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Application News

Efficient Method Development on Pharmaceutical Impurities Using Single Quadrupole Mass Spectrometer

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User Benefits
◆ Peak tracking using an LCMS-2050 single quadrupole mass spectrometer supports highly reliable method development by accurately tracking each impurity that has similar UV spectra.
◆ LabSolutions MD is efficiently able to develop method that provides both excellent resolution and shorter analysis time.

■ Introduction

Since pharmaceutical impurities require strict control to ensure safety, development of highly reliable analytical method is essential. LabSolutions MD, a new Shimadzu software for method development, supports efficient method development based on Analytical Quality by Design (AQbD). AQbD-based analytical method development consists of the phases of initial screening, optimization, and robustness evaluation. This article introduces an example of its use for the development of a robust LC method for impurities on Montelukast (a medication used in the maintenance treatment of asthma). By changing each parameter of gradient program, the resolution of Montelukast and each impurity was evaluated by visualizing through "design space." Though it was previously difficult to accurately track each impurity with similar UV spectra using photodiode array detector (PDA), LCMS-2050 can solve this problem. Furthermore, by utilizing design space of resolution and RT of last eluting peak, it is possible to efficiently develop method that provides both excellent resolution and shorter analysis time.

■ Analytical Conditions

Table 1 shows the analytical conditions used in the optimization study for separation of Montelukast and its impurities (Imp1 to 6: Fig. 1). By varying the final concentration and slope of gradient program, the resolution of Montelukast and its impurities was examined to find the optimal condition. Specifically, final concentration was varied from 75 % to 85 % in increments of 5 % (3 levels), gradient slope from 8 min to 18 min in increments of 5 min (3 levels).

Table 1 Analytical Conditions for Optimization

LC Conditions:	Nexera™ X3 (Method Scouting System)
Mobile Phase: Pump A: 0.15 % formic acid in water Pump B: 0.1 % formic acid in acetonitrile	
Column: Shim-pack™ Scepter Phenyl-120 (100 mm × 3.0 mm I.D., 1.9 μm)*1	
Analytical Conditions	
Initial B Conc.:	45 %
Final B Conc.:	75, 80, 85 % (3 patterns)
Gradient Slope:	8, 13, 18 min (3 patterns)
Time Program:	B Conc. 45 % (0-3 min)→75 % (11 min) →85 % (11.01-13 min) →45 % (13.01-18 min) Note: If final B Conc. is 75 %, gradient slope is 8 min
Column Temp.:	30 °C
Flowrate:	0.5 mL/min
Injection Vol.:	10 μL (1000 mg/L)
Detection (PDA):	238 nm (SPD-M40, UHPLC cell)

*1 P/N: 227-31064-03

MS Conditions:	LCMS-2050
Ionization:	ESI/APCI (DUIS™), positive and negative mode
Mode:	SCAN (m/z 400-800)
Nebulizing Gas Flow:	2.0 L/min
Drying Gas Flow:	5.0 L/min
Heating Gas Flow:	7.0 L/min
DL Temp.:	200 °C
Desolvation Temp.:	450 °C
Interface Voltage:	+3.0 kV / -2.0 kV
Qarray Voltage:	+20 V

■ Accurate Tracking of Peaks by LCMS-2050

LC chromatograms obtained at final gradient concentration of 80 % and gradient slopes of 8 min and 18 min, along with m/z for respective impurities (Imp1 to 6), are shown in Fig. 1. UV spectra for Imp1 to Imp6 are shown in Fig. 2. The similarity between UV spectra for Imp1 and Imp6 (similarity > 0.99), for Imp2 and Imp4 (similarity > 0.9), and for Imp3 and Imp5 (similarity > 0.999) suggests that peak tracking based on UV spectrum would be difficult. In contrast, LabSolutions MD enables peak tracking based on m/z with LCMS-2050 for accurate identification of impurities that have similar UV spectra (Fig. 1).
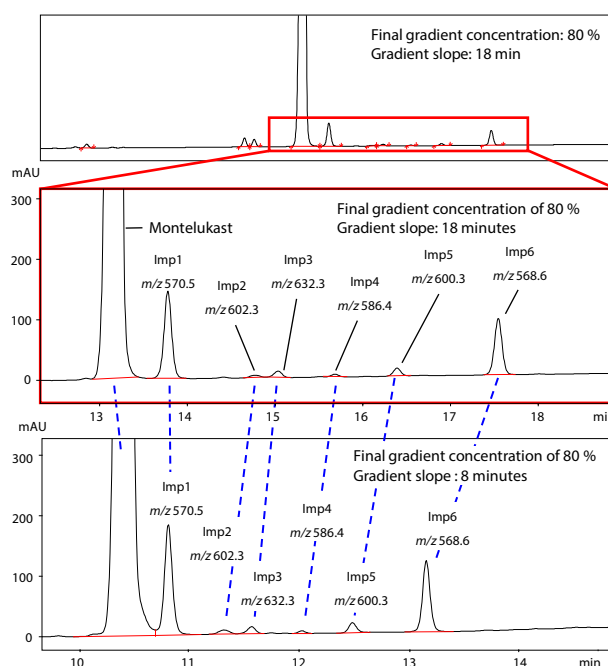

Fig. 1 LC Chromatograms with a Final Gradient Concentration of 80 % and Gradient Slopes of 8 (Lower) and 18 Minutes (Upper)
• Dashed line indicates tracking impurities based on m/z.
• Imp 1 to 6 are different from the impurities indicated in Japanese Pharmacopoeia.
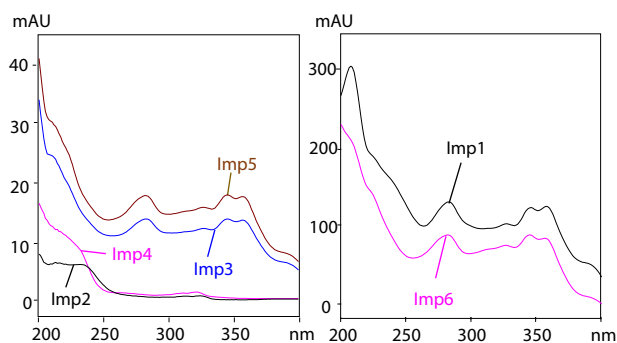
Fig. 2 UV Spectra of Montelukast Impurities (Imp1 to 6)

Next, to identify the optimal analytical condition, the resolution, when the final concentration and gradient slope are changed, is visualized by design space.

■ Design Space Evaluation for Optimal Analytical Condition

Fig. 3 shows the design space for resolution of Montelukast and Imp1. The red region indicates higher resolution, and the blue region indicates lower resolution. By visualizing resolution through design space, it was indicated that lower final concentration and longer gradient slope can achieve better separation.
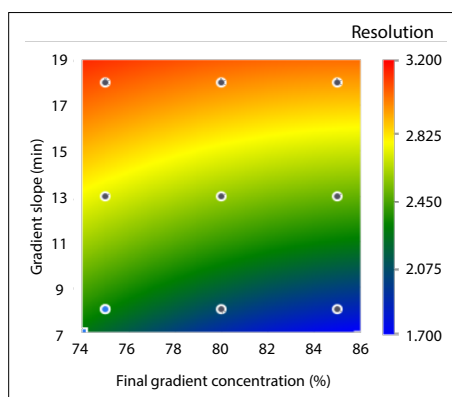
Fig. 3 Design Space for Resolution of Montelukast and Imp1

LabSolutions MD can simplify the search for optimal analytical condition by overlaying design spaces. Fig. 4 shows the area of analytical condition that meets resolution of Montelukast and Imp1 is > 2.6, minimum resolution of each compound is > 1.2, and RT of last eluting peak (Imp6) is < 17 min. The region enclosed by the green line in the figure is the region where minimum resolution is < 1.2, the region enclosed by the blue line is the region where resolution of Montelukast and Imp1 is < 2.6, the region enclosed by the pink line is the region where RT of last eluting peak is > 17 min, and the remaining region (shown by the black hatching) is the condition that satisfies all the criteria. Within the hatched area, the optimal point with the shortest analysis time is around point A, which is circled in red. Thus, by overlaying design spaces of resolution and RT of last eluting peak, optimal condition that provides enough resolution and shorter analysis time can be easily found.

Utilizing design space enables to understand how the LC parameters affect responses such as resolution and RT easily. This means a robust method can be defined without relying on the user experience.
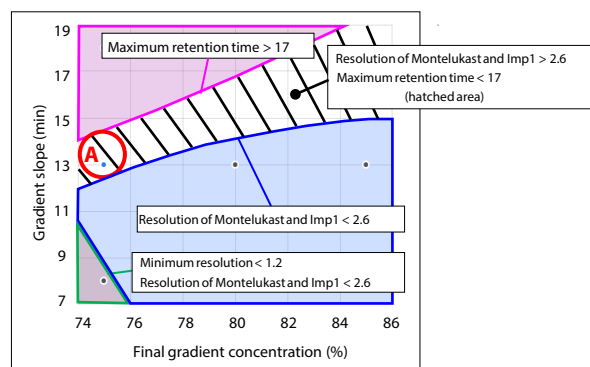

Fig. 4 Overlay of Design Spaces of Resolution and Last Eluting Peak

■ Chromatogram with Optimal Condition

The chromatogram obtained with optimal condition (point A) is shown in Fig. 5. It shows that the resolution of Montelukast and Imp1 is 2.7, minimum resolution of each impurities is 1.4 (Imp3), and RT of last eluting peak is less than 17 minutes, which successfully satisfies the criteria.
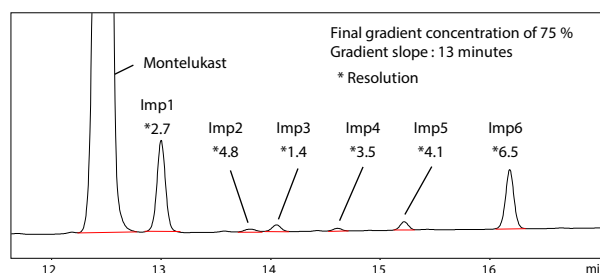

Fig. 5 LC Chromatogram Obtained with Optimized Condition
Final gradient concentration of 75 % and gradient slope of 13 minutes

■ Conclusion

This article introduces efficient method development on pharmaceutical impurities by using LabSolutions MD and LCMS-2050. Peak tracking based on *m/z* enables to identify each impurity accurately even in the case of similar UV spectra. Visualizing and overlaying the patterns of resolution for each compound and RT of last eluting peak enables a more efficient (Resolution) and fast (Analysis Time) method be easily developed regardless of the user experience.

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