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Application News

Software for Efficient Method Development "LabSolutions™ MD"

Efficient Method Development for Pharmaceutical Stability Testing

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User Benefits

- LC conditions that meet a resolution criteria can be found by visualizing resolution through design space.
- Utilizing predicted chromatograms allows for easy verification of separation behavior under various conditions without actual analysis.
- Peaks detected in multiple chromatograms can be combined, and conditions for their separation can be explored.

■ Introduction

Stability tests for drug approval applications include long-term storage tests, accelerated tests, and severe tests, where changes over time in pharmaceuticals are evaluated under various exposure environments to ensure drug safety and determine shelf life. Proper separation of degradants in these environments is required for their characterization and monitoring over time. LabSolutions MD (Technical Report C190-E284), a dedicated software for method development, supports efficient method development based on Analytical Quality by Design (AQbD). This article introduces a case study on efficiently exploring optimal separation conditions for degradants using model samples that simulate different degradants obtained from stability tests.

■ Analytical Conditions and Target Compounds

The analytical conditions and target samples are shown in Table 1. A control sample containing no degradants and three simulated samples with different degradant profiles (mixtures of small molecular drugs as pseudo-degradants, samples A, B, and C) were employed. The degradants in these three samples (degradants 1, 2, and 3 in Fig. 1) were subjected to LabSolutions MD to efficiently explore optimal separation conditions.

Table 1 Analytical Conditions and Target Compounds

System : Nexera™ X3	
Control	: Antipyrine, Benzoic acid, Hydrocortisone, Furosemide, Ketoprofen, Probenecid, Indometacin
Sample A	: Control with added quinidine, phenol, and acetylsalicylic acid
Sample B	: Control with added naproxen
Sample C	: Control with added diclofenac
Mobile phase:	
Pump A : 0.1% formic acid in water	
Pump B : Acetonitrile	
Column : Shim-pack Velox™ SP-C18 (100 mm × 3.0 mmI.D., 1.8 µm)*1	
Analytical conditions	
B Conc.	: 9%(0 min)→50%(5~8 min)→9%(8~13 min)
Column Temp.	: 30 °C
Flow rate	: 0.7 mL/min
Injection Vol.	: 5.0 µL
Detection	: 254 nm (SPD-M40, UHPLC cell)

*1 : 227-32002-02 (Shimadzu GLC product number)

■ Degradant Profiles Obtained from Stability Test

Fig. 1 shows the chromatograms of samples A, B, C, and the control, assuming the degradants generated by the stability test (analytical conditions : Table 1). Degradant 1 was identified as a degradant in sample A, degradant 2 in sample B, and degradant 3 in sample C. Since these degradants were co-eluted with other compounds (indicated by red circles in Fig. 1), separation from the other compounds is necessary for the evaluation of the generated degradants. By using LabSolutions MD to combine the chromatograms of samples A, B, and C, the investigation of conditions that enable the separation of all components, including degradants, was conducted efficiently.

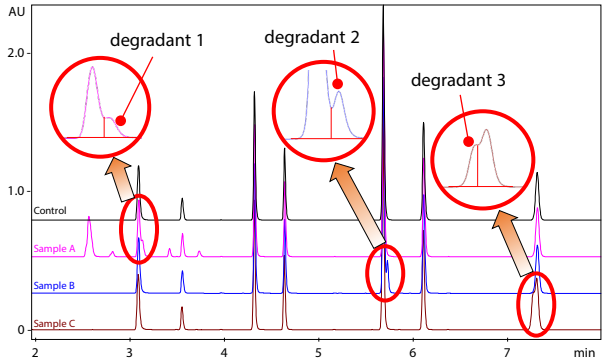

Fig. 1 Chromatograms of Sample A, B, C, and Control

■ Efficiency in Exploring Optimal Separation Conditions Using LabSolutions MD

Optimal separation conditions were explored using LabSolutions MD by varying the final gradient concentrations to 40, 42, 44, 46, and 48% (five levels), the gradient times to 3, 4, 5, 6, and 7 minutes (five levels), and the column oven temperatures to 25, 30, and 35 °C(three levels) for the chromatograms shown in Fig. 1. The results of visualizing the resolution of degradants 1, 2, and 3, using design space are shown in Fig. 2. The vertical axis represents the final gradient concentration, while the horizontal axis represents the gradient time. The red areas in the figures indicate higher resolution, and the blue areas indicate lower resolution. From the design space visualization, it was found that for degradant 1, shorter gradient times provide better separation, and for degradants 2 and 3, lower final gradient concentrations lead to better separation. Additionally, LabSolutions MD can generate predicted chromatograms at any condition within the design space, allowing the separation behavior to be confirmed without actual analysis. For example, the predicted chromatograms for degradant 1 under conditions ① (final gradient concentration : 48%, gradient time : 3.5 min) and ② (final gradient concentration : 48%, gradient time : 7 min) in the design space are shown in Fig. 3.

When the gradient time is changed, degradant 1 shows specific retention behavior compared to the other compounds. In the predicted chromatogram for condition ① (Fig. 3 upper), degradant 1 is separated from the other compounds, whereas in the predicted chromatogram for condition ② (Fig. 3 lower), it is co-eluted. This indicates that condition ①, located in the red area of the design space, provides better separation compared to condition ②, located in the blue area.

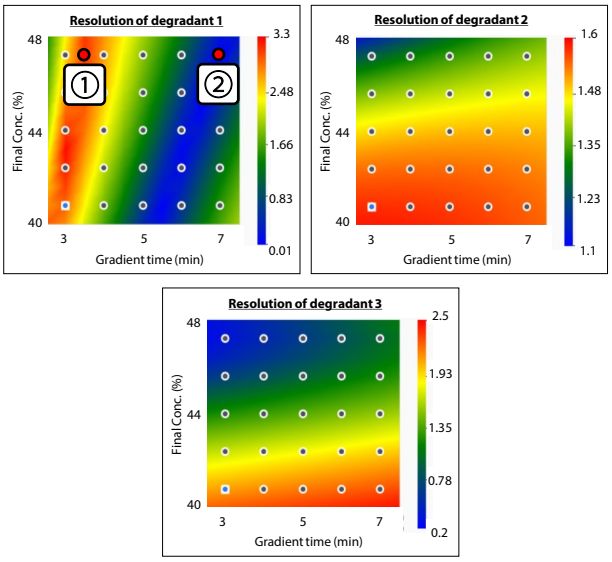

Fig. 2 Design Space for Resolution on Each Degradant

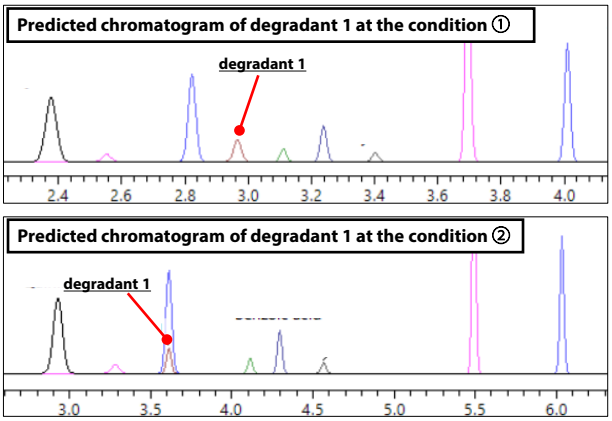

Fig. 3 Predicted Chromatogram of Degradant 1

LabSolutions MD can automatically search for conditions that meet multiple criteria. The chromatograms of samples A, B, and C were combined to search for conditions that would separate all degradants 1, 2, and 3. The criteria for separation were set to a resolution of 1.5 or higher for all degradants, and conditions that met these criteria were explored (Fig. 4). In Fig. 4, the purple area represents regions where the resolution of degradant 1 is less than 1.5, the orange area represents regions where the resolution of degradant 2 is less than 1.5, and the pink area represents regions where the resolution of degradant 3 is less than 1.5. In the remaining areas (black line hatched), point A within the red circle was automatically identified as the optimal condition. The predicted chromatogram for point A is shown in Fig. 5. The predicted chromatogram combines the chromatograms of samples A, B, and C and explores conditions where all degradants 1, 2, and 3 are separated. Thus, by utilizing design space and predicted chromatograms, separation can be optimized without relying on the experience in chromatography.

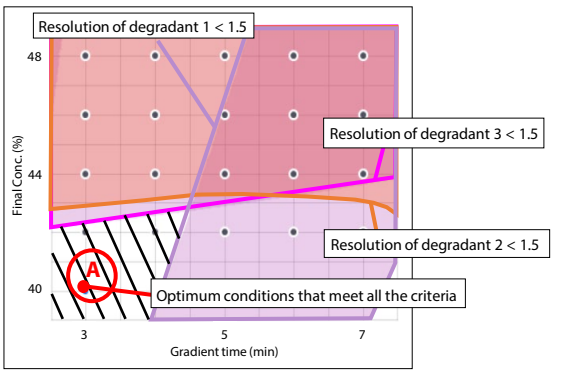

Fig. 4 Result of Exploration of Optimal Condition
(explored by combining chromatograms of samples A, B, and C)

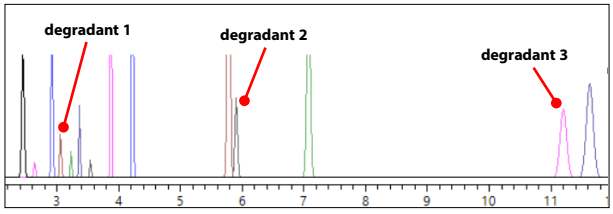

Fig. 5 Predicted Chromatogram Under Optimal Condition (Point A)

■ Chromatogram at the Optimal Condition

Fig. 6 shows the chromatogram obtained from an actual analysis under the optimal condition (point A) explored through the design space. It is confirmed that the separation conditions meet the criteria of a resolution of 1.5 or higher for all degradants 1, 2, and 3.

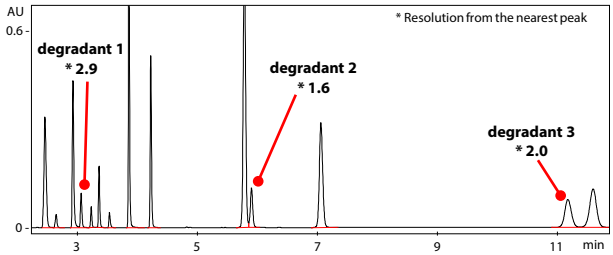

Fig. 6 Actual Chromatogram Under Optimal Condition
(final gradient concentration : 40 %, gradient time : 3 min, column oven temperature : 25 °C)

■ Conclusion

Stability tests are conducted to obtain the necessary information for establishing storage methods and shelf life of pharmaceuticals. Investigating optimal separation conditions for the degradants generated during this process is important to ensure the efficacy and safety of the pharmaceuticals. The design space in LabSolutions MD allows for the visualization of resolutions for multiple degradants. Additionally, the prediction chromatogram enables easy confirmation of separation behavior. These features make it easy for anyone, regardless of chromatographic proficiency, to search for conditions that meet the resolution criteria.

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