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Technical Report

Efficient method development based on Analytical Quality by Design with LabSolutions™ MD software

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Abstract:

This article describes the efficient development of robust analytical methods based on Analytical Quality by Design (AQbD) using LabSolutions MD with small-molecule drugs. Method development based on AQbD consists of 3 phases including screening, optimization, and validation. LabSolutions MD allows efficient method development by supporting every phase with dedicated functions such as experimental design, building of design space by automatic Peak Tracking function, and robustness evaluation.

Keywords: LabSolutions MD, AQbD, method development, method scouting, experimental design

1. Background

The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) suggests AQbD approach for method development. It is recommended to acquire data by efficient experiments such as the use of experimental design and verify the parameters that have a large effect on analytical results and then build a design space to understand the effective domain of the parameters with respect to the analysis results. The risk-based approach ensures the development of robust, low-risk methods without relying on the user experience.

2. Overview of LabSolutions MD

LabSolutions MD supports efficient method development through each phase based on AQbD (Fig. 1). This software includes several functions for creating analysis schedule based on experimental design (Fig. 2) and for data analysis such as building design space and simulation of chromatograms (Fig. 3). It is possible to generate the analysis schedule that can switch mobile phases and columns automatically for screening. Furthermore, by utilizing experimental design, it is possible to find an optimum condition with reduced numbers of analysis. In figure 2, steps (1) to (6) show how to easily and quickly create the analysis schedule.

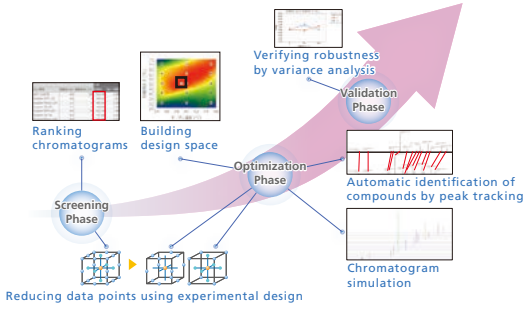


Fig. 1 Efficient Method Development Using LabSolutions MD

Mobile phases and columns can be selected directly by clicking the image in the software and analysis schedule including column equilibration is automatically generated. This doesn't only improve operational efficiency, but also reduces human errors. The experimental design can also be selected with a single click. Moreover, data analysis functions support automatic identification of target compounds by Peak Tracking function, visualization of design space to identify optimum condition, and simulation of chromatograms by predicting different analytical conditions. In addition, it is possible to identify parameters that have a large effect on separation by analysis of variance. This article describes the workflow of method development including screening, optimization, and validation with the sample of small-molecule drugs.

Fig. 2 Functions for Creating Analysis Schedule

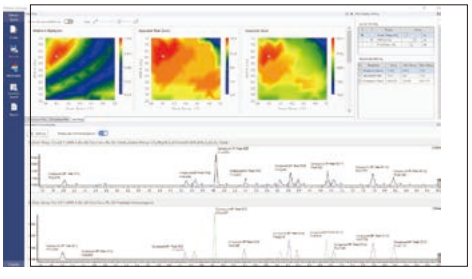


Fig. 3 Functions for Data Analysis

1 Analytical & Measuring Instruments Division

3. Analysis

3-1. Sample Information

Table 1 shows the small-molecule drugs used for method development and their physical properties. As a model case, small-molecule drugs which have different log P and pKa are selected.

Table 1 Sample Information

No.	Compounds	log P	pKa
1	Probenecid	3.21	3.4
2	(S)-(+)-Naproxen	3.18	4.15
3	Acetylsalicylic acid	1.19	3.49
4	Diclofenac sodium	4.51	4.15
5	Papaverine hydrochloride	3	6.4
6	Dibucaine hydrochloride	4.4	8.85
7	Amitriptyline hydrochloride	4.92	9.4
8	Indometacin	4.27	4.5
9	Antipyrine	0.38	1.4
10	Lidocain	2.44	8.01
11	Quinidine	3.44	8.56
12	Metoclopramide	2.62	9.27

3-2. Screening Phase

For screening phase, a total of 36 (2×3×6) data is acquired by using 2 aqueous mobile phases, 3 organic mobile phases, and 6 columns with full factorial design (Table 2). Because mobile phases and columns usually have a large effect on separation, these factors are screened in this initial phase of method development. pH of each aqueous mobile phase (Pump A) is automatically adjusted by Solvent Blending function as well as ratio of organic solvent (Pump B). Mobile phase selection and column switching are automatically implemented during analysis, strongly improving the overall efficiency of the method development phase.

Table 2 Mobile Phases and Columns in Screening Phase

Mobile phase:
- Pump A Buffer *1
  - A1 20 mmol/L (Sodium) phosphate buffer (pH 2.7)
  - A2 20 mmol/L (Sodium) phosphate buffer (pH 6.8)
- Pump B Organic solvent *2
  - B1 Acetonitrile
  - B2 Acetonitrile / Methanol = 50 : 50
  - B3 Methanol

Column:

1	Shim-pack Scepter C18-120	(100 mm × 3.0 mm I.D., 1.9 µm)*3
2	Shim-pack Scepter C8-120	(100 mm × 3.0 mm I.D., 1.9 µm)*4
3	Shim-pack Scepter C4-300	(100 mm × 3.0 mm I.D., 1.9 µm)*5
4	Shim-pack Scepter Phenyl-120	(100 mm × 3.0 mm I.D., 1.9 µm)*6
5	Shim-pack Scepter PFPP-120	(100 mm × 3.0 mm I.D., 1.9 µm)*7
6	Shim-pack GIST C18 AQ HQ	(100 mm × 3.0 mm I.D., 2.0 µm)*8

Analytical conditions:

Time program	:B.Conc. 5%(0 min) → 80%(8.01-11 min) → 5%(11.01-15 min)
Flow rate	:0.7 mL/min
Inj.vol.	:1.0 µL
Temperature	:40 ˚C
Detection	:Max plot 220- 400 nm (SPD-M40)

*1 The aqueous mobile phases below are automatically prepared by solvent blending function.

	Solvent	A1 ratio	A2 ratio
A	50 mmol/L Phosphoric acid water	16%	0%
B	50 mmol/L Sodium dihydrogen phosphate water	24%	24%
C	50 mmol/L Disodium phosphate water	0%	16%
D	Water	60%	60%

*2 The organic mobile phases below are automatically prepared by solvent blending function.

	Solvent	B1 ratio	B2 ratio	B3 ratio
A	Acetonitrile	100%	50%	0%
B	Methanol	0%	50%	100%

*3 P/N 227-31013-03
*4 P/N 227-31034-03
*5 P/N 227-31176-03
*6 P/N 227-31064-03
*7 P/N 227-31054-03
*8 P/N 227-30808-02

3-3. Chromatogram in Screening Phase

Fig. 4 shows chromatograms obtained by Shim-pack™ Scepter Phenyl-120. 14 peaks are eluted, including the impurities of quinidine and acetylsalicylic acid. It is confirmed that retention time and resolution factor of each compound changed significantly depending on pH of aqueous mobile phases, organic mobile phases, and columns.

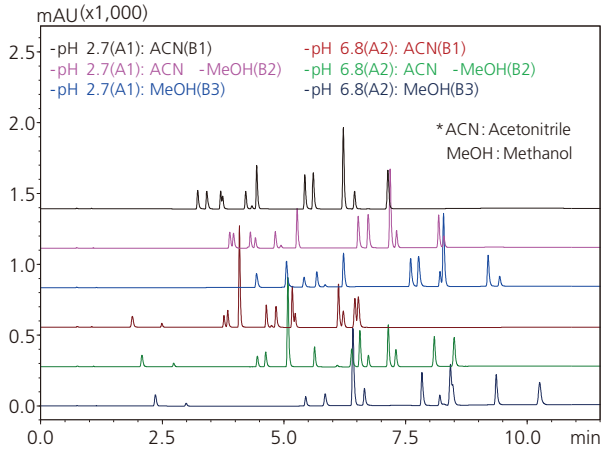


Fig. 4 Chromatogram with Shim-pack Scepter Phenyl-120

3-4. Quickly Find Optimum Condition

Because screening generates as many chromatograms as the number of conditions considered, they must be evaluated to determine which one is optimal. If all chromatograms had to be checked one by one, it would take a lot of time and cost. LabSolutions MD can quickly and easily find optimum condition using equation (1) below to quantitatively estimate the chromatographic separation.

$$(\text{Evaluation Value}) = P \times (Rs_1 + Rs_2 + \ldots + Rsp) \qquad \text{(Equation 1)}$$

Evaluation Value (E) is calculated as the number of peaks detected (P) multiplied by the sum of resolution factor (Rs) for all peaks. Fig. 5 shows Evaluation Value obtained from screening in an order from the highest to the lowest. It was confirmed that the highest Evaluation Value is obtained when using an aqueous mobile phase with pH 6.8, 50:50 acetonitrile/methanol as organic mobile phase, and Shim-pack Scepter Phenyl-120 column (Fig. 4: Green chromatogram).

			Response	
Column Nick Name	MPA pH	MPB A (%)	Evaluation Value	Minimum Resolution
Scepter-Phenyl-120	6.8	50	546.000	3.224
Scepter-C8-120	6.8	0	469.894	0.093
GIST-C18-AQ	2.7	0	465.124	1.075
GIST-C18-AQ	6.8	50	443.580	1.826
Scepter-C8-120	6.8	50	436.241	0.026
Scepter-Phenyl-120	2.7	50	419.659	1.743
Scepter-C18	2.7	0	419.338	1.518
Scepter-C18	6.8	50	396.000	4.326
Scepter-C4-300	2.7	0	394.239	0.402
Scepter-C18	6.8	100	384.553	2.046

Fig. 5 Ranking of the Conditions (mobile phases and columns) by Evaluation Value (Shown from the Highest to the Lowest)

3-5. Identification of the Parameters that have a Large Impact on Separation Using Analysis of Variance

Using the analysis of variance, it is possible to quantify how much each parameter such as mobile phase, columns, etc. affects separation. Identifying the parameters that have a large effect on separation helps to decide which should be further considered in the optimization phase, increasing the efficiency of method development.

Fig. 6 shows the results of analysis of variance with respect to each parameter. P value for "mobile phase A × mobile phase B" and "column" (in red) is both less than 0.05. The parameters with p value of 0.05 or less can be considered to be different (e.g. resolution factor) at each level, suggesting that they have a large effect on separation.

ANOVA Table

Display Plots	Effect	SSR	df	MS	F Value	p Value
☑	MPA pH x MPB B (%)	44817.9	2	22408.9	6.72	0.0141
☑	Column Nick Name	66312.0	5	13262.4	3.98	0.0302
☑	Column Nick Name x MPA pH	35853.2	5	7170.6	2.15	0.142
☑	Column Nick Name x MPB B (%)	50149.0	10	5014.9	1.50	0.265
☑	MPB B (%)	9123.7	2	4561.9	1.37	0.298
☑	MPA pH	3243.6	1	3243.6	0.973	0.347
	Error	33336.5	10	3333.7		
	Total	242835.8	35			

Fig. 6 Results of Analysis of Variance

3-6. Results of Screening

Table 3 shows the best condition obtained in screening phase with the highest Evaluation Value. The condition is obtained with aqueous mobile phase pH 6.8, 50:50 acetonitrile/methanol as organic mobile phase, and Shim-pack Scepter Phenyl-120. In optimization phase (3.7), further examinations are implemented for method optimization, including gradient program of pump and column oven temperature.

Table 3 Conditions with the Highest Evaluation Value

Mobile phase:	
Pump A	Buffer
A2	20 mmol/L (Sodium) phosphate buffer (pH 6.8)
Pump B	Organic solvent
B2	Acetonitrile / Methanol = 50 : 50
Column:	
4	Shim-pack Scepter Phenyl-120
Analytical conditions:	
Time program	:B.Conc. 5% (0 min) → 80% (8.01-11 min) → 5% (11.01-15 min)
Flow rate	:0.7 mL/min
Inj.vol.	:1.0 µL
Temperature	:40 °C
Detection	:Max plot 220- 400 nm (SPD-M40)

3-7. Optimization Phase

After the pH level of aqueous mobile phase and column are selected in the screening phase, analytical condition is further optimized by considering the parameters of mixture ratio of organic mobile phase (30, 40, 50, 60, 70 %), oven temperature (35, 40, 45 °C), and final concentration of gradient program (75, 80, 85 %). The effect on separation of these parameters are shown by design space with the mixture ratio of organic mobile phase on the vertical axis and oven temperature on the horizontal axis.

Building design space makes it possible to visualize the impact of the parameters on minimum resolution comprehensively. LabSolutions MD can suggest the most robust condition among the entire region of design space (black square in Fig. 7), which means a robust analytical method can be defined without relying on the user experience. Based on design space, it is confirmed that the most robust analytical condition is organic solvent mixture ratio of 50 %, oven temperature of 39 °C, and final concentration of gradient program of 80 %. Moreover, by clicking any point in design space (e.g. point A in Fig. 7), simulated chromatogram can be displayed (Fig. 8). This function of chromatogram simulation allows to check how the separation will change through any conditions quickly without running any analysis.

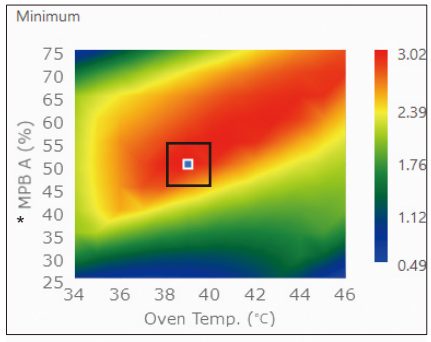


Fig. 7 Design Space of Minimum Resolution (Final Gradient Concentration of 80 %)
* Mobile Phase B A: Acetonitrile

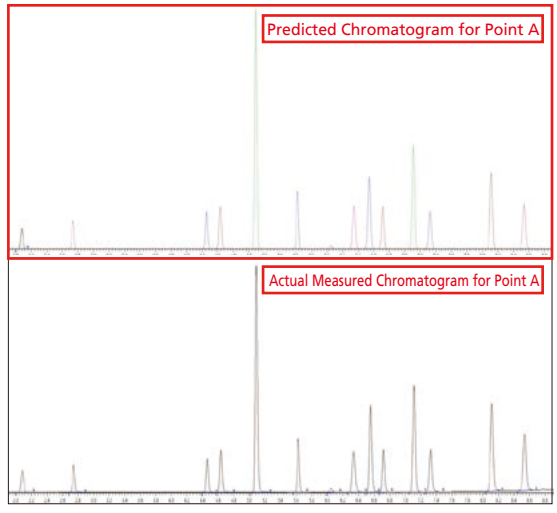


Fig. 8 Simulated Chromatogram and Actual Chromatogram at Point A (Fig. 7)

3-8. Automatic Identification of Compounds Using Peak Tracking

When analytical condition such as mobile phase, oven temperature, gradient program of pump is changed, retention time of each compound can also be different. It's a time consuming process to identify manually each compound through all the acquired data. LabSolutions MD can automatically identify target compounds by Peak Tracking function through all the data (Fig. 9). For example, similarity of UV spectrum, peak area, and other parameters can be used to automatically track each compound, contributing to quick recognition of peaks through all the data without any additional manual work.

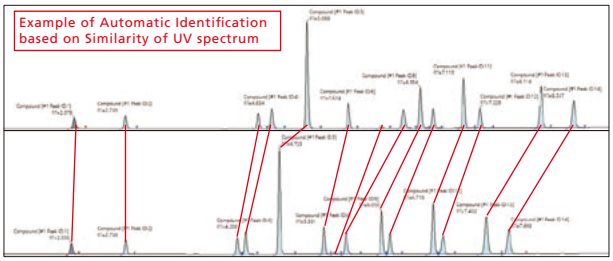


Fig. 9 Automatic Identification of Target Compounds Using Peak Tracking Function

3-9. Results of Optimization

Table 4 shows the condition determined through optimization phase. By building design space, optimum condition is decided with 50:50 acetonitrile/methanol as organic mobile phase, oven temperature of 39 °C, and final concentration of gradient program of 80 %. In the validation phase in 3.10, robustness of the optimized method is evaluated by checking the separation pattern when each parameter is changed in a small range.

Table 4 Optimized Condition

Mobile phase:	
Pump A	Buffer
A2	20 mmol/L (Sodium) phosphate buffer (pH 6.8)
Pump B	Organic solvent
B2	Acetonitrile / Methanol = 50 : 50
Column:	
4	Shim-pack Scepter Phenyl-120(100 mm × 3.0 mm I.D., 1.9 µm)*6
Analytical conditions:	
Time program	:B.Conc. 5%(0 min) → 80%(8.01-11 min) → 5%(11.01-15 min)
Flow rate	:0.7 mL/min
Inj.vol.	:1.0 µL
Temperature	:39 °C
Detection	:Max plot 220- 400 nm (SPD-M40)

3-10. Validation Phase

In Validation phase, LabSolutions MD can create sequential experimental design to perform robustness evaluation. Robustness evaluation is important to understand how the variations of parameters will affect results and ensure the reliability of method. LabSolutions MD creates sequential experimental design automatically by changing the parameters of optimized method in a small range to evaluate the robustness. Specifically, mixture ratio of organic mobile phase by 1 % (49, 50, 51 %) and oven temperature by 1 °C (38, 39, 40 °C) (white circle in Fig. 10) to verify the effect on separation. Fig. 11 shows chromatograms obtained for robustness evaluation. The effect of variation of the parameters on separation is very small, showing robustness of the optimized method constructed by design space. The optimized analytical method based on AQbD approach will be beneficial for next phases of method development.

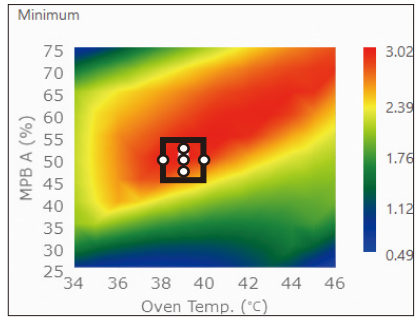
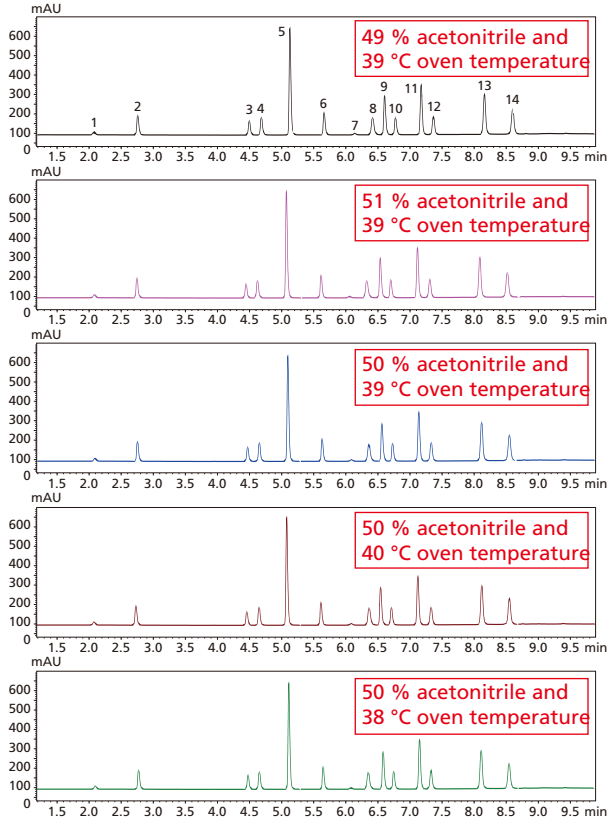


Fig. 10 Data Points of Robustness Evaluation
* Mobile Phase B A: Acetonitrile



1 : Acetylsalicylic acid
2 : Impurity of acetylsalicylic acid
3 : Antipyrine
4 : Metoclopramide
5 : (S)-(+)-Naproxen
6 : Probenecid
7 : Impurity of quinidine
8 : Quinidine
9 : Diclofenac sodium
10 : Indometacin
11 : Papaverine hydrochloride
12 : Lidocain
13 : Dibucaine hydrochloride
14 : Amitriptyline hydrochloride

Fig. 11 Chromatograms of Robustness Evaluation at each Data Point (White circle in Fig. 10)

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