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Technical Report

Ultra Fast Method Scouting
– Maximizing Efficiency for Method Development –

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Abstract:

The traditional process of investigating analytical conditions during method development is often complicated and time-consuming, and only when it becomes possible for anyone, regardless of skill level, to carry out this task easily, quickly, and ideally as an automated procedure, will greater speed and efficiency raise the overall level of analytical operations. This Report introduces the Nexera Method Scouting system, a UHPLC (ultra high performance LC) system for identifying and resolving the limiting factors associated with traditional method development techniques.

Keywords: Method Development, Method Scouting, Nexera Method Scouting, UHPLC, LC/MS, LC/MS/MS

1. Method Development

Designing and validating a new analytical method is an important task in departments responsible for method development and production technology. Although many processes are involved from start to completion, method development can basically be divided into 4 stages, as summarized below and illustrated in Fig. 1.

<<Stage>> 1 Simulation

This is an offline process used to predict a compound's retention behavior. Prior to an actual analysis, retention times and chromatographic patterns are simulated based on compound information such as structural formula, pKa and expected retention based on different column types. Typically, this is conducted when known components are involved.

<<Stage 2>> Method Scouting

This is an exhaustive process of screening columns and mobile phases to determine the run conditions that result in adequate separation of multiple components in the sample. It consists of a search (scouting) for the optimum conditions (column and mobile phase combination) by conducting trial analyses with various columns and mobile phases. In the case of LC/MS, column selection is a critical factor due to the constraints on the types of mobile phase additives that are allowed, i.e., they must be volatile.

<<Stage 3>> Method Optimization

This is the process in which the various parameters are further optimized in order to achieve better resolution between certain sample components or to shorten the run time. Optimization is conducted based on the column and mobile phase selected in Stage 2. In some cases this Stage is omitted, with the process proceeding directly from Stage 2 to Stage 4.

<<Stage 4>> Method Validation

This is the process in which validation is applied to the analytical method to verify the robustness of the method.

2. Rate-Limiting Step in Method Development

Among Stages 1 – 4, the most time-consuming by far is Method Scouting of Stage 2. For example, analysis of impurities or natural substances, or chiral analysis of optical isomers, etc. often involve difficult, in-depth searching (scouting) for suitable analytical conditions, frequently requiring a great deal of trial and error testing. In addition, this stage requires abundant experience and expertise, and the time required for the search greatly depends on the skill of the analyst.

Thus, method scouting is certainly one of the more rate-liming aspects of method development.

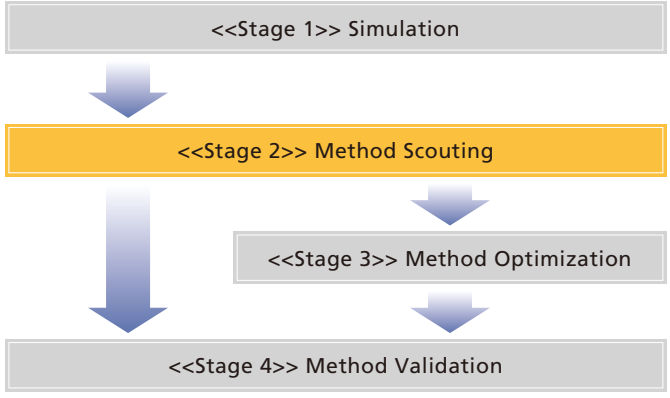


Fig. 1 Steps of Method Development

1 Analytical & Measuring Instruments Division

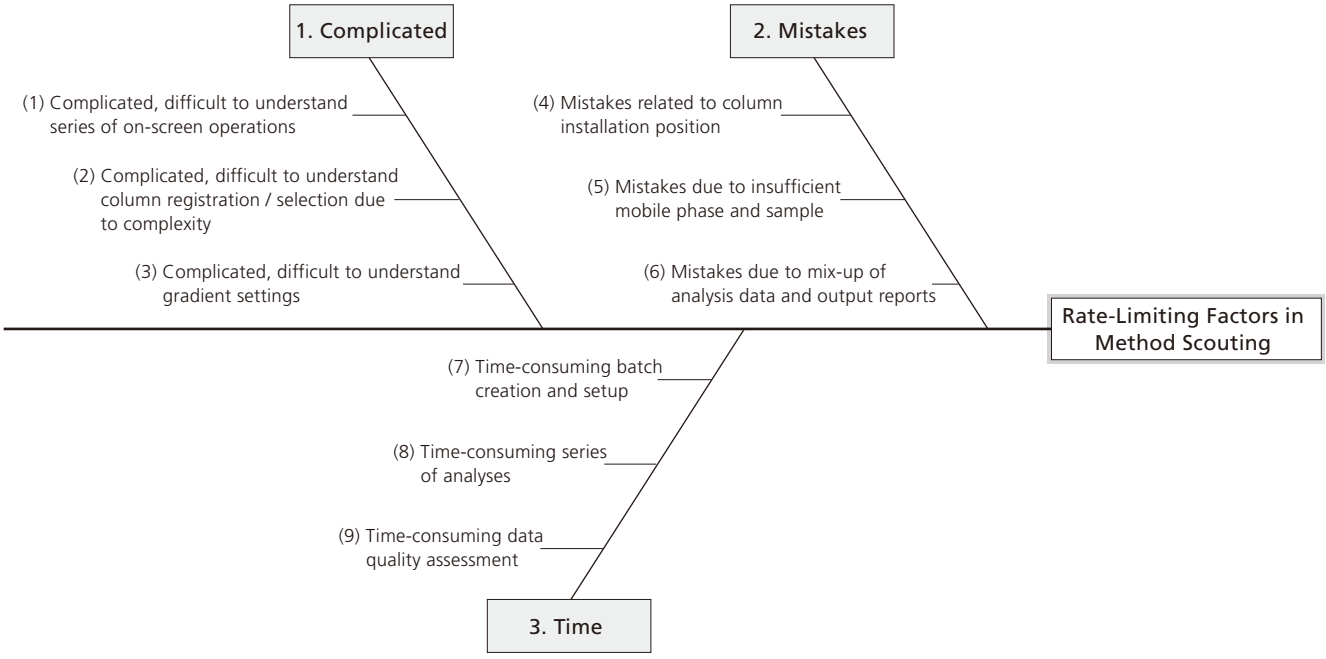


Fig. 2 Method Scouting Rate-Limiting Factors

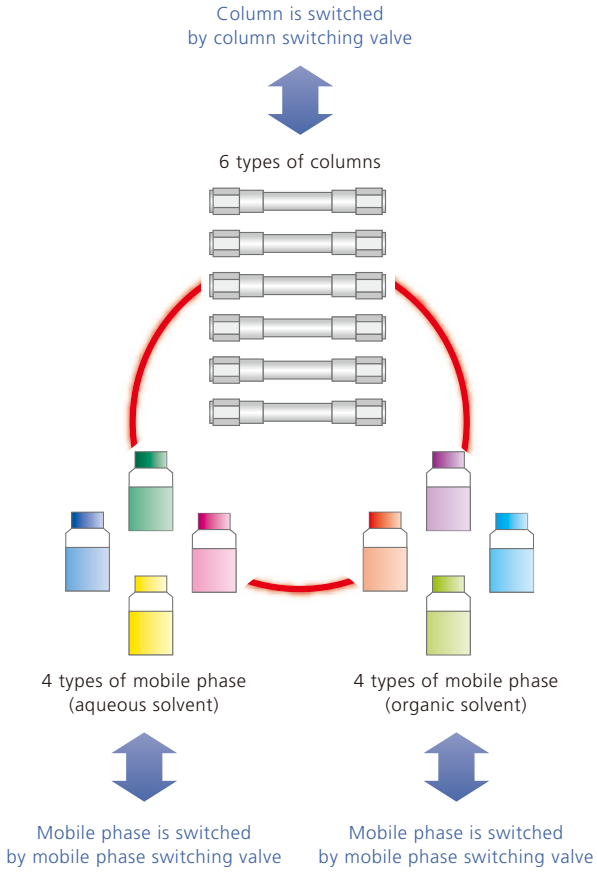


Fig. 3 Mobile Phase and Column Preparation

3. Rate-Limiting Factors in Method Scouting

The primary rate-limiting factors in method scouting are due to the sample as well as the personnel involved, but in addition to these, the system used for method scouting can also become an important factor. For example, when searching for the optimum analytical conditions using various mobile phases (aqueous solvents, organic solvents) and columns, as shown in Fig. 3, adding solvent and column-switching valves to a typical LC system for more column and solvent choices contributes to the rate-limiting factor. Because method scouting entails continuous trial analyses in which parameter values are changed slightly for each run, any additional solvent and column options require sufficient time for analysis…a process which is quite different from routine continuous analysis.

The critical rate-limiting factors associated with method scouting are presented in the outline of Fig. 2.

Here, nine rate-limiting factors are presented and classified into three categories.

1. Complications – Operations that are difficult to understand

→Complicated software operation

2. Mistakes – Missteps in operation

→Missteps at mobile phase preparation stage and/or during data analysis

3. Time – Inefficiencies

→Prolonged work time from analysis preparation to evaluation completion

None of the factors from (1) to (9) of Fig. 2 can afford to be ignored, thus the need to eliminate all of the rate-limiting factors associated with method scouting becomes imperative.

Table 1 Resolving Rate-Limiting Due to Conventional Systems

(A) Problem Points*

*This content is the same as that in Fig. 2.

1. Complicated	
(1)	Complicated, difficult-to-understand series of on-screen operations
(2)	Complicated, difficult-to-understand column registration / selection
(3)	Complicated, difficult-to-understand gradient settings
2. Mistakes	
(4)	Mistakes related to column installation position
(5)	Mistakes due to insufficient mobile phase and sample
(6)	Mistakes due to mix-up of analysis data and output reports
3. Time-consuming	
(7)	Time-consuming batch creation and setup
(8)	Time-consuming series of analyses
(9)	Time-consuming data quality assessment

(B) Solutions

1. Simple	
(1)	Specialized screens without Wizards
(2)	Column selection from registration list
(3)	Gradient setting by selecting start/end values
2. Mistake-free	
(4)	Tags are affixed at installation positions to prevent mistakes
(5)	Required volumes for batch analysis are displayed for mobile phase and sample
(6)	Column and mobile phase information are entered as data name and at top of report
3. Quick	
(7)	Batch creation by clicking vial start/end positions
(8)	Change to UHPLC system
(9)	Quality of result indicated numerically / graphically

4. Resolving Rate-Limiting Due to System

Concrete measures for resolving (1) – (9) of Fig. 2 are shown in Table 1 (B). A system that incorporates such solutions is necessary to achieve successful method scouting, and the system that has achieved this is the Nexera Method Scouting System (ultra high performance liquid chromatograph method scouting system) and its specialized Method Scouting Solution software (*see* Fig. 4).

5. Nexera Method Scouting

The Nexera Method Scouting software allows anyone to easily, quickly, and effectively scout for analytical conditions, thanks to the features presented in Table 1 (B). Figs. 5 to 13 show graphically how the solutions (1) to (9) of Table 1 (B) are implemented, along with detailed descriptions.

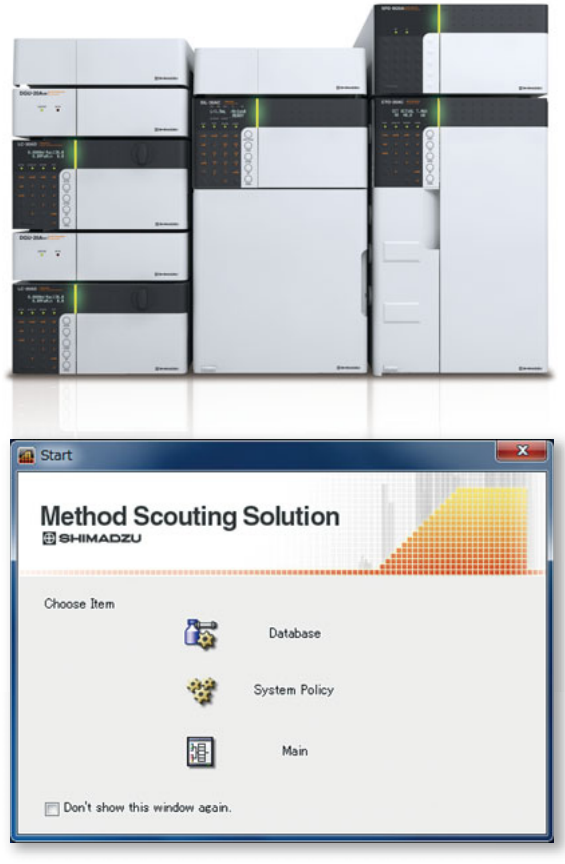


Fig. 4 Nexera Method Scouting

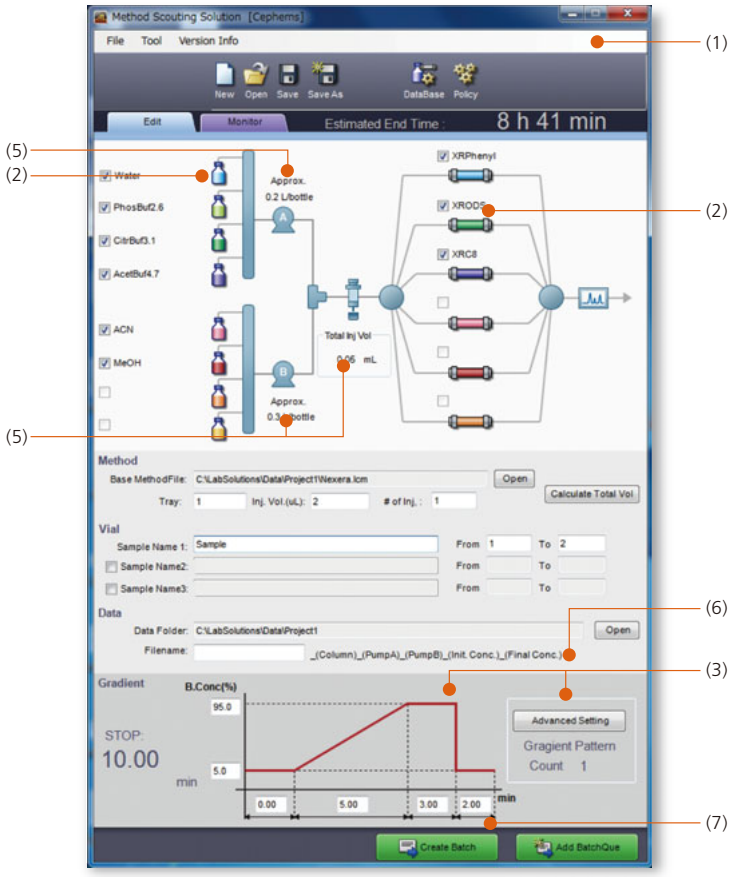


Fig. 5 Main Window of Method Scouting Solution

5-1. Complicated ➡ Simple

(1) Conditions Setting Window

Operations using this system are primarily conducted in the main window of Method Scouting Solution. Unlike other systems that rely on Wizards (a series of windows that interactively guide the user through the parameter settings), this system adopts a straightforward interface where users can set conditions while viewing a flow-line diagram that serves as a useful and convenient reference for all levels of users.

(2) Selection of Mobile Phases and Columns

Simply clicking on an icon of a mobile phase or column in the main window (Fig. 5) automatically activates the [Database] window (column and mobile phase database window) (Fig. 8). The mobile phases (aqueous solvents, organic solvent) and columns to be used in the scouting process are selected from the database. This window is also used to add mobile phases and columns to the database.

(3) Gradient Setup

Gradient conditions are also set in the main window (Fig. 5). To run consecutive trials using multiple gradient conditions, simply click the [Advanced Setting] button to display the [Gradient] window (Fig. 9). This window allows setting of the gradient patterns while viewing them graphically on screen. This ensures that all steps are entered correctly, especially when complex or multi-step gradients are used.

5-2. Mistakes ➡ Mistake-free

In method scouting, the key point is to prevent careless mistakes when dealing with a number of analytical methods which contain slightly different settings.

(4) Connecting Columns

Tubing that is connected to columns can be identified using color-coded tags (Fig. 7). The color of the tag corresponds to the color of the "column icon" on the window, so mistakes in connecting columns can be prevented.

(5) Preparation of Samples and Mobile Phases

The total amounts of sample and mobile phase required for a series of continuous analyses are displayed (Fig. 5(5)). Referring to these during analysis preparation will proactively prevent the depletion of sample or mobile phase during analysis.

(6) Handling of Analysis Data and Reports

Information including the column, mobile phase, and gradient conditions is automatically appended to and saved with the analysis data file name (Fig. 5(6)). More detailed information is automatically provided in the output report (Fig. 11). This can prevent mistakes due to confusion among many different sets of data during analysis.

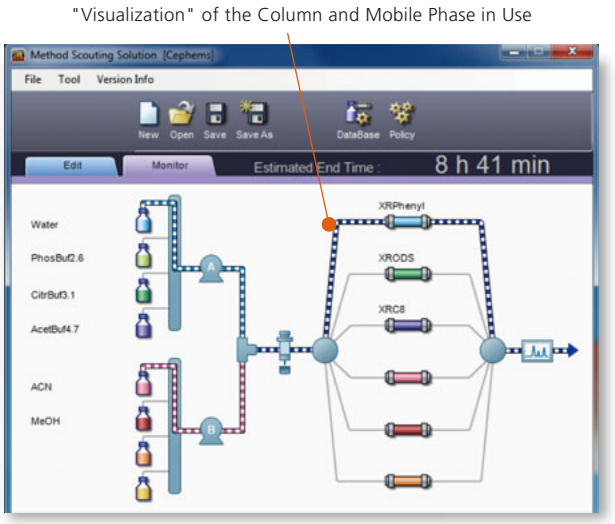


Fig. 6 [Monitor] Window During Analysis

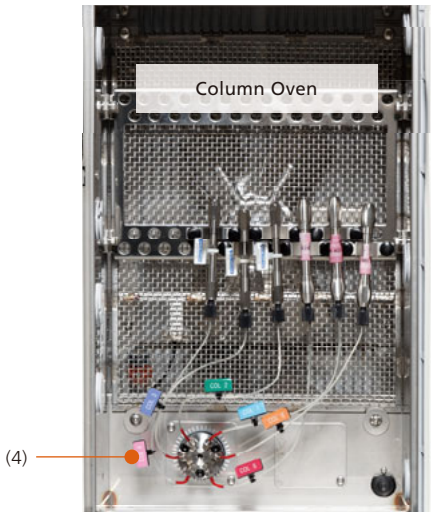


Fig. 7 Identifying Flow Lines

Table 2 Comparison of Time Required for Scouting Preparation

(1) Time Required to Create Method File

Conventional Method Scouting System	Nexera Method Scouting
32 hours(*1) (Creation of 960 method files manually)	5 minutes(*2) One method file

*1: Method file creation time calculated based on 1 file per 2 minutes

*2: Represents time required to enter settings, after which file is instantaneously generated.

(2) Time Required to Create Batch File

Conventional Method Scouting System	Nexera Method Scouting
8 hours(*3) (Created manually, line by line)	5 minutes(*4) Created all at once with Method Scouting Solution

*3: Batch file creation time calculated based on 30 seconds per line.

*4: Represents time required to enter settings, after which file is instantaneously generated.

5-3. Time-consuming ➡ Quick

(7) Batch Setup

Clicking the [Create Batch] button (Fig. 5(7)) automatically generates a batch file (file for continuous analysis) in accordance with settings (1) to (3). For example, as indicated in Table 2, the time required to create 960 different method files would typically take 32 hours by conventional methods, but using this system, it is completed almost instantaneously by generating a single method file. Similarly, it would typically take 8 hours to create a batch file one line at a time by conventional methods, but because it can now be done almost instantaneously, both time and effort are greatly reduced. Further, even after the batch file is automatically generated, settings such as the column, mobile phase, and gradient conditions can be changed, eliminating the need to re-create the batch file.

(8) Analysis Time

This system was built specifically based on the functionality offered with the Nexera UHPLC (ultra high performance LC), and thus dramatically shortens analysis time compared to that obtained with conventional systems. It also supports LCMS analysis, permitting method development for the LCMS-2020 single quadrupole ultra-fast LC/MS, and the LCMS-8030 triple quadrupole ultra fast LC/MS/MS (See Fig. 13).

(9) Scouting Results Assessment

As shown in Fig. 12, using Class-Agent and CLASS-Agent Report software, it is possible to quantitatively assess the scouting results. CLASS-Agent Report automatically extracts the necessary values (number of detected peaks and peak resolution) from the analysis results, and imports the data into a template prepared beforehand to automatically generate a report like that shown in Fig. 12. Because it eliminates the need for such tasks as printing out large quantities of analysis results one by one to compare chromatograms, it promotes labor savings and supports the paperless ecological initiative. For more information on the quantitative evaluation of the scouting results, please refer to Technical Report "Improved R&D Efficiency Through Speedier Method Development (1) / (2)"

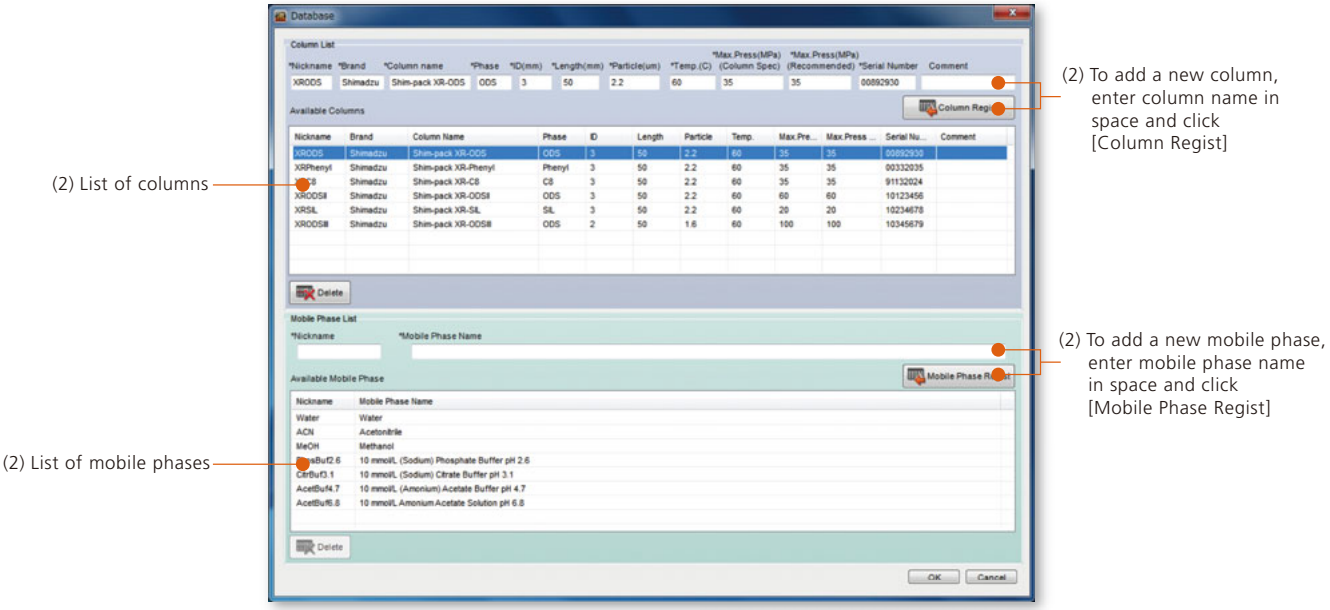


Fig. 8 Column and Mobile Phase Lists ([Database] window)

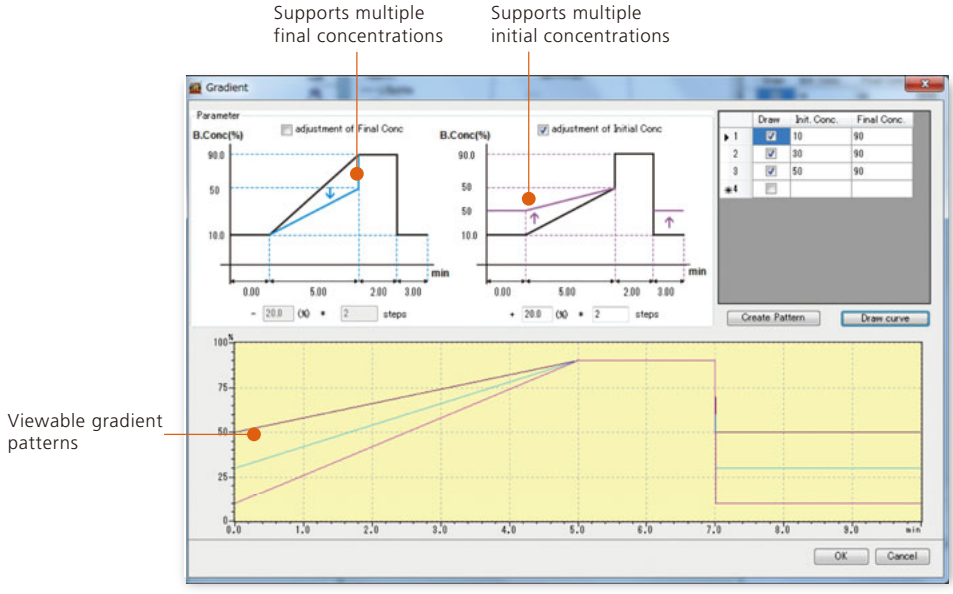


Fig. 9 Gradient Pattern Settings ([Gradient] window)

6. Nexera Method Scouting Application Example

Fig. 10 shows an actual example of the Nexera Method Scouting for analysis of thirteen cephem antibiotics. Nexera Method Scouting supports continuous execution of scouting runs for mobile phases, columns and gradients, however, to ensure thorough investigation of the analytical conditions, the process was divided into two steps (STEP 1, STEP 2), as shown in the figure.

In STEP 1, we prepared 4 aqueous and 3 organic mobile phases, as well as 6 types of columns, and then conducted scouting for the optimum mobile phases and column. The settings that were used are shown in Fig. 5. Then, scouting for the proper gradient conditions was conducted in STEP 2 using a total of 9 gradient patterns. These settings are shown in Fig. 9. The analytical conditions obtained as a result of the scouting process are shown in <<Scouted Analytical Conditions and Chromatogram>> in Fig. 10.

Utilizing the UHPLC (ultra high performance LC) performance of the Nexera, the analysis time was greatly shortened compared to that of conventional LC, to the extent that work previously requiring one week to complete was shortened to just one day.

A detailed description of this example is provided in Technical Report "Improved R&D Efficiency Through Speedier Method Development (3)".

<< Scouting Analytes: 13 Cephem Antibiotics>>

1. Cefsulodin 2. Cefadroxil 3. Cephapirin 4. Cefaclor 5. Cephalexin
6. Cephradine 7. Cefotaxime 8. Cefazolin 9. Cefuroxime 10. Cefmetazole
11. Cefoxitin 12. Cefoperazone 13. Cephalothin

Scouting was conducted for a method to conduct simultaneous analysis of these 13 compounds.

<<STEP 1: Mobile Phase and Column Scouting>>

Mobile Phases: (A) (a) Sodium phosphate buffer solution (pH 2.6)
(b) Sodium citrate buffer solution (pH 3.1)
(c) Ammonium acetate buffer solution (pH 4.7)
(d) Ammonium acetate buffer solution (pH 6.7)
(B) (a) Acetonitrile
(b) Methanol
(c) Acetonitrile / methanol = 50/50 (v/v)

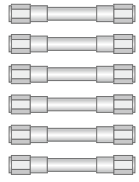
Aqueous: 4 types

Organic: 3 types

Columns:

(1)	Shim-pack XR-ODS	(50 mmL. × 3.0 mmI.D., 2.2 µm)
(2)	Shim-pack XR-C8	(50 mmL. × 3.0 mmI.D., 2.2 µm)
(3)	Shim-pack XR-Phenyl	(50 mmL. × 3.0 mmI.D., 2.2 µm)
(4)	Kinetex C18	(50 mmL. × 3.0 mmI.D., 2.6 µm)
(5)	Kinetex XB-C18	(50 mmL. × 3.0 mmI.D., 2.6 µm)
(6)	Kinetex PFP	(50 mmL. × 3.0 mmI.D., 2.6 µm)

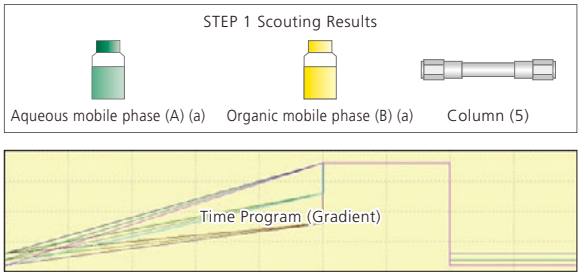
Columns: 6 types



Total of 72 combinations executed

The combination of aqueous mobile phase (A) (a), organic mobile phase (B) (a), and column (5) generated the best results.

<<STEP 2: Scouting of Gradient Conditions>>



Time Program:

(1) B Conc. 5% (0 min)→40% (5.01–7 min)→5% (7.01–9 min)
(2) B Conc. 5% (0 min)→65% (5.01–7 min)→5% (7.01–9 min)
(3) B Conc. 5% (0 min)→90% (5.01–7 min)→5% (7.01–9 min)
(4) B Conc. 10% (0 min)→40% (5.01–7 min)→10% (7.01–9 min)
(5) B Conc. 10% (0 min)→65% (5.01–7 min)→10% (7.01–9 min)
(6) B Conc. 10% (0 min)→90% (5.01–7 min)→10% (7.01–9 min)
(7) B Conc. 15% (0 min)→40% (5.01–7 min)→15% (7.01–9 min)
(8) B Conc. 15% (0 min)→65% (5.01–7 min)→15% (7.01–9 min)
(9) B Conc. 15% (0 min)→90% (5.01–7 min)→15% (7.01–9 min)

Total of 9 Gradient Patterns Executed

(2) showed the best results.

<<Scouted Analytical Conditions and Chromatogram>>

Mobile phase	: (A) Sodium phosphate buffer solution (pH 2.6)
	(B) Acetonitrile
Column	: Kinetex XB-C18 (50 mmL. × 3.0 mmI.D., 2.6 µm)
Time Program	: Conc. 5% (0 min)→65% (5 min)→90% (5.01-7 min)
Flow rate	: 1.0 mL/min
Injection volume	: 5 µL
Column temperature	: 40°C
Detection wavelength	: 260 nm (SPD-M20A)

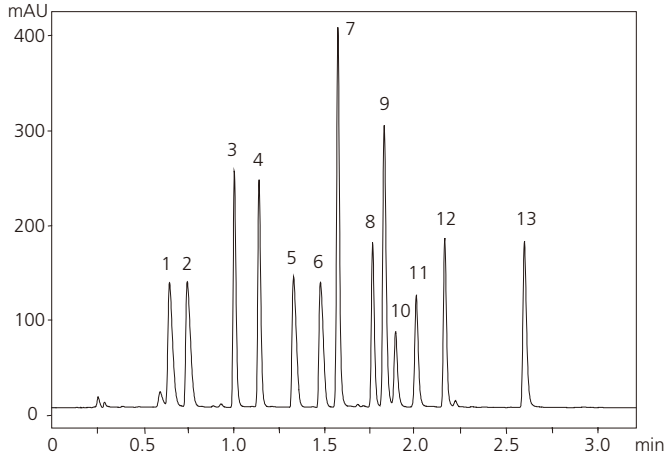


Fig. 10 Method Scouting Example for 13 Cephem Antibiotics

7. Effectiveness of Nexera Method Scouting

As described above, the Nexera Method Scouting system can resolve the rate-limiting factors (Fig. 2) associated with conventional method scouting. Its ease of use allows anyone, regardless of skill level. to easily, quickly, and without error conduct scouting of analytical conditions, making this system superbly effective for building analytical methods.

Further, introduction of "Nexera Method Scouting" will not only improve operational efficiency, it is a system that will effectively reduce solvent consumption and waste solution emissions, thereby reducing the environmental impact.

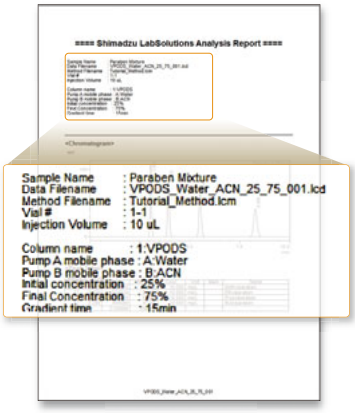


Fig. 11 Analytical Conditions Automatically Published in Output Report

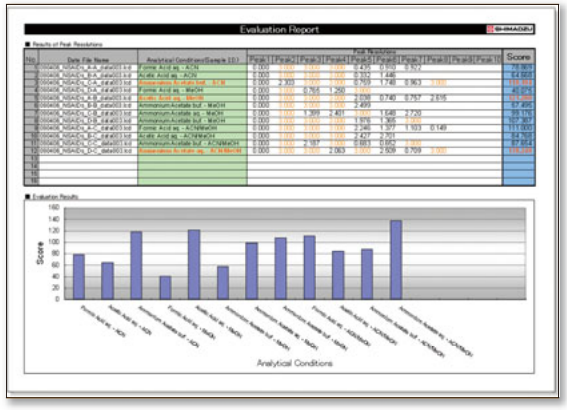


Fig. 12 Assessment of Scouting Results

Nexera Method Scouting

Nexera Method Scouting
+
LCMS-2020 (Single Quadrupole Mass Spectrometer)

Nexera Method Scouting
+
LCMS-8030 (Triple Quadrupole Mass Spectrometer)

Fig. 13 Nexera Method Scouting and Extension to LCMS

References

Technical Report: Improved R&D Efficiency Through Speedier Method Development (1) (C190-E128)
Technical Report: Improved R&D Efficiency Through Speedier Method Development (2) (C190-E130)
Technical Report: Improved R&D Efficiency Through Speedier Method Development (3) (C190-E159)

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