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Application News

LabSolutions™ MD : efficient method development based on Analytical Quality by Design

Efficient Method Development through Design Space Evaluation on Different Brand of Columns

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User Benefits

- Shim-pack Arata™ C18 can improve separation that is difficult to do with other C18 columns because of its unique selectivity.
- By using LabSolutions MD it is possible to easily identify the difference of selectivity of various columns by evaluating the design space.
- LabSolutions MD makes it efficient to search for the best column by comparing the design spaces of resolution.

■ Introduction

Since the column selection in method development has large effect on separation patterns of analytes, an efficient screening of various columns is often required. LabSolutions MD, a new Shimadzu software for method development, supports efficient method development based on Analytical Quality by Design (AQbD). AQbD-based analysis method development consists of different phases: initial screening, optimization and robustness evaluation. This article introduces an example of column screening using the design space evaluation concept. The selectivity of six brands of C18 column was visualized by design spaces considering different mobile phases composition and different gradient program. This makes it possible to understand the impact of the parameters on separation, enabling to search for the best column with a smaller number of analyses. In this article, it was found that the Shimadzu Shim-pack Arata C18 column has unique selectivity in comparison with the other C18 columns.

■ Analysis Conditions

The differences of selectivity of six brands of C18 column were evaluated through visualization of design spaces (Table 1 shows analysis conditions). In order to evaluate the difference of selectivity between the columns, a basic compound (amitriptyline), an acidic compound (benzoic acid), and a neutral compound (phenol) were used as the target compounds. The analyses were carried out by isocratic separation using a 0.1% formic acid in water as the aqueous mobile phase, and a mixed solution of acetonitrile and methanol as the organic solvent. The ratio of acetonitrile in the organic solvent was varied from 30% to 70% in increments of 20% (3 levels), and the initial concentration of organic solvent was also varied from 40% to 50% in increments of 5% (3 levels), and design spaces for minimum resolution were built for each column based on the results of total of 9 analyses (3 × 3 combinations).

Table 1 Analysis Conditions

System : Nexera™ X3 (Method Scouting System)	
Compound :	
Amitriptyline	
Benzoic acid	
Phenol	
Mobile Phase:	
A) 0.1% formic acid in water	
B) Acetonitrile/Methanol = 30 : 70, 50 : 50, 70 : 30 (3 levels)	
Column :	
1 : Shim-pack Arata C18	(100 mm × 3.0 mm I.D., 2.2 µm)*1
2 : Column A (C18 column)	(100 mm × 3.0 mm I.D., 1.8 µm)
3 : Column B (C18 column)	(100 mm × 3.0 mm I.D., 2.5 µm)
4 : Column C (C18 column)	(100 mm × 3.0 mm I.D., 3 µm)
5 : Column D (C18 column)	(100 mm × 3.0 mm I.D., 3 µm)
6 : Column E (C18 column)	(100 mm × 3.0 mm I.D., 3 µm)
Analytical Conditions (Isocratic):	
B Conc.	: 40, 45, 50% (3 levels)
Column Temp.	: 40 ˚C
Flow Rate	: 0.5 mL/min
Injection Vol.	: 1 µL (80 mg/L)
Detection	: 254 nm (SPD-M40, UHPLC cell)

*1 P/N 227-32802-03

■ Visualization of Resolution by Design Space

The design spaces of minimum resolution between the three types of compounds, amitriptyline, benzoic acid, and phenol, were drawn for the six brands of C18 columns, as shown in Fig. 1, where the vertical line shows the initial concentration of organic solvent and the horizontal line shows the ratio of acetonitrile in the organic solvent. In these figures, the red and blue regions represent high resolution and small resolution, respectively.

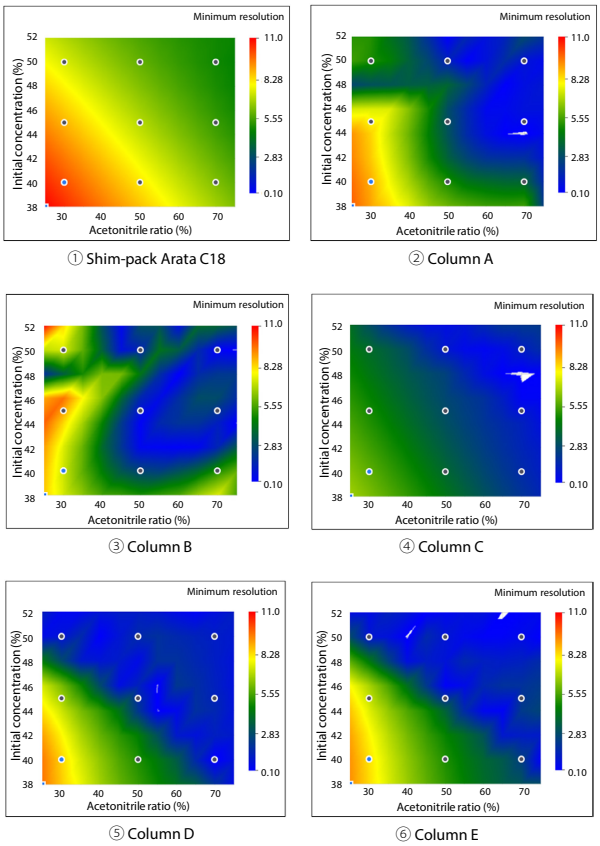

Fig. 1 Design Space for Minimum Resolution of Columns (6 Brands)
* The black dots (total 9 points) in the figure are points where analyses were conducted.
* The white regions (②, ④, ⑥) in the figure are regions where resolution is below the lower limit (0.1).

The design space of ① Shim-pack Arata C18 has a larger red region (higher resolution) compared to other columns, and there is no blue region (lower resolution) in the entire design space. This suggests that Shim-pack Arata C18 provides excellent resolution, as it has different selectivity compared to the other columns over the entire region. As demonstrated by this experiment, the difference of selectivity of each column can be understood easily by visualizing its pattern through design spaces, enabling higher efficiency when searching for the optimum column and analysis condition.

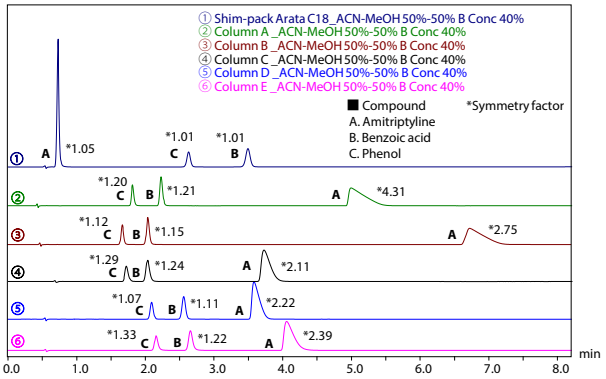

Fig. 2 Chromatograms Obtained with Each Column
Organic solvent: acetonitrile/methanol = 50 : 50,
initial concentration of organic solvent: 40%

Fig. 2 shows the chromatograms obtained with the conditions of acetonitrile ratio in organic solvent as 50% and initial concentration of organic solvent as 40%. With Shim-pack Arata C18, amitriptyline (Peak A) has an excellent peak shape (symmetry factor: 1.05), but with the other columns, deterioration of peak symmetry (symmetry factors: 2.11 to 4.31) can be seen due to interaction with the silanol on the surface of packing material. Moreover, Shim-pack Arata C18 also shows superior peak symmetry to those of the other columns for both the benzoic acid (Peak B) and phenol (Peak C). This experiment also revealed that Shim-pack Arata C18 column shows different elution pattern between acidic and basic compounds, and thus has different selectivity in comparison with the other columns. Because Shim-pack Arata C18 has this unique selectivity, it is possible to maintain better resolution, even in cases where the ratio of acetonitrile in the organic solvent is increased to shorten the analysis time (Fig. 3).

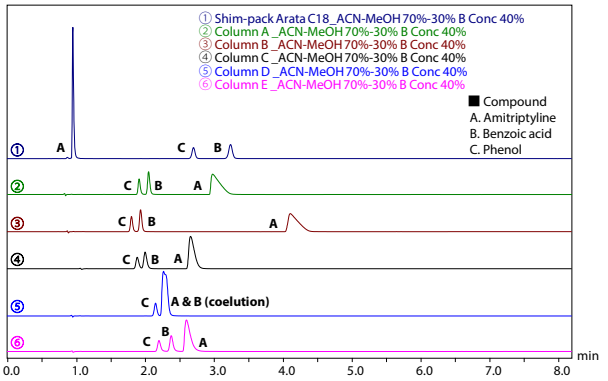

Fig. 3 Chromatograms Obtained with Each Column
Organic solvent: acetonitrile/methanol = 70 : 30,
initial concentration of organic solvent: 40%

Fig. 3 shows the chromatogram for the analyses with a 70% acetonitrile ratio in organic solvent and 40% initial concentration of organic solvent. When compared with Fig. 2, due to the increase of acetonitrile ratio, resolution of benzoic acid (Peak B) and phenol (Peak C) gets worse in chromatograms obtained with columns ② to ⑥, but Shim-pack Arata C18 column keeps enough resolution. Thus, with Shim-pack Arata C18, an excellent peak shape of basic compound and improved separation due to its unique selectivity can be expected in comparison with the other C18 columns.

■ Simplify the Search for Optimum Condition

LabSolutions MD can simplify the search for optimum analysis condition by overlapping design spaces. Fig. 4 shows the area of analysis condition that meets both minimum resolution ≥6 and resolution for amitriptyline ≥18 for each column. In the case of ① Shim-pack Arata C18, the region enclosed by the blue line in the figure is the region where minimum resolution <6 and the region enclosed by the red line is the region where resolution for amitriptyline is <18, and the remaining region (shown by the black hatching) is the region of conditions that satisfies both criteria. Among the six brands of columns, the results show that Shim-pack Arata C18 has the largest region that satisfies both criteria. As shown here, overlapping design spaces enables a quick and simple search for the region that satisfies the criteria of resolution set freely for multiple peaks.

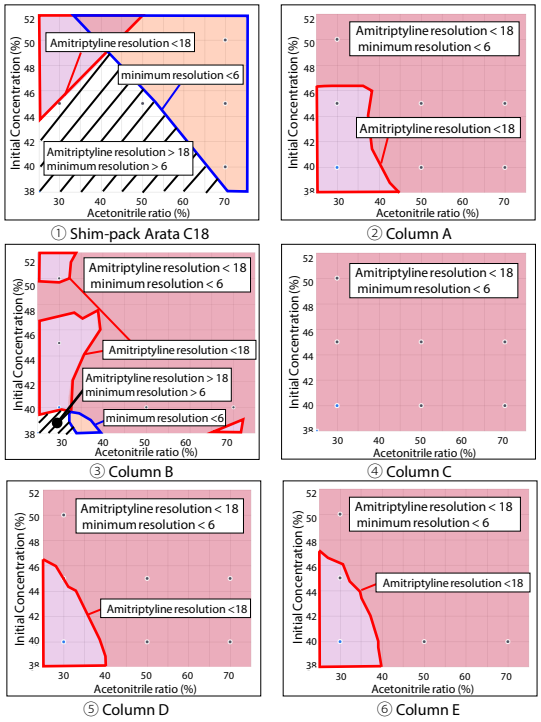

Fig. 4 Overlap of Design Spaces
of Minimum Resolution <6 and Resolution for Amitriptyline <18

■ Conclusion

LabSolutions MD makes it possible to achieve high efficiency in searches for the optimum column and analysis condition, without relying on the user experience, by visualizing and comparing the difference of selectivity between multiple columns through design space.

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