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Application News

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

Efficient Method Development on Pharmaceutical Impurities Based on Analytical Quality by Design

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User Benefits

- Visualizing the resolution of API and impurities in the range of analysis conditions such as composition of mobile phase and column oven temperature comprehensively makes it efficient to find optimum analysis condition.
- Visualizing the area of analysis condition that satisfies robustness through different lot of columns makes robustness evaluation efficient.

■ Introduction

Since pharmaceutical impurities requires strict control to ensure safety, development of highly reliable analysis methods is necessary. 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 the phases of initial screening, optimization, and robustness evaluation. This article introduces an example of its use in optimization and robustness evaluation of the column and mobile phase selected in the initial screening in order to realize high efficiency in the development of a robust LC method for impurities on ketoprofen.

The resolution of each compound was evaluated by visualizing a "design space" after changing mobile phase composition, oven temperature and flow rate. In the step of robustness evaluation following optimization, resolution of each lot of columns was visualized and compared by design space to efficiently evaluate robustness among different lot of columns.

■ Analysis Conditions

Table 1 shows the analysis conditions used in the optimization study for separation of ketoprofen and its impurities. By varying the composition of mobile phase, column oven temperature and flow rate, the resolution of ketoprofen and its impurities was examined to find the optimal condition. Specifically, the acetonitrile ratio (B Conc.) was varied from 40% to 60% in increments of 5% (5 levels), the column oven temperature from 35 ˚C to 45 ˚C in increments of 5 ˚C (3 levels), and flow rate from 0.6 mL/min to 0.8 mL/min in increments of 0.1 mL/min (3 levels).

Table 1 Conditions of Optimization Study

System : Nexera™ X3 (Method Scouting System)	
Mobile Phase :	
Pump A : 0.1% formic acid in water	
Pump B : Acetonitrile	
Column :	
Shim-pack™ Velox C18 (100 mm × 3.0 mm I.D., 2.7 µm)*1	
Analytical Conditions (Isocratic)	
B Conc. (Acetonitrile)	: 40, 45, 50, 55, 60% (5 levels)
Column Temp.	: 35, 40, 45 ˚C (3 levels)
Flow Rate	: 0.6, 0.7, 0.8 mL/min (3 levels)
Injection Vol.	: 0.1 µL
Detection (PDA)	: 254 nm (SPD-M40, UHPLC cell)

*1 P/N: 227-32010-03

■ Separation of Ketoprofen and Impurities

First, Fig. 1 and Fig. 2 show the chromatograms when the acetonitrile ratio is at 60% and 40% (here, the column oven temperature and flow rate are constant at 40 ˚C and 0.7 mL/min, respectively). Imp1 to Imp3 in the chromatograms indicate impurities. When the acetonitrile ratio is 60%, Imp2 and Imp3 are eluted on the foot of the peak of ketoprofen, but at 40%, these peaks are mutually separated, suggesting that the acetonitrile ratio in the mobile phase has a large effect on separation. Next, the resolution when the acetonitrile ratio, column oven temperature, and flow rate are changed is visualized by design space.

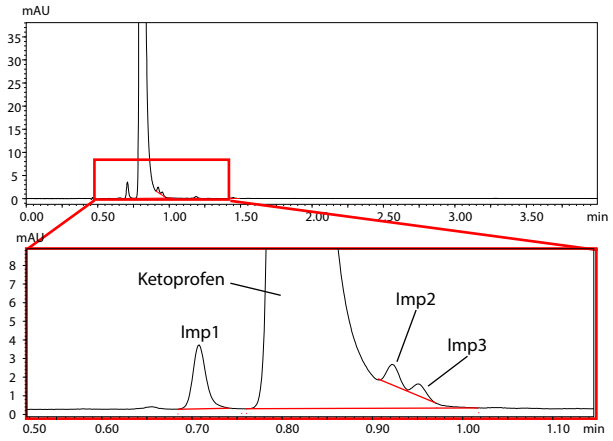

Fig. 1 Chromatogram at B Conc. (Acetonitrile) 60%, Oven Temperature 40 ˚C, Flow Rate 0.7 mL/min

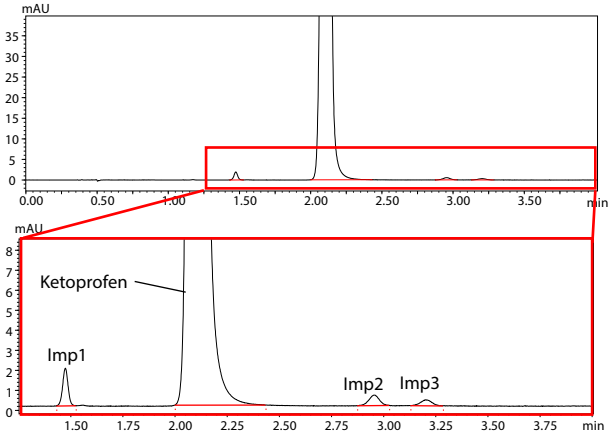

Fig. 2 Chromatogram at B Conc. (Acetonitrile) 40%, Oven Temperature 40 ˚C, Flow Rate 0.7 mL/min

■ Visualization of Resolution by Design Space

Fig. 3 shows the design spaces for resolution of ketoprofen and Imp2 (left) and also, Imp2 and Imp3 (right). The red region indicates higher resolution, and the blue region indicates lower resolution. LabSolutions MD is able to suggest the condition that provides better resolution as well as robustness. In Fig. 3, LabSolutions MD shows that the optimum condition for resolution of ketoprofen and Imp2 as well as, Imp2 and Imp3 is acetonitrile ratio at 40%, column oven temperature at 35 ˚C, and flow rate at 0.6 mL/min (blue square at the lower left in Fig. 3). By using design space, the effect of parameters on resolution can be understood easily to find the optimum condition.

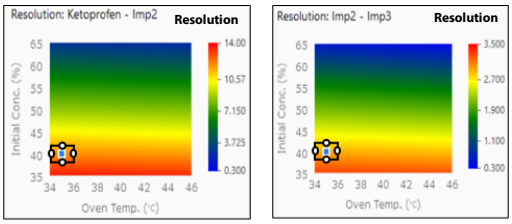

Fig. 3 Design Space for Resolution of Ketoprofen and Imp2 (Left) Imp2 and Imp3 (Right)
*Flow rate: 0.6 mL/min (optimum value suggested by LabSolutions MD),
*Initial Conc.: Acetonitrile ratio, *Executed with column of Lot 1

■ Robustness Evaluation

A robustness evaluation was carried out at the optimum condition (acetonitrile ratio at 40%, column oven temperature at 35%, flow rate at 0.6 mL/min).

The acetonitrile ratio was varied in 1% increments (39%, 40%, 41%) and the column oven temperature in 1 ˚C increments (34 ˚C, 35 ˚C, 36 ˚C) (shown in Fig. 3 by the blue point and 4 white circles, total of 5 points), and the effect on resolution was examined. Fig. 4 is the design spaces visualizing the resolutions of ketoprofen and Imp2 (left) and also, Imp2 and Imp3 (right). The resolution is higher than 8 (Ketoprofen and Imp2) and than 2 (Imp2 and Imp3) (shown by orange and red) within the entire region identified by Design Space. Furthermore, as shown in Fig. 5 and Fig. 6, design spaces are built in the same manner for additional two different column lots in order to assess robustness as well. The chromatograms obtained are shown in Fig. 7. In Figs. 4 to 6, it is found that resolution is higher than 8 (Ketoprofen and Imp2) and than 2 (Imp2 and Imp3) in the entire region, showing the optimized analytical method ensures robustness of all column lots.

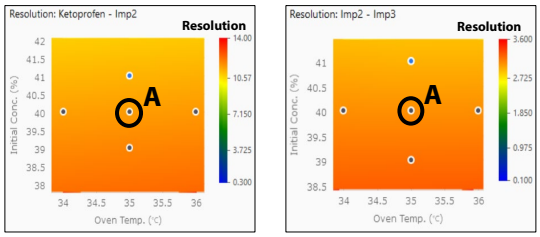

Fig. 4 Design Space for Resolution of Ketoprofen and Imp2 (Left) Imp2 and Imp3 (Right)
*The black dots (total: 5 points) in the figure are points where the analysis is executed (Column lot 1)

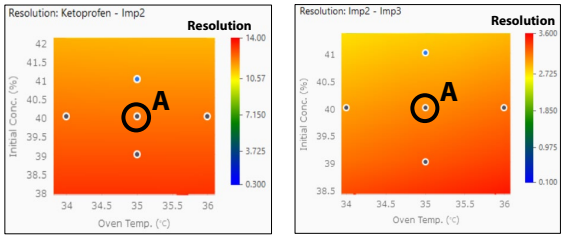

Fig. 5 Design Space for Resolution of Ketoprofen and Imp2 (Left) Imp2 and Imp3 (Right)
*The black dots (total: 5 points) in the figure are points where the analysis is executed (Column lot 2)

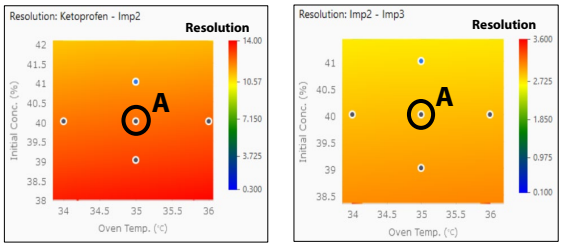

Fig. 6 Design Space for Resolution of Ketoprofen and Imp2 (Left) Imp2 and Imp3 (Right)
*The black dots (total: 5 points) in the figure are points where the analysis is executed (Column lot 3)

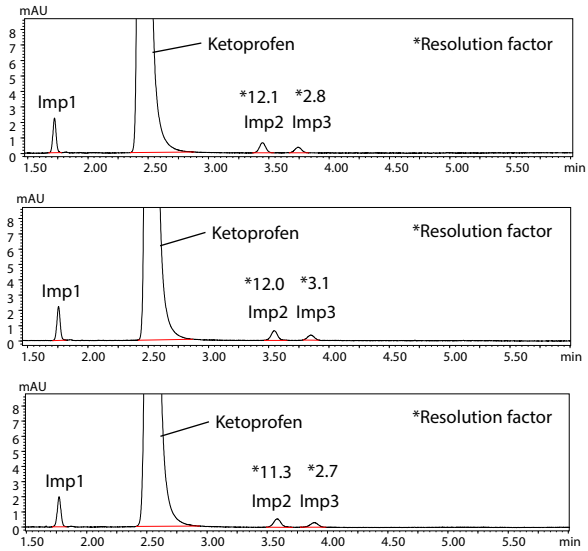

Fig. 7 Chromatograms Acquired with Columns of Different Lots (Point A in Fig. 4 to Fig. 6)
(Top: Lot 1, Middle: Lot 2, Bottom: Lot 3)

■ Conclusion

This article introduces efficient method development on pharmaceutical impurities by using LabSolutions MD. Visualizing resolution of API and impurities by design space enables efficient optimization and robustness evaluation of analytical method without relying exclusively on the user experience. It is also possible to evaluate robustness using different column lots by building design spaces for each lot. The process of development of pharmaceutical impurities introduced in this article can be also efficiently applicable to support method validation.

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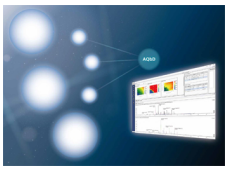

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