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Technical Report

Efficient Method Development by automated pH Screening with LabSolutions MD

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Abstract:

Because the pH level of mobile phase can affect the retention time of ionic compounds, determining the optimal pH level is an important part for developing LC method. The pH screening requires preparing multiple mobile phases with different pH levels. However, preparing these mobile phases manually is not only a time-consuming process but also prone to the operator errors. Automated pH screening process improves the method development efficiency and also the reliability of analysis. This article describes an example of using LabSolutions MD, a dedicated software for supporting method development, to automate pH screening by varying the mobile phase pH from 2.5 to 8.5 to evaluate the optimal pH level for separating 12 small-molecule drugs.

Keywords: LabSolutions MD, pH screening, AQbD, analysis method development, method development

1. Background

The retention behavior of target compounds against mobile phase pH is known to reflect the *pKa* value. Mobile phase pH screening is performed during the initial stage of method development to evaluate the effect of pH level on the separation. However, pH screening requires preparing mobile phases with multiple pH levels and automating this process improves the efficiency of the method development and avoid preparation errors. With a dedicated database for pH screening and functionality for blending mobile phases, LabSolutions MD can automate the entire pH screening process by preparing any mobile phase pH level online. This article describes an example of using LabSolutions MD to evaluate optimal pH level more efficiently by automating the pH screening process for the separation of 12 small-molecule drugs.

2. Automated pH screening with LabSolutions MD

LabSolutions MD automatically generates an analysis schedule by setting each parameter, such as mobile phase and column (steps ① to ⑤ in Fig. 1) . Mobile phases and columns can be simply selected with a single click and switched automatically during the analysis. The condition for pH screening for the optimal separation of 12 small-molecule drugs is shown in Table 1. As the operation of LabSolutions MD, click the pH levels of mobile phase to automatically generate the analysis schedule (Fig. 1). Then the mobile phase blending function automatically prepares the 13 mobile phases with the selected pH levels by varying the pH from 2.5 to 8.5 in 0.5 pH steps (for the case of Table 1). This significantly reduces the amount of work required for manual preparation and prevents preparation mistakes. In addition, all these 13 solutions at different pH levels are prepared from three types of mobile phase (stock solutions) (refer to *1 in Table 1), which results in using fewer mobile phase bottles and reducing liquid wastes compared with preparing all pH levels of mobile phase manually.

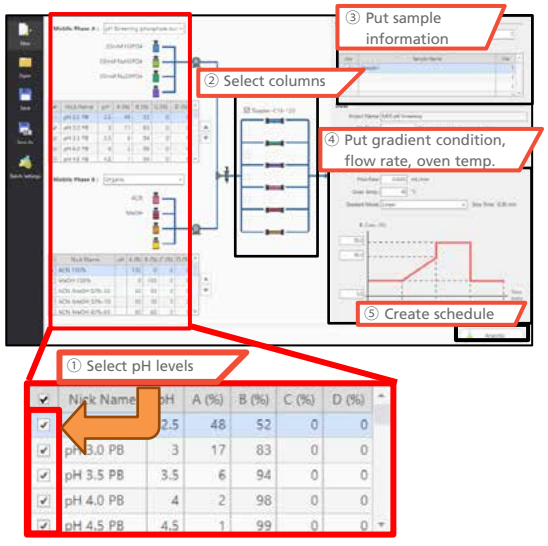

Fig. 1 Steps for Creating Analysis Schedule for pH Screening

Table 1 Analysis Conditions

System : Nexera™ X3 (Method Scouting System)
Sample : 12 small-molecule drugs (each compound shown in Fig. 2)
Mobile phase: Pump A Aqueous (20 mmol/L sodium phosphate aq.)*1 A1) pH 2.5 A6) pH 5.0 A11) pH 7.5 A2) pH 3.0 A7) pH 5.5 A12) pH 8.0 A3) pH 3.5 A8) pH 6.0 A13) pH 8.5 A4) pH 4.0 A9) pH 6.5 A5) pH 4.5 A10) pH 7.0 Pump B Acetonitrile
Column : Shim-pack Scepter™ C18-120 (100 mm × 3.0 mmI.D., 1.9 µm)*2
Analytical conditions : B Conc. : 5% (0 min) → 80% (8.01-11 min) → 5% (11.01-15 min) Column Temp. : 40 ˚C Flow rate : 0.7 mL/min Injection Vol. : 1.0 µL Detection : Max plot 220-400 nm (SPD-M40, UHPLC cell)

*1 Mobile phase blending function is used to automatically prepare the different pH levels (A1 to A13) by blending the three types of mobile phase (stock solutions) indicated below.
(1) 20 mmol/L phosphoric acid in water
(2) 20 mmol/L sodium dihydrogenphosphate in water
(3) 20 mmol/L disodium hydrogenphosphate in water

*2 P/N 227-31013-03

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3. pH Screening Results and Summary

Chromatograms of a sample containing 12 small-molecule drugs at different pH levels (pH 2.5 to 8.5) are shown in Fig. 2. A total of 13 peaks were eluted, because acetylsalicylic acid (peak 1) contained an impurity (peak 2). The results show that some of the compounds were coeluted at pH 2.5, but all compounds were separated at pH 7.0, 7.5, and 8.5. LabSolutions MD can quantitatively evaluate and rank the separation pattern for each pH level, based on Equation 1 below, to determine optimal pH level without relying on the user experience.

$$(\text{Evaluation Value}) = P \times (Rs_1 + Rs_2 + \ldots Rs_P) \quad \ldots(\text{Eq. 1})$$

Evaluation Value is calculated as the number of peaks detected (P) multiplied by the sum of resolution factor (Rs) for all peaks. Fig. 3 shows Evaluation Value obtained through pH screening, listed in order from highest to lowest. It indicates that pH 7.5 provides the highest value. The benefits of using pH screening for method development include being able to check how retention behavior changes for each compound as mobile phase pH is continuously varied and being able to estimate the *pKa* values for unknown compounds. Consequently, robust analysis methods can be developed by setting pH level while considering estimated *pKa* for each compound. This also helps improve the efficiency of various phases of method development after the phase of pH screening, such as optimization and robustness evaluation. This article showed an example of automated pH screening process during method development, but LabSolutions MD can also be used to increase the efficiency of other phases of method development, including optimization and robustness evaluation. For more details, refer to the Technical Report entitled "Efficient method development based on Analytical Quality by Design with LabSolutions™ MD software (C190-E284)."

Use this QR code to access the Technical Report C190-E284.

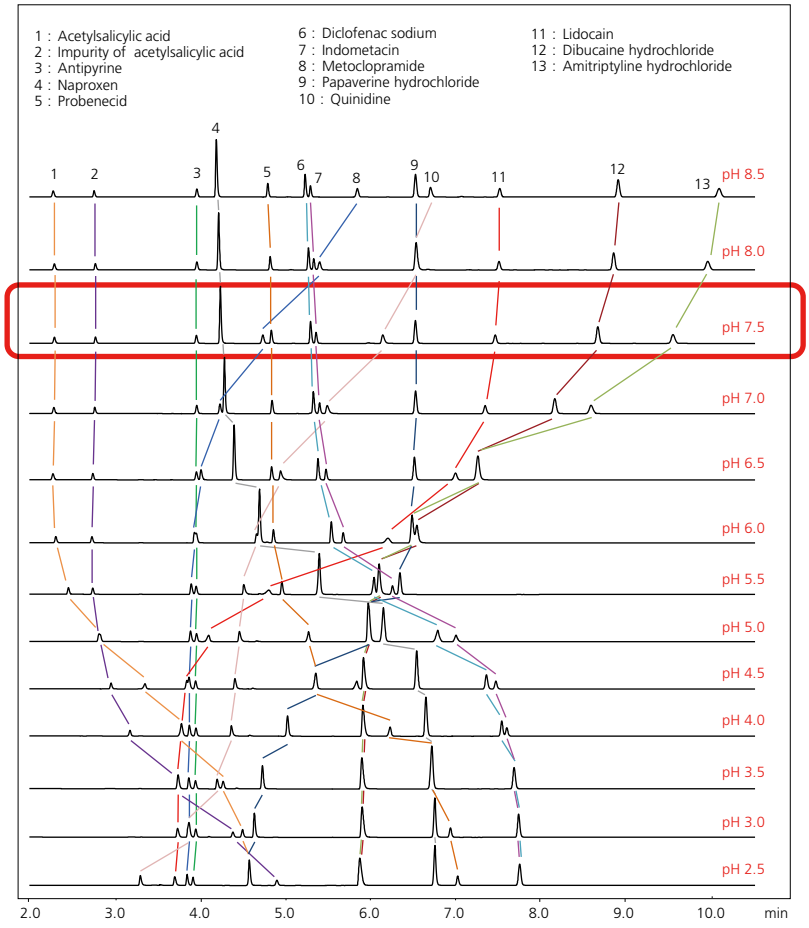

Fig. 2 pH Screening Results for Small-Molecule Drugs (12 Types)

	Response
MPA pH	Evaluation Value
7.5	233.977
8.5	233.717
7	230.157
8	196.311
6.5	194.094
5.5	190.046
4.5	182.656
6	162.67
4	160.325
2.5	135
3	135
5	135
3.5	107.326

Fig. 3 Ranking at each pH level by Evaluation Value (Listed from Highest to Lowest)

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