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Technical Report

Ultra Fast Method Scouting (2)
—Maximizing the Efficiency of Method Development

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Abstract:

Due to the large amounts of time required for determining analytical conditions (method scouting), users are looking for ways to minimize the time required for method scouting.
This report describes an example of using a Nexera Method Scouting system in combination with dedicated method scouting software to determine analytical conditions for simultaneous analysis of nine types of sulfa drugs. The dedicated method scouting software, Method Scouting Solution, is able to use the multi-data report functionality in LabSolutions DB/CS software to rapidly find optimal analytical conditions from huge amounts of analytical results. Therefore, this report also describes the multi-data report function.

Keywords: Nexera Method Scouting, Method Scouting Solution, LabSolutions DB/CS, Multi-Data Report

1. Introduction

Developing analytical methods for new compounds mainly involves four steps.

(1) Simulation
Retention behavior is predicted offline based on information about the structural and other properties of target compounds being analyzed.

(2) Method Scouting
Column and mobile phase candidates are comprehensively scouted by performing trial analyses using different combinations of various columns and mobile phases.

(3) Method Optimization
The columns and mobile phases selected during method scouting are used to optimize various parameters.

(4) Method Validation
This step evaluates the robustness of analytical methods.

The Nexera Method Scouting system is based on the Nexera X2 ultra high performance liquid chromatograph, which features a 130 MPa pressure tolerance, and is used for applications corresponding to Step 2 on the left. The system is able to comprehensively search for analytical conditions by automatically trying up to 192 combinations of eight types of mobile phases and twelve types of columns.

This report describes an example of using the Nexera Method Scouting system, Method Scouting Solution software, and multi-data report functionality included in LabSolutions DB/CS software to determine optimal analytical conditions by comparing results measured using a variety of analytical conditions.

Fig. 1 Nexera Method Scouting System

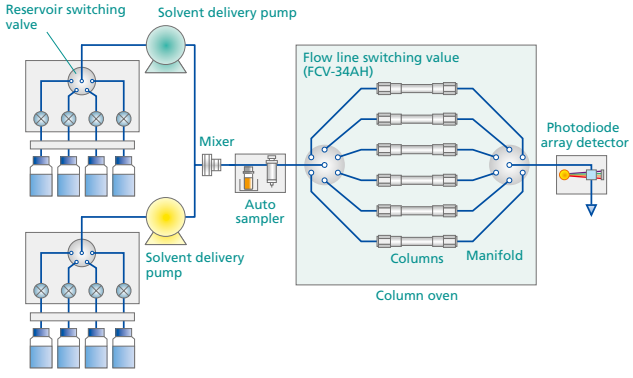


Fig. 2 Flow Line Diagram of Nexera Method Scouting System (Shown with Six Types of Columns)

1 Analytical & Measuring Instruments Division

2. Simultaneous Analysis of Sulfa Drugs Using Method Scouting Solution

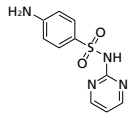
This section describes an example of scouting conditions for simultaneous analysis of sulfa drugs, which are used in synthetic antibacterial agents, anticancer agents, and other pharmaceuticals. The initial conditions considered for the sulfa drugs are shown in Figure 3. In this case, a total of four types of mobile phases were considered by combining either of two types of (sodium) phosphate buffer solutions with different pH values as solvent A and acetonitrile or methanol as solvent B. Six types of columns were considered, including three Shim-pack XR series columns. These four mobile phases and six columns were used to test a total of 24 analytical condition combinations.

2-1. Method Scouting Solution

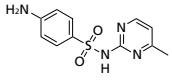
The main window displayed in Method Scouting Solution is shown in Figure 4. It shows the various parameters for columns, mobile phases, sample information, gradient conditions, and so on, and corresponding icons that can be clicked to easily change the respective parameters.

Using the dedicated software, batch analysis can be performed (scouting started) easily by (1) selecting the columns and mobile phases preregistered in the database, (2) specifying gradient conditions, (3) entering sample information and other parameters, and (4) clicking the [Create Batch] button. By working in conjunction with LabSolutions software, the Method Scouting Solution software is able to automate all analysis processes involved in method scouting, from preparing the system for analysis (startup) to shutting down the system after the analysis is finished (shutdown). Consequently, the software can significantly reduce the amount of time and effort required to specify conditions and execute analyses for method scouting.

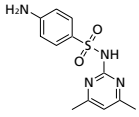
Analytes: Nine Sulfa Drug Compounds



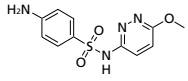
1. Sulfadiazine



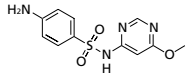
2. Sulfamerazine



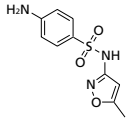
3. Sulfadimidine



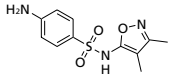
4. Sulfamethoxypyridazine



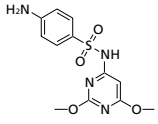
5. Sulfamonomethoxine



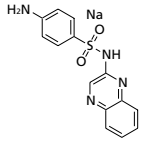
6. Sulfamethoxazole



7. Sulfisoxazole



8. Sulfadimethoxine



9. Sulfaquinoxaline sodium

Mobile Phases: 2 Aqueous × 2 Organic = 4 Mobile Phases

(A) (a) 10 mmol/L (sodium) phosphate buffer, pH 2.6
(b) 10 mmol/L (sodium) phosphate buffer, pH 6.9

(B) (a) Acetonitrile
(b) Methanol

Columns: 6 Types

1. Shim-pack XR-ODS (75 mm L. × 3.0 mm I.D., 2.2 µm)
2. Shim-pack XR-C8 (75 mm L. × 3.0 mm I.D., 2.2 µm)
3. Shim-pack XR-Phenyl (75 mm L. × 3.0 mm I.D., 2.2 µm)
4. Column A-ODS (75 mm L. × 3.0 mm I.D., 2.0 µm)
5. Column B-C8 (75 mm L. × 3.0 mm I.D., 2.0 µm)
6. Column C-Phenyl (75 mm L. × 3.0 mm I.D., 2.0 µm)

4 types of mobile phases × 6 types of columns → Total 24 combinations

Other Analytical Conditions

Flow rate : 0.8 mL/min
Injection volume : 2 µL
Column temperature : 45 °C
Detection : UV detection (265 nm)
Time program : B Conc. 10 % (0 min) → 35 % (6.50 min) → 50 % (6.51 to 7.50 min) → 10 % (7.51 to 11.00 min)

Fig. 3 Scouting Analytical Conditions for Simultaneous Analysis of Sulfa Drugs

(1) Select mobile phase and column.

(2) Select base method and create gradient conditions.

(3) Select vial and enter sample information.

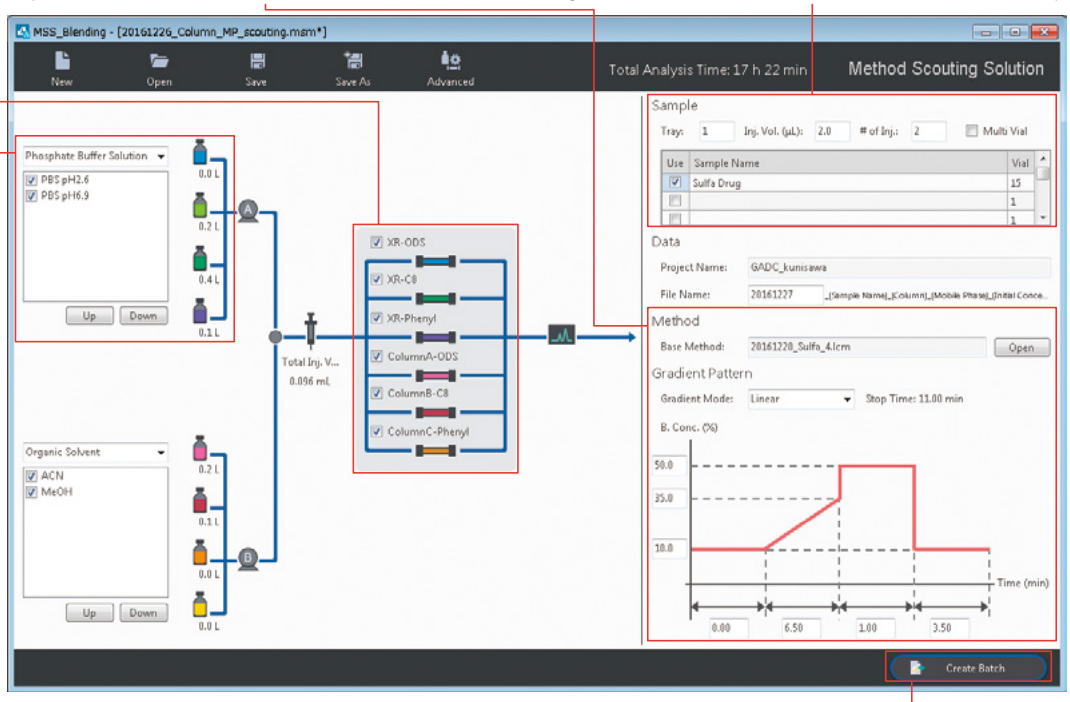


Fig. 4 Method Scouting Solution (Main Window)

(4) Create batch and start scouting.

2-2. Selecting Columns and Mobile Phases and the Automatic Mobile Phase Preparation Function (Mobile Phase Blending Function)

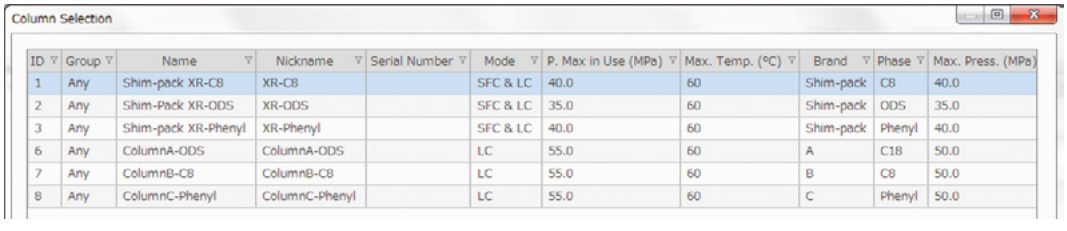
Clicking on a mobile phase or column icon in Method Scouting Solution displays a list of columns or mobile phases registered in the database (Fig. 5). This window is used to select the mobile phases or columns used to scout for analytical conditions.

This step eliminates the need to prepare each mobile phase being considered by the system when multiple mobile phases with different buffer solution pH levels or salt concentration levels are being considered. The system includes an automatic mobile phase preparation function (mobile phase blending function) that is able to automatically mix combinations of up to four types of solvents for each mobile phase sent to two delivery pumps (Fig. 6). Using this mobile phase blending function, the mobile phase pH level, salt concentration, quantity of acid added, and so on, can be adjusted automatically, which reduces the number of mobile phases that must be prepared before starting an analysis. The window for specifying the 10 mmol/L sodium phosphate buffer solution used to analyze the sulfa drugs is shown in Figure 7. If a sodium phosphate buffer solution is used for analysis, the following solvents are placed in the aqueous solvent delivery pump (Pump A).

A: Water
B: 10 mmol/L phosphoric acid
C: 10 mmol/L sodium dihydrogen phosphate
D: 10 mmol/L disodium hydrogen phosphate

Due to the pH 2.6 sodium phosphate buffer solution used for this sulfa drug analysis, conditions were specified for automatically blending solvents B and C at a 50:50 ratio*. Conditions were also specified for blending solvents C and D at a 60:40 ratio if a pH 6.9 sodium phosphate buffer solution is prepared. Furthermore, the salt concentration of the buffer solution can be adjusted after mixing by changing the ratio of water in A. By using the mobile phase blending function in this way, the tedious process of preparing mobile phases can be performed automatically by the software. This saves on time normally required for preparing mobile phases, avoids the risk of operating errors, and eliminates the need to prepare extra mobile phase. Mobile phases specified as a set of solvents can be selected as one mobile phase, in the same manner as a single solvent.

*: The pH value after adjustment is indicated as an example based on theory. The actual pH value may differ from the specified value, depending on the surrounding environment.



ID	Group	Name	Nickname	Serial Number	Mode	P. Max in Use (MPa)	Max. Temp. (°C)	Brand	Phase	Max. Press. (MPa)
1	Any	Shim-pack XR-C8	XR-C8		SFC & LC	40.0	60	Shim-pack	C8	40.0
2	Any	Shim-Pack XR-ODS	XR-ODS		SFC & LC	35.0	60	Shim-pack	ODS	35.0
3	Any	Shim-pack XR-Phenyl	XR-Phenyl		SFC & LC	40.0	60	Shim-pack	Phenyl	40.0
6	Any	ColumnA-ODS	ColumnA-ODS		LC	55.0	60	A	C18	50.0
7	Any	ColumnB-C8	ColumnB-C8		LC	55.0	60	B	C8	50.0
8	Any	ColumnC-Phenyl	ColumnC-Phenyl		LC	55.0	60	C	Phenyl	50.0

Fig. 5 Column Selection Window

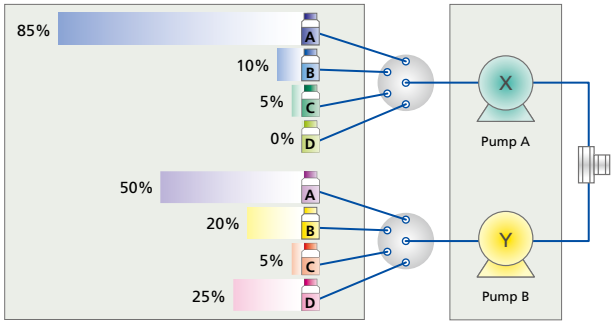


Fig. 6 Mobile Phase Blending Function

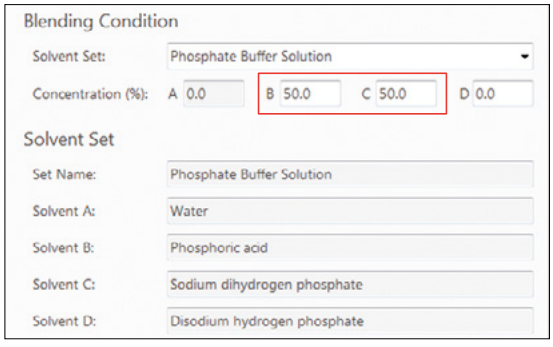


Fig. 7 Buffer Solution Prepared Using the Mobile Phase Blending Function (example of settings for a pH 2.6 (sodium) phosphate buffer solution)

2-3. Specifying Analytical Condition Settings and Creating Gradient Programs

In addition to the existing linear gradient mode, Method Scouting Solution also supports multilinear, stepwise, and isocratic gradient modes (Fig. 8). After selecting the gradient mode, if the organic solvent concentration at respective points in time are entered, then the software creates the gradient profile automatically. In this example of sulfa drug analysis, the linear gradient mode was used, as indicated for Step (2) in Figure 4.

By selecting the basic method created in LabSolutions, the Method Scouting system uses the basic parameters required for analysis (such as flow rate, oven temperature, and detection wavelength). Therefore, analytical conditions can also be scouted using the gradient conditions specified in the basic method.

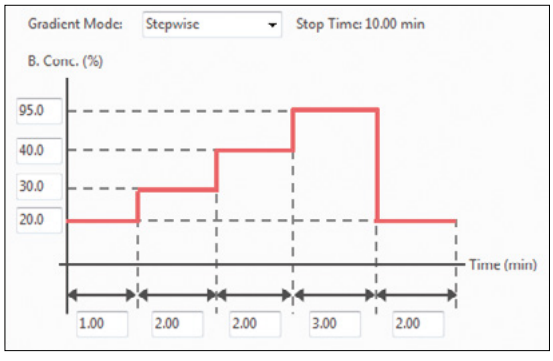
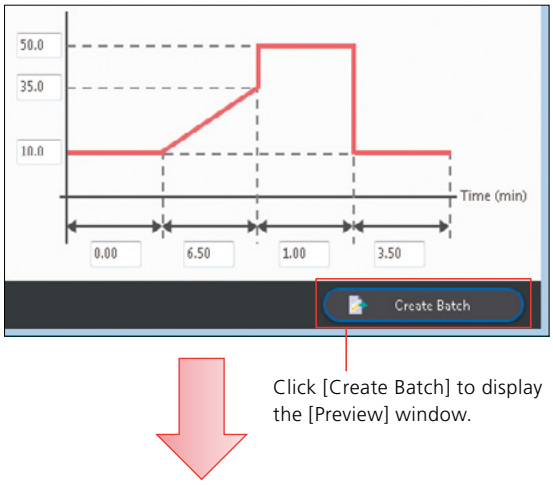


Fig. 8 Stepwise Mode

2-4. Starting Mobile Phase and Column Scouting

After selecting the mobile phases and columns and specifying gradient conditions, sample information, and other settings, clicking the [Create Batch] button in the lower right corner of the window displays the batch creation preview window (Fig. 9). After checking the preview, clicking the [Create Batch & Run] button starts the scouting process. Analysis processes can be even further automated utilizing functionality included in LabSolutions, such as the automatic mobile phase switching and baseline stability check functions.



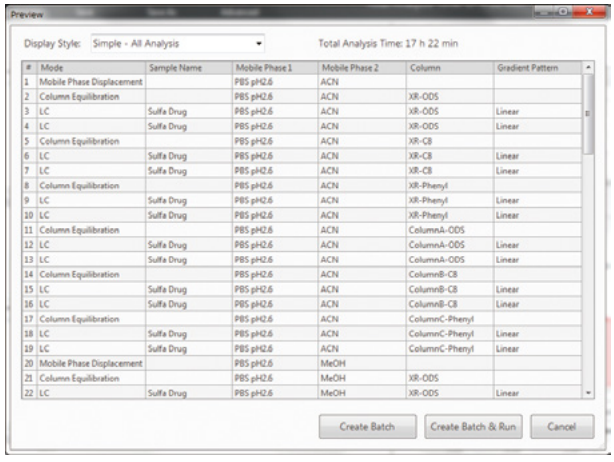


Fig. 9 Batch Creation Preview Window

3. Screening Mobile Phases and Columns Using the Multi-Data Report and Browser Functions

Determining the optimal mobile phase and column combination based on the huge amounts of analytical results generated by method scouting is not easy. However, by using the multi-data report and data browser functionality included in LabSolutions DB/CS, the optimal conditions can be determined quickly. This section shows 24 method scouting results obtained from simultaneous analysis of the sulfa drugs.

The following equation was used to quantitatively evaluate the resolution status in the chromatograms obtained. (For more details, refer to Technical Report C190-E159: Improved R&D Efficiency Through Speedier Method Development (3)).

$$E = P\,(R_{S1} + R_{S2} + \ldots + R_{SP}) \qquad \text{(eq. 1)}$$

Equation 1 was configured to evaluate resolution based especially on peak resolution, where E, the evaluation value, is calculated using P, the number of peaks detected in the chromatogram, and R_S, the resolution.

An example of an evaluation results report for scouting conditions for sulfa drugs is shown in Figure 10. The table in the upper part of the report indicates the number of peaks detected for each set of analytical conditions (data file name) used for measurements, the resolution, and the evaluation value and corresponding rank calculated based on equation 1. Results can either be listed in order the analyses were performed, as shown in Figure 10 (a), or in descending order of evaluation value, as shown in Figure 10 (b). The lower part of the report shows a bar graph of the evaluation values for each set of analytical conditions, which allows evaluation values to be judged visually. By using the multi-data report function in this way, candidates for determining the optimal analytical conditions can be identified from huge amounts of analytical results.

Fig. 10 Results from Method Scouting Using the Multi-Data Report Function

A summary of the chromatograms measured using each set of analytical conditions can be viewed using data browser. Figure 11 shows an example of using data browser to display all the chromatograms obtained. Optimal analytical conditions can be determined not only based on the evaluation values displayed in the multi-data report, but also by displaying a summary of the peak elution status for each chromatogram. Furthermore, data browser can also display only chromatograms for a candidate's optimal analytical conditions, selected based on evaluation value results using the multi-data report.

As a result, optimal conditions for simultaneous analysis of nine sulfa drugs were successfully established based on optimal conditions determined using the multi-data report and data browser functionality. Details are shown in Figure 12.

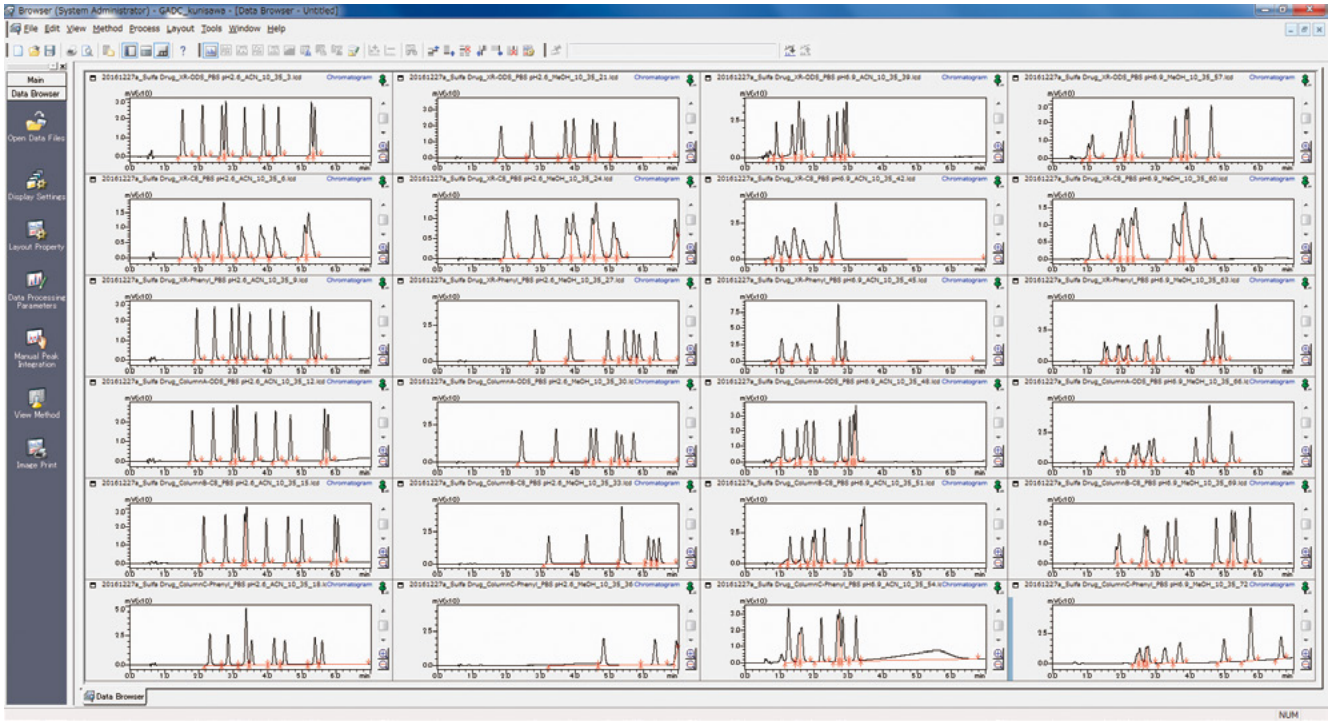
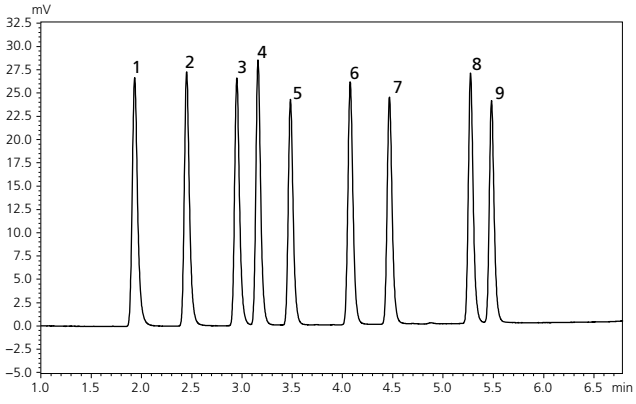


Fig. 11 Comparison of Chromatograms Using the Data Browser Function



Peaks:

1. Sulfadiazine, 2. Sulfamerazine, 3. Sulfadimidine,
4. Sulfamethoxypyridazine, 5. Sulfamonomethoxine, 6. Sulfamethoxazole,
7. Sulfisoxazole, 8. Sulfadimethoxine, 9. Sulfaquinoxaline sodium

Analytical Conditions

Analytical column	: Shim-pack XR-Phenyl (75 mm L. × 3.0 mm I.D., 2.2 µm)
Mobile phase A	: 10 mmol/L (sodium) phosphate buffer solution (pH 2.6)
Mobile phase B	: Acetonitrile
Initial concentration	: 10 % B
Flow rate	: 0.8 mL/min
Temperature	: 45 °C
Detection	: UV detection (265 nm)
Injection volume	: 2 µL
Time program	: B Conc. 10 % (0 min) → 35 % (6.50 min) → 50 % (6.51 to 7.50 min) → 10 % (7.51 to 11.00 min)

Fig. 12 Optimal Combination of Column and Mobile Phases for Simultaneous Analysis of Sulfa Drugs Determined by Method Scouting and Corresponding Chromatogram

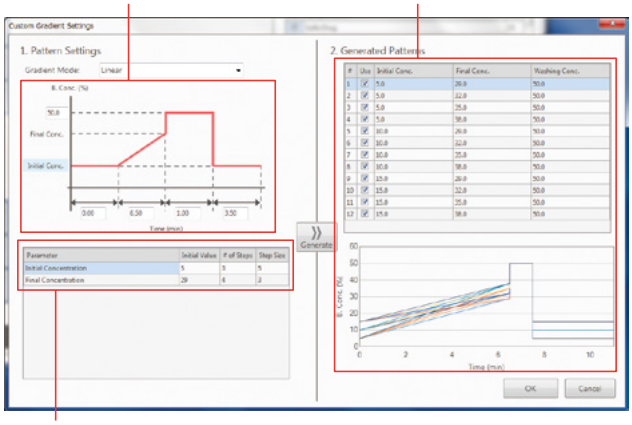
4. Optimizing Gradient Conditions

After determining the optimal combination of mobile phases and column, method scouting can also involve optimizing gradient conditions for the purpose of improving resolution or increasing speed. This section describes an example of using the mobile phases and column determined in section 3 to optimize gradient conditions.

Multiple gradient conditions can be created easily by selecting 'custom' as the gradient mode in the main Method Scouting Solution window. In the window shown in Figure 13, specify the initial concentration, final concentration, washing concentration, and time program settings. In addition, multiple concentration patterns can be specified for the initial and final concentration settings. In this example, the initial concentration was set to 5, 10, and 15 % and the final concentration was set to 29, 32, 35, and 38 %. Using all of these patterns for analysis results in 12 gradient condition configurations. Evaluation results obtained using these 12 gradient condition configurations are shown in Table 1. The resulting optimal analytical conditions and chromatogram are shown in Figure 14. Optimizing the initial and final gradient concentrations resulted in improved resolution between sulfadimidine and sulfamethoxypyridazine and between sulfadimethoxine and sulfaquinoxaline sodium.

Specifies the base parameter (time)

The gradient pattern based on the specified settings is displayed on the right side.



Specifies the initial value, number of steps, and concentration increase level for each concentration.

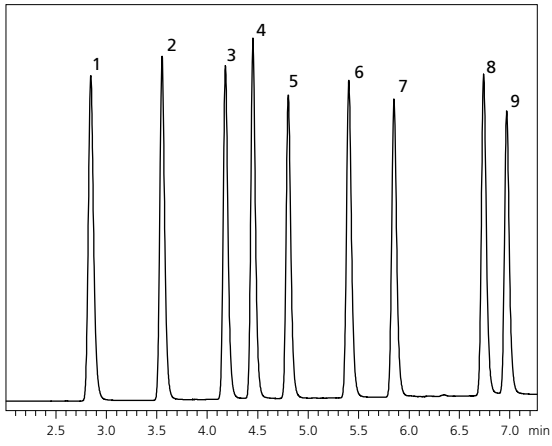
Fig. 13 Gradient Condition Settings Window

Table 1 Evaluation Results for Gradient Conditions

Initial Conc. (%)	Final Conc. (%)	Evaluation Value	Rank
5	29	317.048	1
5	32	306.421	3
5	35	299.231	5
5	38	293.354	7
10	29	309.955	2
10	32	302.475	4
10	35	296.530	6
10	38	290.660	8
15	29	273.424	9
15	32	266.630	10
15	35	264.443	11
15	38	259.044	12

5. Multi-Data Reports

In addition to normal analytical results reports, the multi-data report function in LabSolutions can also be used to prepare regulatory testing reports using MS Excel-like operability, such as for content uniformity tests and related substance tests. The window for creating multi-data report templates and the corresponding report output are shown in Figure 15. The template creation window is configured similar to spreadsheet software, with the ability to perform various calculations by entering or specifying various formulas or functions in respective cells. Field (1) in Figure 15 is for entering a function that ranks the data obtained. The retention time, area, theoretical plates, resolution, and other chromatogram data can be configured in the desired layout by selecting them on the left side of the window, as shown in (2) of Figure 15, and dragging them to the desired cell. Frequently used functions, such as statistical functions or character string functions, can also be selected on the left side of the window to avoid the trouble of having to enter the functions directly. Reports can be output automatically after analysis, either on paper or as a PDF file, by specifying the created template file in advance.



Peaks:

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4. Sulfamethoxypyridazine, 5. Sulfamonomethoxine,
6. Sulfamethoxazole, 7. Sulfisoxazole,
8. Sulfadimethoxine, 9. Sulfaquinoxaline sodium

Analytical Conditions

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Initial concentration	: 5 % B
Flow rate	: 0.8 mL/min
Temperature	: 45 °C
Detection	: UV detection (265 nm)
Injection volume	: 2 µL
Time program	: B Conc. 5 % (0 min) → 29 % (6.50 min) → 50 % (6.51 to 7.50 min) → 10 % (7.51 to 11.00 min)

Fig. 14 Optimized Gradient Conditions and Chromatogram

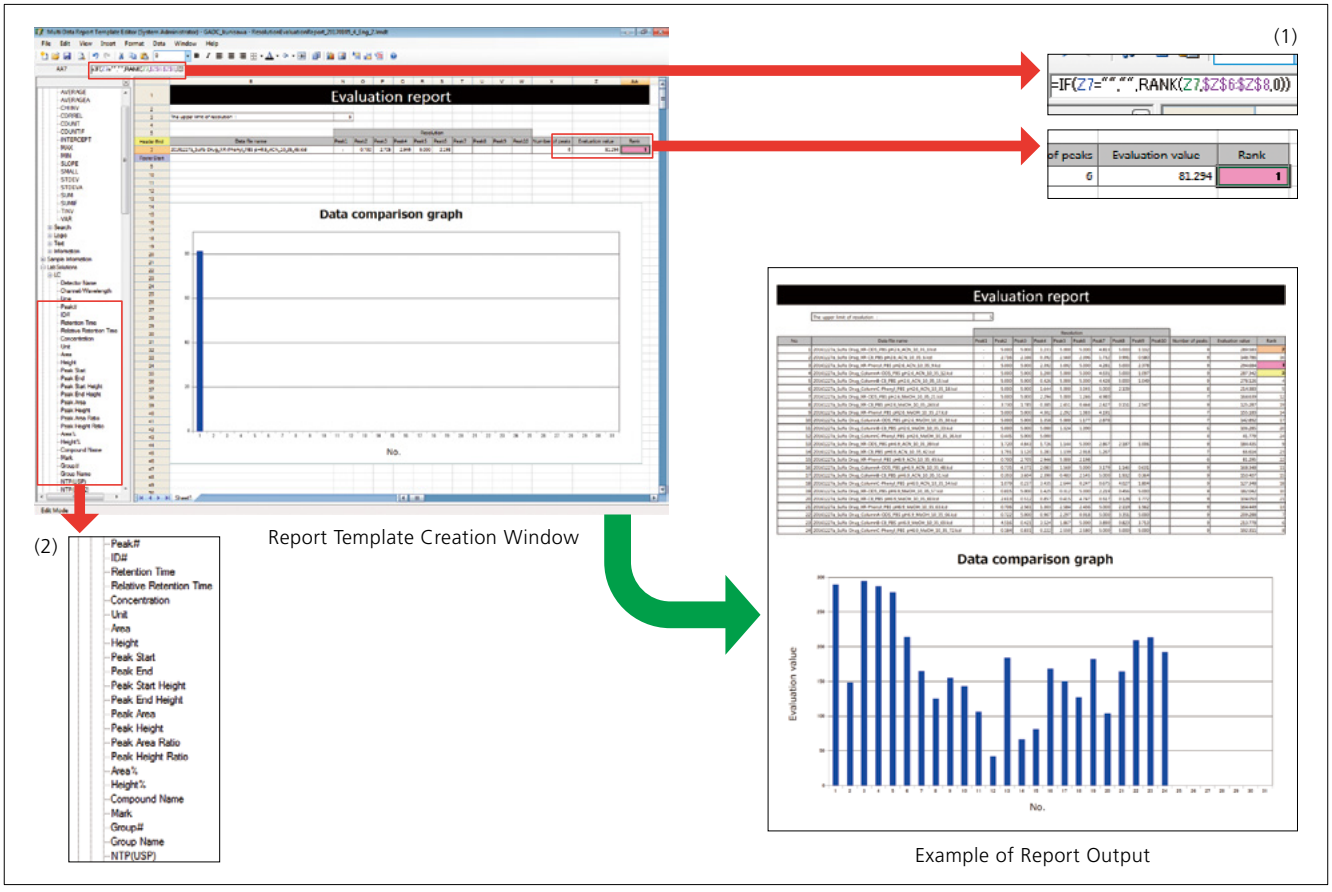

Fig. 15 Creating Multi-Data Reports

6. Conclusion

Using the Nexera Method Scouting system, it is possible to easily and quickly determine the optimal analytical conditions. In addition, it is easy to view a summary of evaluation values and chromatograms for the analytical results obtained. The system can also be used for determining analytical conditions for new compounds, checking the robustness of developed analytical conditions, testing the differences between different lots of analytical columns, and other applications, which can be expected to significantly reduce the time and effort required for analytical operations.

In addition to Nexera Method Scouting systems, the dedicated Method Scouting Solution software is also compatible with Nexera Quaternary systems (low-pressure gradient systems) and i-Series integrated LC systems. That means existing systems can be expanded to method scouting systems.

Nexera Quaternary i-Series

Fig. 16 Low-Pressure Gradient System Controllable by Method Scouting Solution Software

Note: The Nexera Quaternary and i-Series systems do not currently support the mobile phase blending function.

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