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Application News

Enhancing Method Development Efficiency Using MS Peak Tracking with a Triple Quadrupole Mass Spectrometer

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User Benefits

- Method development is generally performed using LC detectors such as a photodiode array (PDA) detector. However, incorporating mass information obtained from a mass spectrometer enables more accurate peak tracking.
- By visualizing resolution within the design space, the search for optimal separation conditions in method development can be streamlined without relying on intuition or experience.

■ Introduction

In the process of separation optimization during method development, optimal analytical conditions are explored by varying LC parameters such as mobile phase composition, gradient profile, and column oven temperature. However, accurate peak tracking across multiple chromatograms can be challenging because differences in separation conditions may cause variations in retention times or co-elution of compounds. Although peak tracking based on differences in UV spectra obtained with a PDA detector can be applied, accurate identification of impurities and co-eluting peaks is often difficult using UV information alone. In contrast, combining a PDA detector with a mass spectrometer can improve tracking accuracy, particularly for low-abundance compounds and impurities. This article describes a case study in which the combination of LabSolutions™ MD, a dedicated software for supporting method development, and LCMS-8060RX triple quadrupole mass spectrometer enabled efficient exploration of optimal separation conditions through accurate MS-based peak tracking for the simultaneous analysis of six small-molecule pharmaceutical compounds.

■ Analytical Conditions

Table 1 summarizes the analytical conditions used for optimizing the simultaneous analysis of six small-molecule pharmaceutical compounds. Gradient elution was performed using 0.1% formic acid in water as the aqueous mobile phase and acetonitrile as the organic mobile phase. The separation of each compound was optimized by varying the gradient conditions. Specifically, gradient times of 4, 5, and 6 minutes (three conditions) were combined with final gradient concentrations of 50%, 60%, and 70% (three conditions), resulting in a total of nine analytical conditions.

Table 1 Analytical Conditions

Sample	: (1) Quinidine, (2) Lidocaine, (3) Metoclopramide, (4) Papaverine, (5) Dibucaine, (6) Amitriptyline
Mobile phase	: Pump A : 0.1% formic acid in water
	: Pump B : Acetonitrile
Column	: Shim-pack Scepter™ C18-120 (100 mm × 3.0 mm I.D., 1.9 µm)*1
Injection Vol.	: 0.1 µL (80 mg/L)
LC Conditions	
System	: Nexera™ X3
Time program	: B Conc. 20%(0 min)→X*2 %(Y min) →20%(Y*3 - Y+4 min)
Column Temp.	: 30 °C
Flow rate	: 0.7 mL/min
Detection (PDA)	: 254 nm (SPD-M40, UHPLC cell)
MS Conditions	
System	: LCMS-8060RX
Ionization	: ESI, positive mode
Mode	: MRM and Q3 SCAN (*m/z* 100-1000)
Nebulizing gas flow	: 3 L/min
Heating gas flow	: 15 L/min
Interface Temp.	: 400 °C
Desolvation Temp.	: 650 °C
DL Temp.	: 250 °C
Block heater Temp.	: 400 °C
Drying gas flow	: 3.0 L/min
Interface voltage	: +1.0 kV
Notes	: Flow split 1:4 (MS : waste) prior to MS

*1 P/N: 227-31013-03
*2 X = 50、60、70
*3 Y = 4、5、6

■ MS-Based Peak Tracking with LCMS-8060RX

Fig. 1 shows automatic peak tracking of each compound performed by LabSolutions MD, using chromatograms (partially shown) obtained under different gradient conditions and mass information acquired with LCMS-8060RX. LabSolutions MD enables accurate tracking of impurities and co-eluting compounds based on the pre-registered MRM transitions of each compound (Table 2). For compounds without predefined MRM transitions, peak tracking can also be performed by using mass information from scan measurements (full scan without fragmentation within a specified *m/z* range), allowing all compounds to be tracked based on mass information. As shown in Fig. 1, although the retention times and separation of compounds varied with changes in gradient time and final gradient concentration, all compounds were accurately tracked. Compound (2) in Fig. 1 is an impurity of quinidine. Because its UV spectrum showed a high similarity (≥0.99) to that of quinidine (Fig. 2), peak tracking using UV spectra alone was considered difficult. However, it was correctly identified using mass information (*m/z* 327.1; Fig. 3) obtained from scan measurements. In addition, dibucaine (Fig. 1, (6)) and amitriptyline (Fig. 1, (7)) were co-eluted, but accurate tracking was achieved even for these unresolved peaks by using product ion obtained from MRM measurements. The MRM chromatograms of dibucaine (Fig. 1, (6)) and amitriptyline (Fig. 1, (7)) are shown in Fig. 4 for reference.

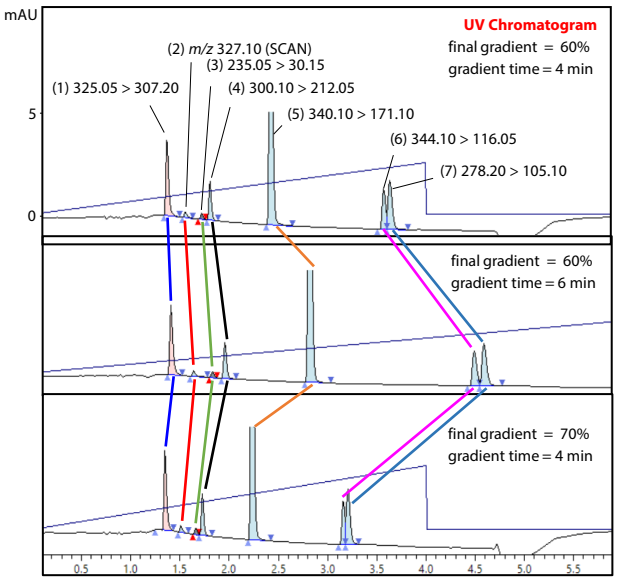

Fig. 1 MS-Based Peak Tracking
(1) Quinidine, (2) Impurity of Quinidine (3) Lidocaine
(4) Metoclopramide, (5) Papaverine, (6) Dibucaine, (7) Amitriptyline

Table 2 MRM Transitions for Each Compound

	Compound	MRM transition
(1)	Quinidine	325.05 > 307.20
(2)	Impurity of Quinidine	-
(3)	Lidocaine	235.05 > 30.15
(4)	Metoclopramide	300.10 > 212.05
(5)	Papaverine	340.10 > 171.10
(6)	Dibucaine	344.10 > 116.05
(7)	Amitriptyline	278.20 > 105.10

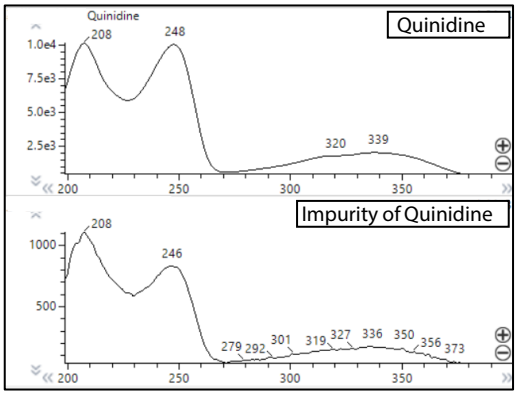

Fig. 2 Comparison of UV Spectra between Quinidine and Its Impurity

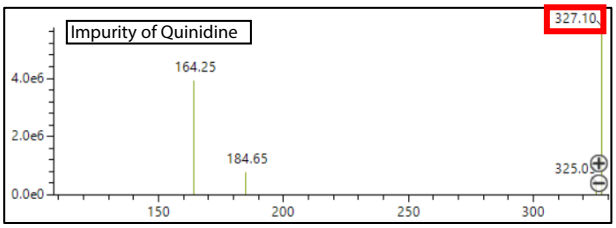

Fig. 3 MS Spectrum of Impurity of Quinidine by Scan Measurement

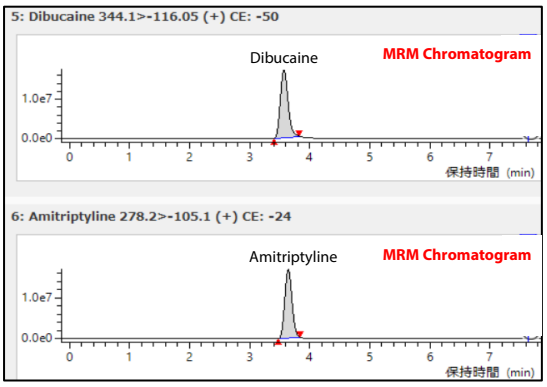

Fig. 4 MRM Chromatograms
Dibucaine (Upper) and Amitriptyline (Lower)

■ Visualization of Resolution by Design Space

LabSolutions MD streamlines the search for optimal separation conditions by visualizing resolution within a design space. Fig. 5 shows the design space for the resolution between dibucaine and amitriptyline—the most challenging pair to separate—with gradient time plotted on the vertical axis and final gradient concentration on the horizontal axis. The red area in the figure represents regions of high resolution, while the blue area represents regions of low resolution. The design space evaluation indicates that better separation is obtained with longer gradient times and lower final gradient concentrations. Point A was identified as the optimal separation condition (chromatogram at point A: Fig. 6).

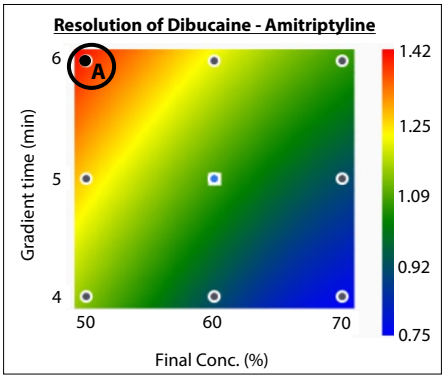

Fig. 5 Design Space of Resolution between Dibucaine and Amitriptyline

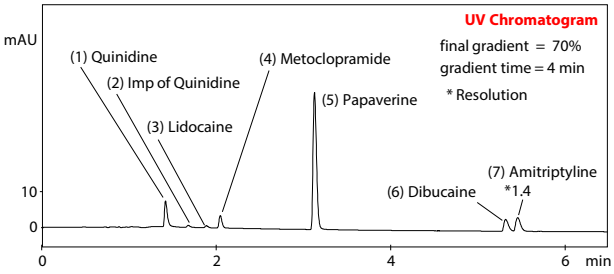

Fig. 6 Chromatogram under the Optimal Conditions

■ Conclusion

The efficient method development using LabSolutions MD and LCMS-8060RX triple quadrupole mass spectrometer is presented. By utilizing the mass information obtained from LCMS-8060RX, precise peak tracking can be achieved even for impurities and co-eluted compounds. In addition, visualization of resolution within a design space allows efficient exploration of optimal separation conditions without relying on the intuition and experience of the analyst.

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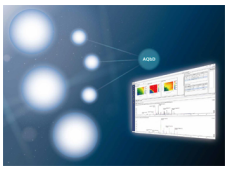

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