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Software for Efficient Method Development

Application News

Enhancing Efficiency of Method Development with Integrated LC System

Shinichi Fujisaki

User Benefits

- A comprehensive analysis schedule can be automatically generated by varying LC parameters such as gradient conditions and column oven temperatures.
- By utilizing Evaluation Value provided by LabSolutions™ MD, optimal separation conditions can be quickly identified from a large amount of data.

■ Introduction

Developing appropriate separation conditions in LC method development often requires considerable time. For example, creating an analysis schedule to explore a wide range of conditions can be a labor-intensive process. In addition, evaluating which conditions provide the desired separation requires reviewing all chromatograms obtained, resulting in significant effort for data analysis. This article introduces an approach for simplifying and accelerating the process of optimization of separation conditions by combining the integrated LC system, i-Series (Fig. 1), with LabSolutions MD, a dedicated software for supporting method development. Specifically, a mixture of nine small-molecule compounds was used as a model sample, and an analysis schedule was automatically generated to systematically vary parameters such as gradient conditions and column oven temperatures. Furthermore, Evaluation Value calculated by LabSolutions MD were applied to the large volume of acquired data, enabling the rapid identification of optimal separation conditions.

■ Analytical Conditions and Target Compounds

The analytical conditions and target compounds are shown in Table 1. In this article, a mixture of nine small-molecule compounds was used as the model sample. Separation conditions were optimized by comprehensively varying gradient conditions and column oven temperatures.

Table 1 Analytical Conditions and Target Compounds

System : LC-2080C 3D	
Sample : (1) Antipyrine, (2) Benzoic acid, (3) Salicylic acid (4) Hydrocortisone, (5) Furosemide, (6) Ketoprofen (7) Naproxen, (8) Probenecid, (9) Diclofenac	
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.	: X *2 %(0 min)→80%(Y *3 min) →X%(Y~Y+4 min)
Column Temp.	: 25, 35, 45 °C
Flow rate	: 0.7 mL/min
Injection Vol.	: 5 µL
Detection	: 254 nm (STD cell)

*1 P/N : 227-31013-03
*2 : X = 10, 15, 20 (3 patterns)
*3 : X = 5, 10, 15 (3 patterns)

Fig. 1 Integrated LC System "i-Series" (LC-2080C 3D)

■ Automatic Generation of Analysis Schedules

LabSolutions MD enables error-free automatic generation of analysis schedules (steps (1) to (4) in Fig. 2) under a comprehensive set of conditions. For example, when varying the initial gradient concentration (highlighted in red in Fig. 2), the input center value of 15% is automatically adjusted by ±1 step at 5% intervals, resulting in a schedule covering 10%, 15%, and 20%. In this article, an analysis schedule was automatically generated to explore separation conditions comprehensively by varying the initial gradient concentration (10%, 15%, 20% : 3 levels, Fig. 3), gradient time (5, 10, 15 min : 3 levels, Fig. 3), and column oven temperature (25, 35, 45 °C : 3 levels).

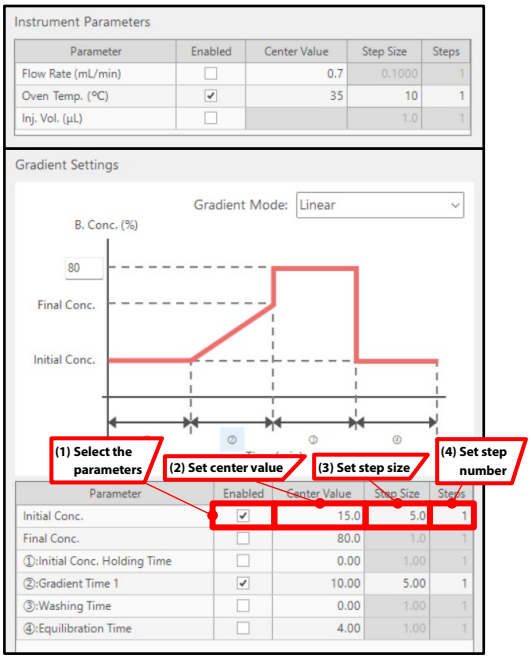

Fig. 2 Analysis Schedule Creation Screen

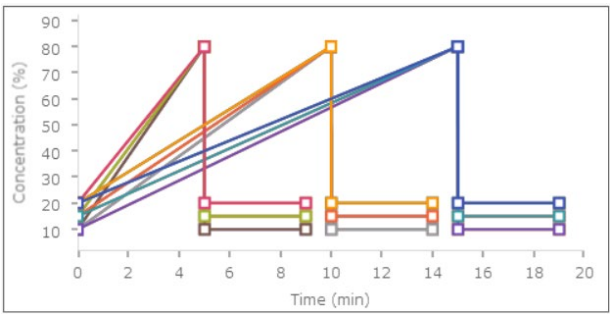

Fig. 3 Variation of Gradient Conditions

■ Quickly Exploring Optimal Conditions

Representative chromatograms obtained by varying gradient conditions and column oven temperatures comprehensively are shown in Fig. 4 and 5. As shown in Fig. 4, longer gradient times tended to improve the resolution between peaks. In addition, Fig. 5 indicates that lower column oven temperatures resulted in better resolution between ketoprofen and naproxen.

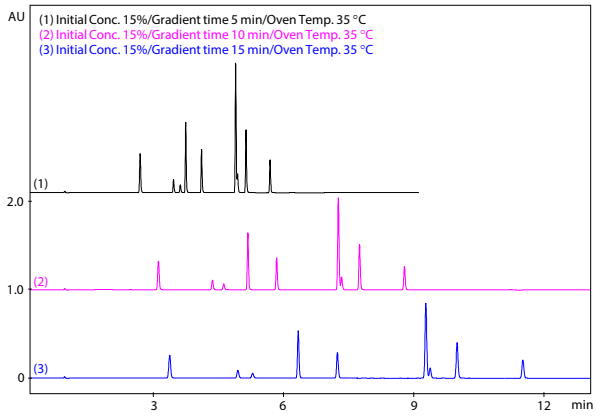

Fig. 4 Chromatograms with Different Gradient Times
5 min (1), 10 min (2), 15 min (3)

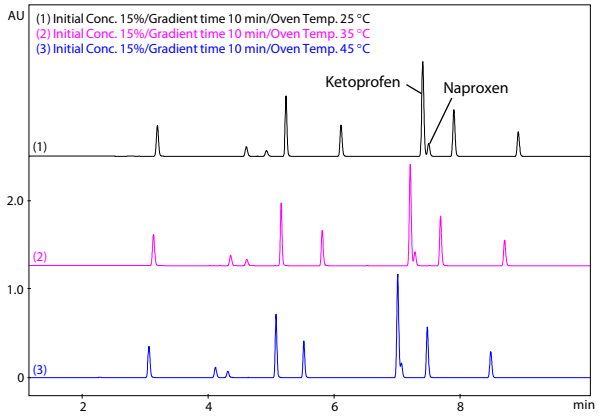

Fig. 5 Chromatograms with Different Column Oven Temperatures
25 °C (1), 35 °C (2), 45 °C (3)

In the process of optimizing separation conditions, varying multiple parameters results in a large number of chromatograms, making it a time-consuming and labor-intensive task to determine which conditions provide the desired separation. This process typically requires both substantial effort and a certain level of chromatographic expertise. LabSolutions MD enables users to quickly and easily identify optimal conditions by quantitatively evaluating the separation performance for each condition using the equation shown below (Eq. 1), eliminating the need to rely on intuition or experience.

$$(\text{Evaluation Value}) = P \times (Rs_1 + Rs_2 + \ldots + Rs_{P-1}) \qquad \text{(Eq. 1)}$$

Evaluation Value is calculated as the number of peaks detected (P) multiplied by the sum of resolution factor (Rs) for all peaks. Fig. 6 shows the Evaluation Values obtained from the investigation of gradient conditions and column oven temperatures, ranked in descending order. The highest Evaluation Value was observed under the condition with an initial gradient concentration of 15%, a gradient time of 15 minutes, and a column oven temperature of 25 °C. The chromatogram obtained under this condition is shown in Fig. 7, where good separation (Rs ≥ 1.5) was achieved between all peaks.

Initial Conc. (%)	Duration 2 (min)	Oven Temp. (°C)	Evaluation Value
15	15	25	139.857
10	15	25	139.761
20	15	25	139.640
15	10	25	138.972
20	10	25	138.626
10	10	25	138.600
20	15	35	137.734
15	15	35	137.587
10	15	35	137.257
15	10	35	137.161

Fig. 6 Ranking of Each Condition by Evaluation Value
(Top 10 Chromatograms Listed from the Highest to the Lowest)

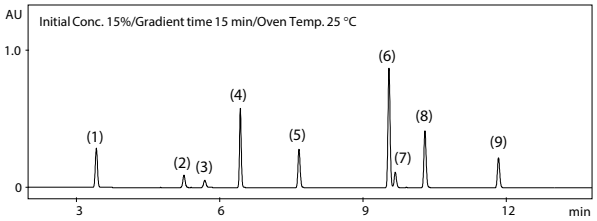

Fig. 7 Chromatogram with Highest Evaluation Value
(Peak numbers correspond to those listed in Table 1)

■ Conclusion

This article introduced a case study using i-Series and LabSolutions MD to streamline the method development process for a mixture of nine small-molecule compounds. With LabSolutions MD, analysis schedules that comprehensively cover various LC parameters can be automatically generated, reducing the risk of errors and improving efficiency compared to manual scheduling. In addition, by applying Evaluation Values to a large amount of data obtained, optimal separation conditions can be quickly and easily identified. While this article focused on the optimization of gradient conditions and column oven temperatures, LabSolutions MD also supports the streamlining of the entire method development workflow including column and mobile phase screening as well as robustness evaluation. For more information, please refer to the Technical Report (C190-E284).

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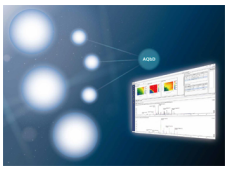

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