algorithm automatically optimized the separation of all compounds through correction analyses. For Sample 1, isocratic elution at 33% enabled all compounds to achieve a resolution of 1.5 or higher while keeping the retention time of the last peak (compound E) within 5 minutes. Similarly, for Sample 2, isocratic elution at 45% allowed all compounds to reach a resolution of 1.5 or higher, with the retention time of the last peak (compound E) also within 5 minutes.

■ Conclusion

Automatic optimization of separation conditions under isocratic elution using the AI algorithm of LabSolutions MD was applied to a mixture of low-molecular-weight compounds as a model sample. As a result, conditions meeting the criteria (“minimum resolution: 1.5” and “elution time of the last peak: less than 5 minutes”) were successfully explored automatically. By using LabSolutions MD, separation conditions satisfying the set criteria can be automatically identified, which is expected to significantly reduce the workload of method development. In addition, optimization under isocratic elution facilitates method transfer between different systems. While this article introduces a case of automatic optimization of separation conditions, LabSolutions MD also supports the entire workflow of method development, including the screening and robustness evaluation phases. For further details, 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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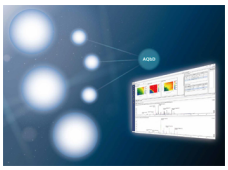

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