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Application News

High Performance Liquid Chromatograph Nexera™ Method Scouting System

High Efficiency Method Scouting for Small Molecule Drugs

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User Benefits

- Nexera Method Scouting System enables the labor-saving and improvement of efficiency in HPLC method development.
- Complicated analytical conditions changes can be automated by simple operations.
- The multi-data report function enables quantitative evaluation of scouting results and easy determination for the optimum conditions.

■ Introduction

The columns (stationary phase) and mobile phases used are parameters which have a large effect on retention and separation of target compounds in HPLC analysis. Conducting method scouting first to narrow the options of columns and mobile phases is an extremely effective approach for improving the efficiency of method development. However, in the process of method scouting, it is necessary to actually perform the analyses under various conditions in order to determine the optimum method for the target compounds from among the large number of possible mobile phases (buffer solution pH, salt concentration, organic solvent ratio) and columns (ODS, C8, phenyl). Moreover, considerable time, labor, and skill are also required in work such as the switching of columns and preparation/setting of the mobile phases.

This article introduces the Nexera Method Scouting System and the dedicated software "Method Scouting Solution", which were developed to automate the method scouting workflow, through a example of scouting conditions for simultaneous analysis of small molecule drugs.

■ Overview of System and Dedicated Software

Fig. 1 and Fig. 2 show the appearance of the Nexera Method Scouting System and the Method Scouting Solution screen, respectively. Although the appearance of the system is no different from that of standard high pressure gradient systems, analytical conditions can be scouted by automatically switching mobile phases and columns as shown in the flow path diagram in Fig. 2.

In the scouting of mobile phases, it is possible to automate the scouting of conditions for a maximum of 16 combinations of mobile phases by installing a reservoir switching valve on each pump. Automatic preparation of buffer solutions with different pH values is also possible by using the mobile phase blending function.

In the scouting of columns, the switching for a maximum of 12 columns (Fig. 2 shows the case of 6 columns) can be automated by installing a flow line switching valve in the column oven.

Scouting conditions can be easily set on the Method Scouting Solution software.

Fig. 1 Nexera Method Scouting System

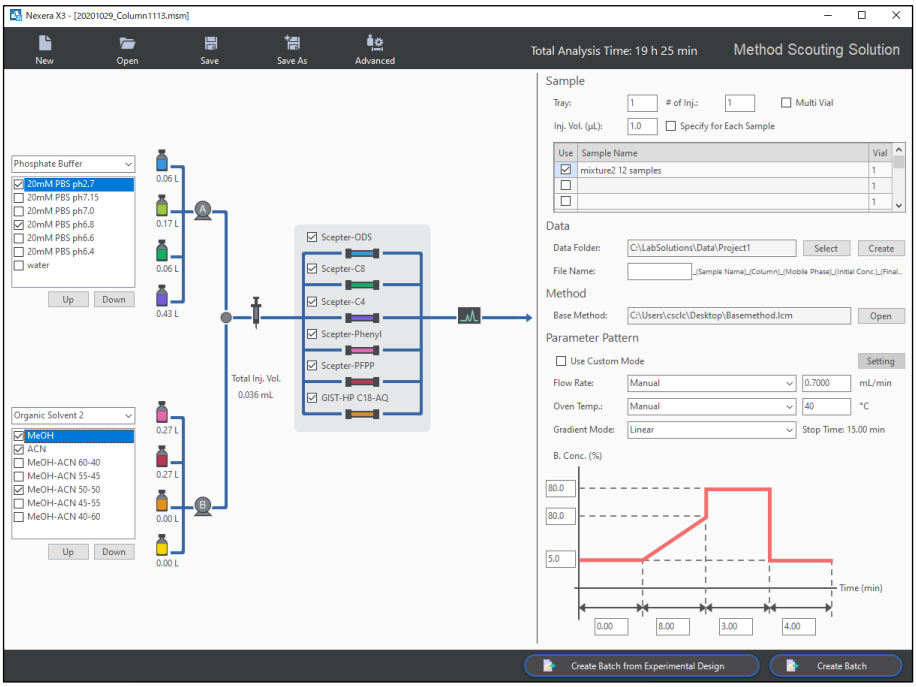

Fig. 2 Method Scouting Solution

■ Target Compounds

Table 1 shows the target compounds and their physical properties for this demonstration.

Table 1 Target Compounds

No.	Compound	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

■ Scouting Conditions and Chromatograms

Table 2 shows the mobile phase and column scouting conditions. Acidic and neutral buffer solutions were prepared automatically by using the blending function. Similarly, the online blending function was also used in mixing of the organic solvents. Blending and column settings can be set easily by selecting items preregistered in the Method Scouting Solution database. Manually changing the conditions require a great amount of work, including preparation/setting of the mobile phases, exchanges of the columns, and creating methods and sequences for scouting. However, all of this work can be automated by a simple operation when using the Nexera Method Scouting System and Method Scouting Solution.

In this article, scouting of a total of 36 sets of analytical conditions, consisting of combinations of 2 types buffer solutions, 3 types of organic solvents, and 6 types of columns, was carried out automatically.

The chromatograms for each column are shown in Fig. 3 to Fig. 8. Because quinidine and acetylsalicylic acid contain impurities, a maximum of 14 peaks were detected. Large differences in retention and separation can be confirmed, depending on the column, the pH of the mobile phase, and the composition of the organic solvent.

Table 2 Mobile Phase and Column Scouting Conditions

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 = 1 : 1
  - 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 condition:
- 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 buffer solutions were prepared automatically by online blending of the following solvents.

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 solvents were prepared automatically by online blending of the following solvents.

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

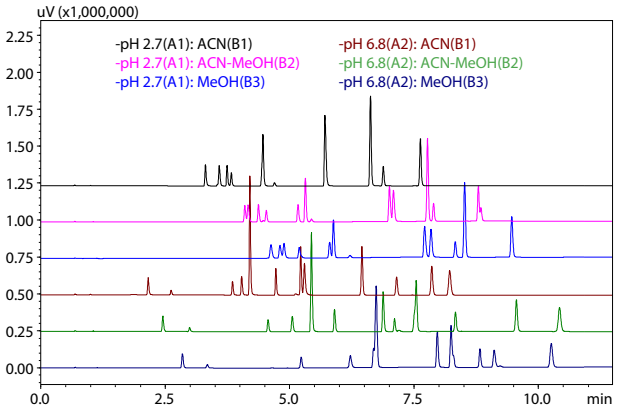

Fig. 3 Chromatograms of Shim-pack Scepter C18-120

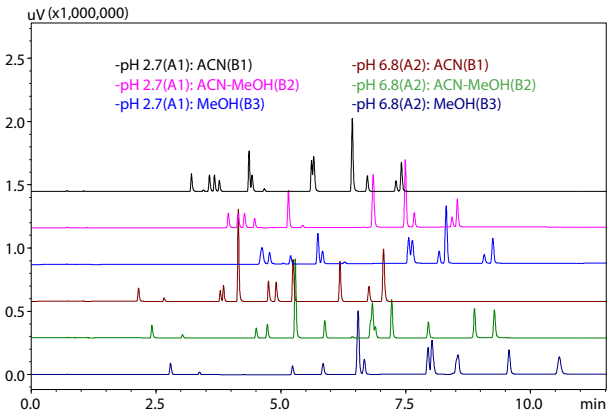

Fig. 4 Chromatograms of Shim-pack Scepter C8-120

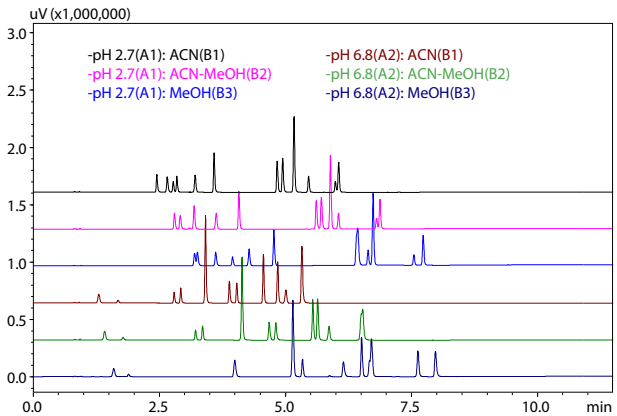

Fig. 5 Chromatograms of Shim-pack Scepter C4-120

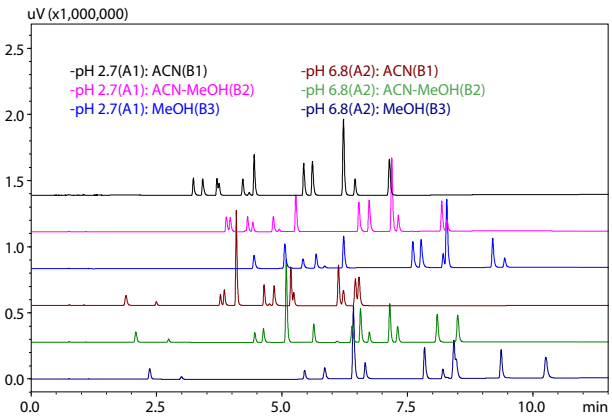

Fig. 6 Chromatograms of Shim-pack Scepter Phenyl-120

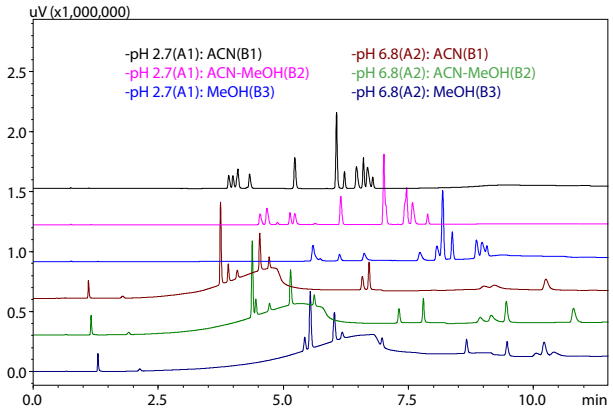

Fig. 7 Chromatograms of Shim-pack Scepter PFPP-120

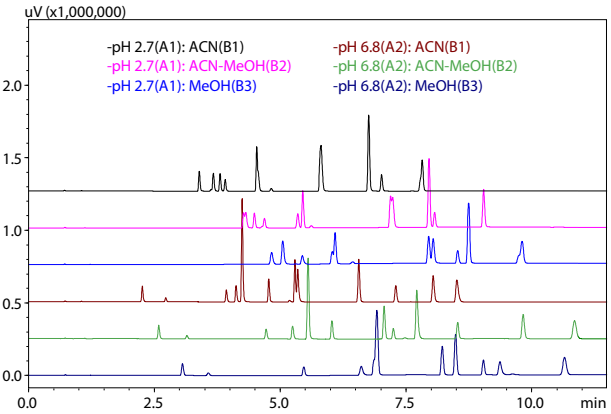

Fig. 8 Chromatograms of Shim-pack GIST C18 AQ

■ Quantitative Evaluation of Chromatograms

Since the number of chromatograms obtained is equal to the number of scouting conditions, an evaluation to determine which sets of conditions achieve the desired separation is necessary. It takes a lot of time and effort to check and verify all of the chromatograms.

In this article, the results of separation (resolution) under each set of analytical conditions were evaluated quantitatively by using the following equation. The evaluation values were calculated by using the multi-data report function of LabSolutions™ based on the data file acquired by scouting.

$$E = P \times (Rs1 + Rs2 + \ldots RsP) \quad \cdots \quad (1)$$

The evaluation value (E) is calculated using the product of the number of detected peaks (P) and resolution (Rs; however, with an upper limit of 3.0).

The multi-data report function can automatically extract the parameters used in the evaluation, such as the resolution of chromatograms, obtained from scouting and carry out spreadsheet calculations. The calculation results can be displayed automatically by outputting on a report template prepared in advance.

Fig. 9 shows a bar graph of the evaluation values for each set of scouting conditions. In this way, the optimum analytical conditions which provide the best separation can be judged visually and verified easily.

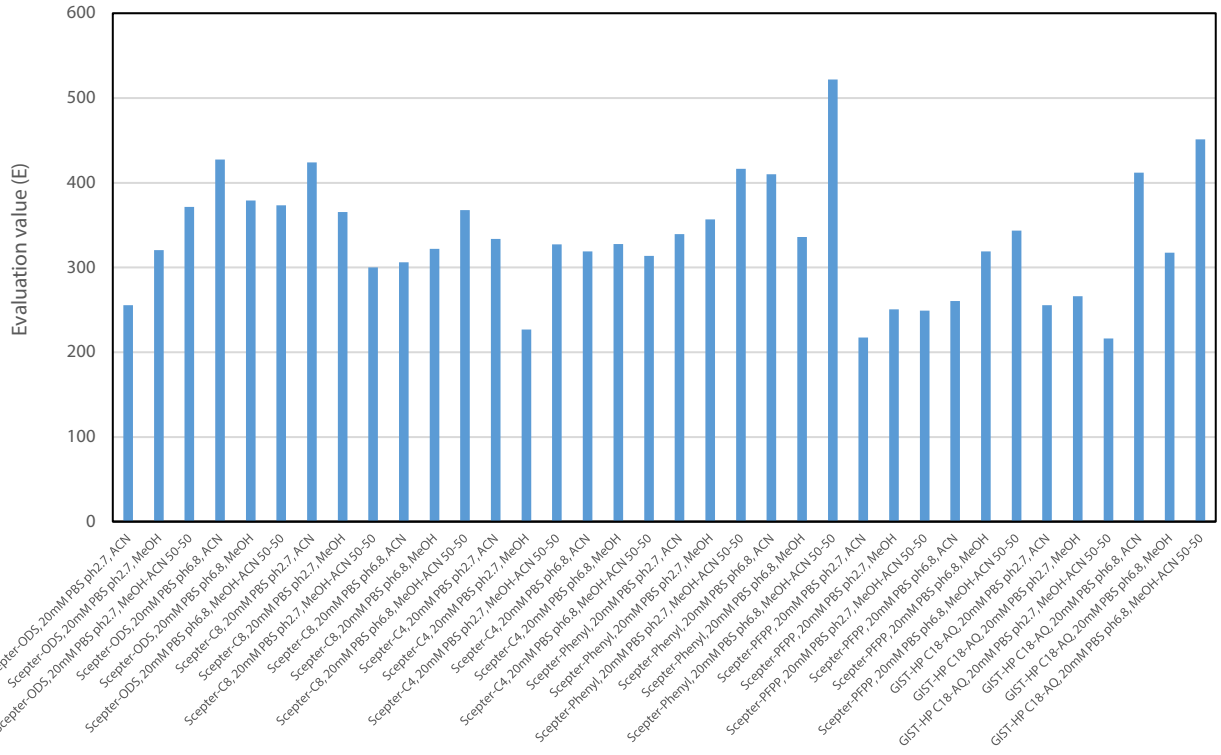

Fig. 9 Analytical Conditions and Evaluation Values

■ Scouting Results

Table 3 shows the analytical conditions with the highest evaluation value obtained by the multi-data report function. Fig. 10 shows the chromatogram obtained under the optimum analytical conditions, and Table 4 shows the peak parameters.

Because separation with resolution of 2.3 or more was obtained for all peaks, the scouting was completed by these analytical conditions. Even if scouting results show insufficient separation, the optimization of gradient conditions, column temperature, and other parameters can be carried out by using the combination of the column with the best separation and the mobile phase.

After the optimum analytical conditions were determined, the peaks were identified by analyzing the standards of each compound.

Table 3 Analytical Conditions with Highest Evaluation Values

Mobile phase:	
Pump A	Buffer
A2	20 mmol/L (Sodium) phosphate buffer (pH 6.8)
Pump B	Organic solvent
B2	Acetonitrile / Methanol = 1 : 1
Column:	
4	Shim-pack Scepter Phenyl-120 (100 mm × 3.0 mm I.D., 1.9 µm)
Analytical condition:	
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)

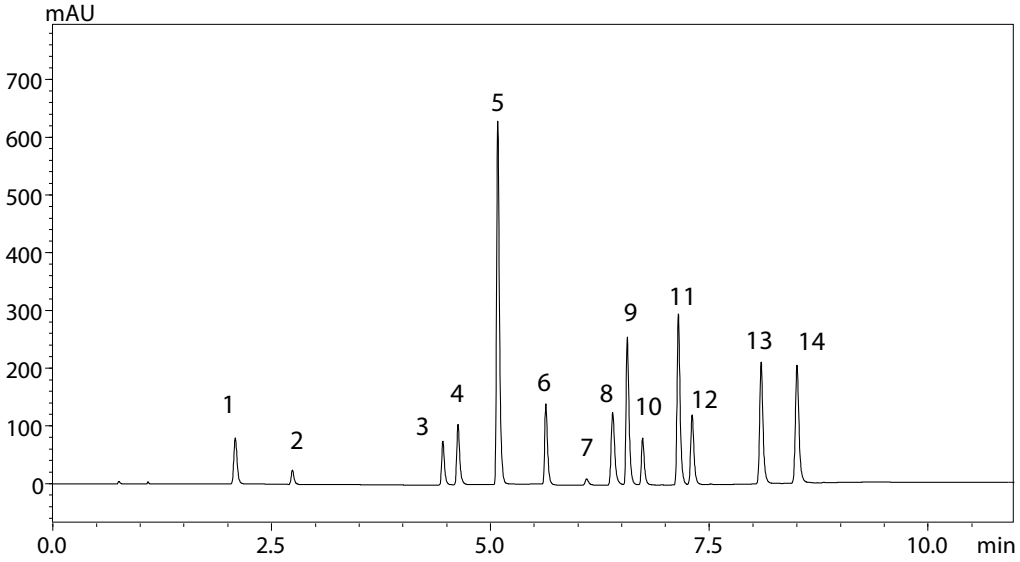

Fig. 10 Chromatogram Obtained with Optimum Analytical Conditions

Table 4 Peak Parameters Obtained with Optimum Analytical Conditions

Peak No.	Compound	R.T. (min)	Area	Height	Resolution (USP)	Symmetry factor
1	Acetylsalicylic acid	2.085	207910	79624	--	1.243
2	Impurity of acetylsalicylic acid	2.736	54637	24698	10.0	1.367
3	Antipyrine	4.456	169100	75912	28.7	1.378
4	Metoclopramide	4.629	251454	104804	2.8	1.424
5	(S)-(+)-Naproxen	5.083	1408900	628679	7.3	1.493
6	Probenecid	5.632	317401	139466	9.0	1.475
7	Impurity of quinidine	6.099	31171	10936	6.9	1.47
8	Quinidine	6.397	364149	125731	4.0	1.378
9	Diclofenac sodium	6.563	612211	256567	2.4	1.509
10	Indometacin	6.739	190695	81710	2.8	1.401
11	Papaverine hydrochloride	7.147	709025	295553	6.4	1.385
12	Lidocain	7.304	317027	120963	2.3	1.294
13	Dibucaine hydrochloride	8.093	570512	211125	11.2	1.296
14	Amitriptyline hydrochloride	8.501	593951	204085	5.5	1.326

■ Conclusion

This article introduced an automatic method scouting workflow through an example of the method scouting for a simultaneous analysis of small molecule drugs. The results showed that the separation patterns of compounds with different physical properties, such as logP and pKa, differ greatly due to the influence of the mobile phase and stationary phase.

The combination of the column and mobile phase used in an analysis has a large impact on separation/retention of the target compounds. Method scouting, by comprehensively changing the combinations of columns and mobile phases and confirming their separation performance, is effective in the initial stage of method scouting. The Nexera method scouting system and the dedicated software "Method Scouting Solution " provide automatic method creation and data acquisition, greatly reducing the labor and improving the efficiency of HPLC method development.

A quantitative evaluation of the separation status of chromatograms obtained under each set of analytical conditions is also possible by using the multi-data report function of LabSolutions. The multi-data report function is an effective tool for narrowing the optimum analytical conditions, as the scouting results can be visualized in graph form.

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