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Application News

Software for Efficient Method Development "LabSolutions™ MD"

Efficient Method Development Using Single Quadrupole Mass Spectrometer -Automatic Detection of Co-eluted Peaks-

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User Benefits

- LCMS-2050 single quadrupole mass spectrometer provides not only accurate peak tracking based on *m/z* but also automatic detection of co-eluted peaks for efficient method development.
- LabSolutions MD enables easy searching for method operatable design region that satisfies the criteria of resolution for multiple compounds.

■ Introduction

In the process of separation optimization in LC method development, optimal analytical conditions are searched by varying parameters such as mobile phase composition, gradient curve, and column oven temperature. However, accurate peak tracking among obtained chromatograms is generally challenging due to variations in elution times and co-eluted compounds caused by different analytical conditions through optimization. A PDA (photodiode array) detector can provide peak tracking using differences in UV spectra, but for co-eluted peaks and related substances such as impurities and degraded products, accurate identifications by UV spectra may be difficult. On the other hand, the use of a mass spectrometer in addition to a PDA detector is expected to improve the accuracy of determining co-eluted peaks and tracking related substances based on *m/z*. This article describes how to improve the efficiency of separation optimization in method development through accurate peak tracking and automatic detection of co-eluted peaks by utilizing LCMS-2050 and LabSolutions MD, a dedicated software for supporting method development.

■ Analytical Conditions

Table 1 shows the analytical conditions used to optimize the simultaneous analysis of six small molecular pharmaceuticals. Gradient elution was applied using a 0.15% formic acid aqueous solution as the aqueous mobile phase and a mixture of acetonitrile and methanol as the organic mobile phase. The separation of individual compounds was investigated comprehensively by varying the mobile phase composition of organic solvent and column oven temperature. Specifically, the acetonitrile ratio in the organic mobile phase was varied from 0% to 100% in 10% increments (eleven levels), and the column oven temperature was varied from 30 °C to 40 °C in 5 °C increments (three levels).

Table 1 Analytical Conditions and Target Compounds

System	: Nexera™ X3 (Method Scouting System)
Sample	: Quinidine, Lidocaine, Metoclopramide, Papaverine, Dibucaine, Amitriptyline
Mobile phase	: Pump A : 0.15% formic acid in water
	: Pump B : Acetonitrile/Methanol = X : (100 - X)
	*X = 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100
Column	: Shim-pack Scepter™ C18-120 (100 mm × 3.0 mm I.D., 1.9 µm)*1
Injection Vol.	: 0.5 µL (80 mg/L)
LC Conditions	
Time program	: B Conc. 20%(0 min)→75%(12min)
	→90%(12.01-14min)→20%(14.01-17 min)
Column Temp.	: 30, 35, 40 °C
Flow rate	: 0.7 mL/min
Detection (PDA)	: 254 nm (SPD-M40, UHPLC cell)

*1 P/N: 227-31013-03

MS Conditions	
System	: LCMS-2050
Ionization	: ESI/APCI (DUIS™), positive and negative mode
Mode	: SCAN (*m/z* 150-400)
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

■ Peak Tracking based on m/z by LCMS-2050

Fig. 1 displays chromatograms obtained by varying the acetonitrile ratio (from 0% to 100% in 10% increments) in the organic mobile phase and the column oven temperature (from 30 °C to 40 °C in 5 °C increments). In the chromatogram at 60% acetonitrile (highlighted in red in Fig. 1), five peaks were detected. Notably, two combinations of impurities, Quinidine (②)/Lidocaine (③) and Dibucaine (⑥)/Amitriptyline (⑦), were co-eluted, and these un-separated peaks might have gone unnoticed. When co-elution of peaks is suspected, LabSolutions MD highlights the target peak in orange (as indicated by the black circle in Fig. 1) by using mass information from LCMS-2050. It also provides *m/z* for multiple co-eluted compounds. For instance, the peak suspected of co-elution at the acetonitrile ratio of 60% contained compounds each with m/z values of 344.25 and 278.23 respectively (Fig. 2), suggesting co-elution of Dibucaine and Amitriptyline. Peak tracking using *m/z* revealed the presence of up to seven compounds in the sample. It showed that impurities of Quinidine (②) and Lidocaine (③), as well as Dibucaine (⑥) and Amitriptyline (⑦), were eluted in a different order depending on the variation in the acetonitrile ratio in the organic mobile phase. In this way, LabSolutions MD can eliminate the possibility of missing un-separated peaks and provide a more efficient workflow for searching for optimal separation conditions, thanks to accurate peak tracking using *m/z*, as well as automatic determination and highlighting of co-eluted peaks.

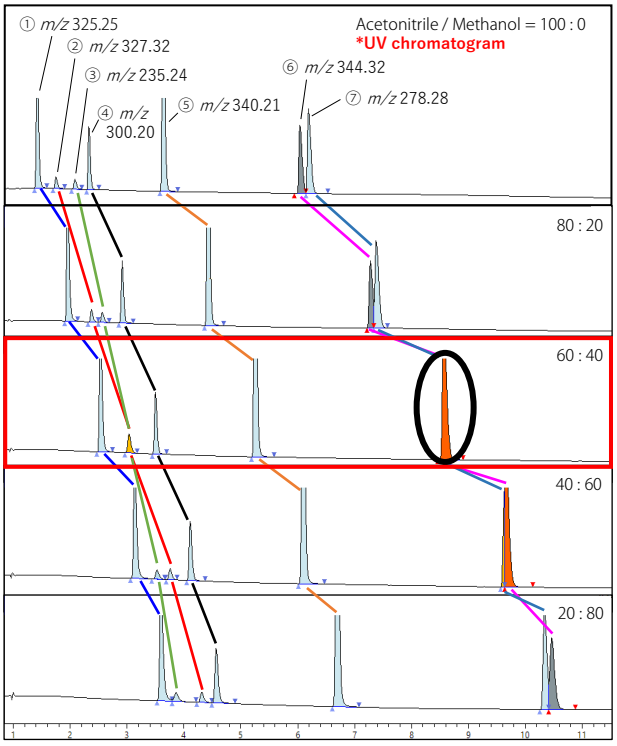

Fig. 1 Peak Tracking based on *m/z*

①Quinidine, ②Impurity of Quinidine ③Lidocaine, ④Metoclopramide, ⑤Papaverine, ⑥Dibucaine, ⑦Amitriptyline

* Acetonitrile ratios in organic solvent are 100, 80, 60, 40, and 20% from the top
* 30 ℃ of column temperature for all the chromatograms

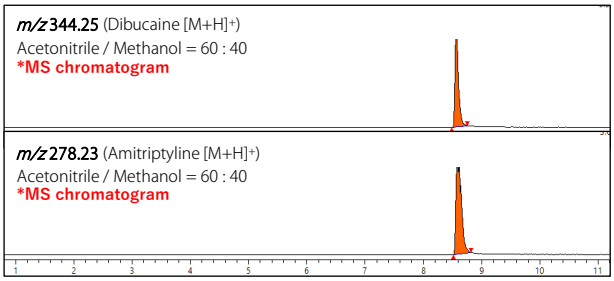

Fig. 2 MS Chromatograms and *m/z* of Peaks Suspected to be Co-eluted (black circled in Fig. 1).

■ Visualization of Resolution by Design Space

LabSolutions MD provides an efficient workflow for searching optimal separation conditions by visualizing resolutions for each compound through design space. The lower limits of resolution (criteria) for the targeted compounds are shown in Table 2. The Method Operatable Design Regions (MODRs), considered to have higher resolutions than the respective criteria, are shown as black hatched area in Fig. 3. The vertical axis represents the acetonitrile ratio in the organic mobile phase, and the horizontal axis represents column oven temperature. The colored area is considered to have lower resolutions than criteria and the remaining MODR is expected to provide higher resolutions than criteria. The conditions with higher acetonitrile ratio and lower column oven temperature (point A in Fig. 3) are expected to offer optimal separation. The visualization of resolution by design space enables easy and quick identification of MODRs that meet the criteria for individual peaks. This allows efficient optimization and an understanding of the relationship between various parameters and resolutions without depending on user experience.

Table 2 Criteria of Resolution for Each Compound

No.	Compound	Lower limit of resolution
①	Quinidine	3.0
②	Impurity of Quinidine	1.5
③	Lidocaine	1.5
④	Metoclopramide	1.5
⑤	Papaverine	1.5
⑥	Dibucaine	1.0
⑦	Amitriptyline	1.0

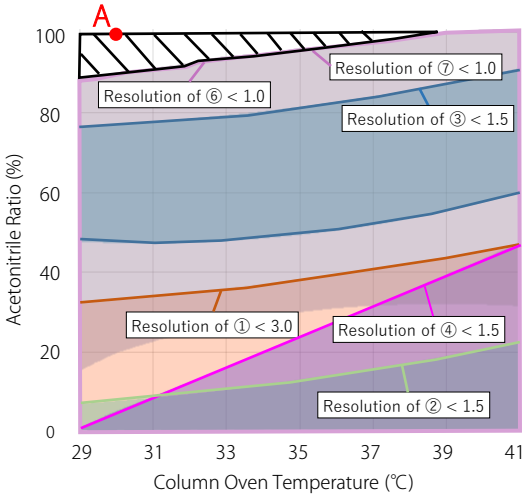

Fig. 3 Design Space of Resolution for Each Compound
* MODR is a black hatched area (upper left).

■ Chromatogram at Optimized Conditions

Fig. 4 shows a chromatogram at point A (acetonitrile ratio : 100%, column oven temperature : 30 °C), indicating the optimal separation conditions identified within the design space. It was confirmed that the resolutions for respective compounds under the optimized conditions met the criteria in Table 2.

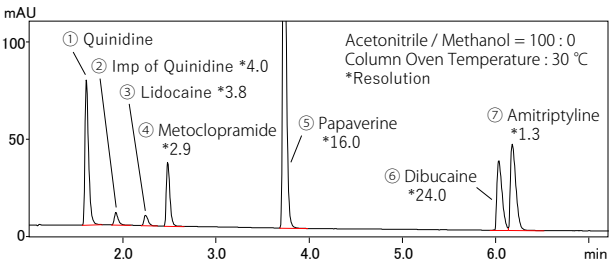

Fig. 4 Chromatogram at Optimized Conditions

■ Conclusion

The efficient optimization of separation conditions with LabSolutions MD and LCMS-2050 was introduced. Employing the mass information of LCMS-2050 can contribute to eliminate the possibility of missing co-eluted peaks and performing accurate peak tracking. Additionally, the visualization of resolutions using design space facilitates the easy identification of MODR that meets the resolution criteria for multiple compounds.

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