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Application News

High Performance Liquid Chromatograph LC-2050C 3D

Optimization of Ion Analytical Conditions in Pharmaceuticals Using LabSolutions MD

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User Benefits

- Using software, each parameter can be varied comprehensively and easily, enabling efficient analysis method development.
- Resolution and analysis parameter relationships can be visually assessed and the valid parameter areas can be confirmed.

■ Introduction

The physicochemical and pharmacokinetic properties of active pharmaceutical ingredients change depending on the counterion. In the drug development stage, various counterions are tested and selected as appropriate salts. Residual inorganic impurities such as catalysts and ions used the synthesis stage can affect product safety, solubility, and stability, so it is very important to analyze ions as impurities.

In this article, an analysis example using ion exclusion chromatography is described. Formic acid, acetic acid, fumaric acid, and maleic acid, which are organic acids frequently used for drug counterions, were analyzed in the example. Response was visualized by drawing design spaces while comprehensively changing each parameter, and analytical conditions were optimized by using LabSolutions™ MD and LC-2050C 3D.

■ Analytical Conditions

In ion exclusion chromatography, retention strength mainly depends on column temperature and acid concentration. In addition, depending on the analytical conditions, there are components that greatly change the retention time, so it is necessary to consider analytical conditions. In this article, we examined the analytical conditions that can achieve good separation of the four components of formic acid, acetic acid, fumaric acid, and maleic acid by using LabSolutions MD. Table 1 shows the analytical conditions used for the separation study of each component.

The resolution of 4 organic acids was comprehensively examined by changing the column temperature and acid concentrations in the mobile phase that affect separation. Acid concentration was changed from 1 to 5 mmol/L in 1 mmol/L increments, and column temperature from 30 °C to 50 °C in 5 °C increments.

Table1 Analytical Conditions

Mobile Phase A:	Water
Mobile Phase B:	10 mmol/L perchloric acid
Column:	Shim-pack™ Fast-OA (100 mm × 7.8 mm I.D., 5 µm)*1 × 2
	Shim-pack Fast-OA (G) (10 mm × 4.0 mm I.D., 5 µm)*2
B Conc.:	10, 20, 30, 40, 50 % (5 patterns)
Column Temp.:	30, 35, 40, 45, 50 °C (5 patterns)
Flowrate:	0.8 mL/min
Vial:	SHIMADZU LabTotal™ for LC 1.5 mL, Glass*3
Injection Vol.:	10 µL
Detection:	PDA at 210 nm

*1 P/N: S228-59942-41
*2 P/N: S228-59942-42
*3 P/N: 227-34001-01

■ Peak Tracking

LabSolutions MD has a function to identify peaks using multiple parameters. In this article, each peak was identified and peak tracking was performed by combining the two parameters of height % and peak elution number for each component (Fig. 1).

It was found that the retention time of fumaric acid changed significantly compared with other peaks. In this case, each peak could be identified automatically by filtering by peak number and peak height % (maleic acid), or by peak height % only (other components). It was also possible to automatically identify each peak for fumaric acid, for which the peak number changed.

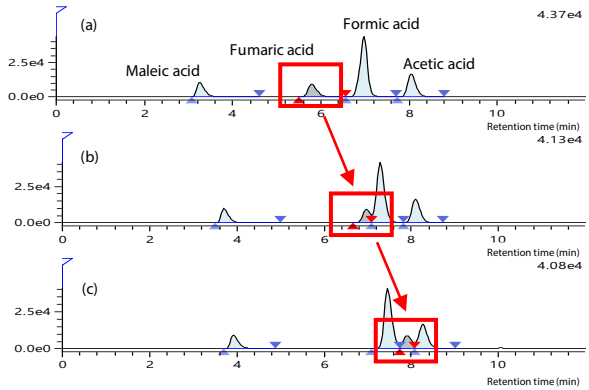

Fig. 1 Chromatogram for Each Analytical Condition

a. Column Temp. : 50 °C, B Conc. : 10 %
b. Column Temp. : 50 °C, B Conc. : 30 %
c. Column Temp. : 35 °C, B Conc. : 40 %

■ Visualizing Separation by Design Space

LabSolutions MD can visually evaluate the relationship between analytical conditions and separation by drawing design spaces. Based on the identified retention time, a design space was produced that shows the minimum separation of each peak in the height direction, with mobile phase B concentration in the vertical axis and the column oven temperature on the horizontal axis (Fig. 2). The warm areas indicate a high response and minimum resolution, allowing the effective analytical conditions to be visually determined.

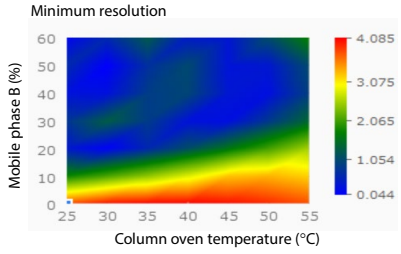

Fig. 2 Design Space for Parameters and Responses

LabSolutions MD also can describe design spaces that focus on specific compounds. Fig. 3 shows the design space when the lower limit of resolution for formic acid and acetic acid is set to 1.5. For formic acid, the region with a resolution of 1.5 or higher is confirmed in the upper left, but with acetic acid, it can be seen that the resolution from other components is not good in the corresponding region. In this way, it is also possible to evaluate the resolution and analytical conditions for each component. In addition, by overwriting the resolution with 2D contour lines, it is possible to evaluate the effective area from multiple perspectives. This time, it was possible to visually confirm the relationship between the effective region showing a resolution of 1.5 or more and the relationship for each component (Fig. 4).

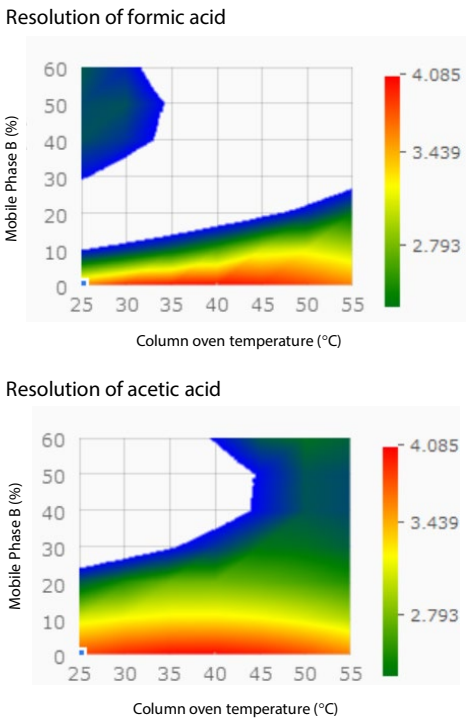

Fig. 3 Design Space for the Resolution of Each Component

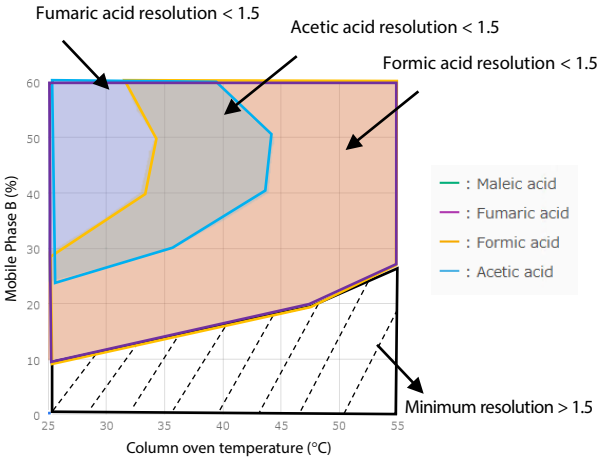

Fig. 4 Overlay 2D Contour Lines

■ Proposal of Optimal Analytical Conditions

LabSolutions MD has a function to search for optimal conditions based on model analysis results. By using this function, it is possible to propose analytical conditions with good separation and high robustness in the entire variation region of various analysis parameters. This time, a search was performed for the optimal point for the minimum resolution, and the corresponding parameters were confirmed (Fig. 5). The predicted and measured chromatograms for the presented analysis parameters are shown in Fig. 6. It was confirmed that there were no large discrepancies in the separation and retention time of each component.

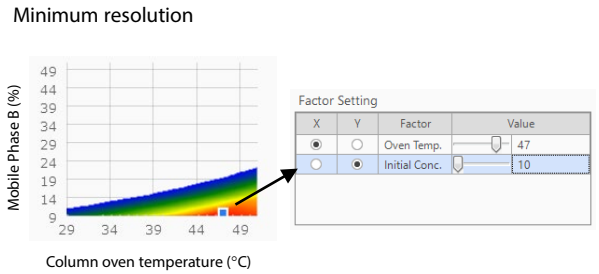

Fig. 5 Optimal Analysis Parameters Proposed

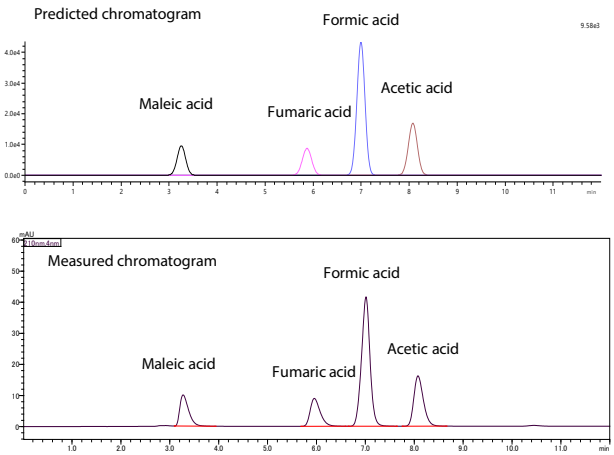

Fig. 6 Predicted and Measured Chromatogram

■ Conclusion

The analytical conditions of the four organic acid components were examined using LabSolutions MD. By using a complex of parameters, it was possible to automatically identify peaks for components whose peak elution order changed. In addition, by drawing the design space, it was possible to visually determine the effect on the resolution of varying various parameters. It was also possible to confirm the optimal analytical conditions.

Using LabSolutions MD makes it possible to optimize analytical conditions based on scientific evidence without depending on the analyst's experience or intuition.

01-00358-EN First Edition: Sep. 2022

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