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Application News

High Performance Liquid Chromatograph i-Series／LC-2060C 3D

Method Scouting for Simultaneous Analysis of Twelve Tar Dyes

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User Benefits

- Batch schedules for scouting different analytical conditions can be created automatically using Method Scouting Solution.
- Multiple mobile phases and columns can be switched automatically, enabling the effective use of nighttime and holidays.
- Data obtained from the method scouting can be evaluated quantitatively using a multi-data report function which allows rapid determination of the optimal conditions.

■ Introduction

In HPLC analysis, column and composition of mobile phase greatly affect the retention and the separation of the target compounds. Many mobile phase compositions of different pH, salt concentration and organic solvent ratio need to be confirmed to determine the optimal analytical conditions for the target compounds. Columns with different stationary phases (ODS, C8, Phenyl, etc.) should also be considered. It requires considerable labor, time, and skill.

By adding a flow line switching valve to the integrated HPLC i-Series, up to six columns can be used to search for analytical conditions (method scouting) in combination with four mobile phase solvents.

The system also has a mobile phase blending function that can automatically mix up to four solvents at any desired ratio. With only a few solvents prepared in advance, the optimal analytical conditions for the target compounds can be quickly discovered.

This article introduces a method scouting workflow of the simultaneous analysis of twelve tar dyes using i-Series method scouting system.

■ Scouting for Analytical Conditions of Simultaneous Analysis

Fig. 1 shows the appearance of i-Series method scouting system, and Fig. 2 shows its flow path diagram . A flow line switching valve for switching the column is built in the column oven.

Table 1 shows the analytical conditions, and Table 2 shows the compound names of the twelve tar dyes used in this article. Six columns with different stationary phases were used. Two types of aqueous solutions and two types of organic solvents were set as mobile phases. And the respective third aqueous and organic mobile phases were prepared using the mobile phase blending function. Totally nine types of mobile phases were used for the scouting. Using the above six types of columns and nine mobile phases, a total of 6 x 9 = 54 analytical conditions were automatically evaluated.

Table 1 Analytical Conditions

System	: LC-2060C 3D
Column	: (1)Shim-pack Scepter™ C18-120*1 (50 mm x 3.0 mm I.D., 1.9 µm) (2)Shim-pack Scepter C8-120*2 (50 mm x 3.0 mm I.D., 1.9 µm) (3)Shim-pack Scepter Phenyl-120*3 (50 mm x 3.0 mm I.D., 1.9 µm) (4)Shim-pack Scepter C4-300*4 (50 mm x 3.0 mm I.D., 1.9 µm) (5)Shim-pack Scepter PFPP-120*5 (50 mm x 3.0 mm I.D., 1.9 µm) (6)Shim-pack™ GIST C18-AQ HP*6 (50 mm x 3.0 mm I.D., 1.9 µm)
Vial	: SHIMADZU LabTotal™ Vial for LC 1.5 mL, Glass*7
Mobile phase A	
Aqueous mobile phase:	A1: 10 mmol/L Acetic acid aqueous solution A2: 10 mmol/L Ammonium acetate aqueous solution A3: A1/A2=50:50 Ammonium acetate buffer solution prepared by blending function
Mobile phase B	
Organic mobile phase:	B1: Acetonitrile(ACN) B2: Methanol(MeOH) B3: B1/B2=50:50 ACN-MeOH prepared by blending function
Flow rate	: 0.7 mL/min
Column temp.	: 40 ˚C
Injection volume	: 2 µL
Detection	: 254 nm (LC-2060 PDA)
Time program	: B.Conc 5%(0 min)→90%(5 min)→90%(5.01-7 min)→5%(7.01-10 min)

*1. P/N: 227-31013-01, *2. P/N: 227-31034-01
*3. P/N: 227-31064-01, *4. P/N: 227-31176-01
*5. P/N: 227-31054-01, *6. P/N: 227-30808-01
*7. P/N: 227-34001-01

Fig. 1 Appearance of i-Series Method Scouting System

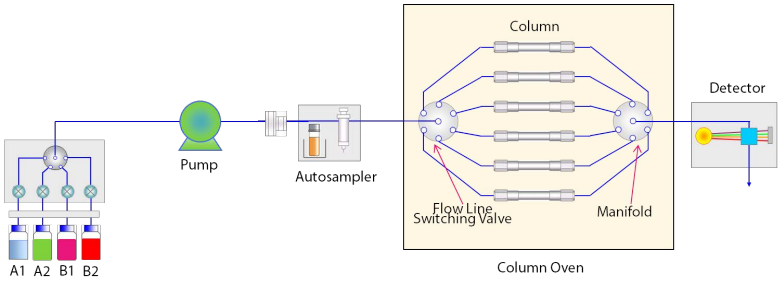

Fig. 2 Flow Path Diagram of i-Series Method Scouting System

Table 2 Compound Names of Twelve Tar Dyes

Compound name
Y4 (Tartrazine)
R2 (Amaranth)
B2 (Indigo Carmine)
R102 (New Coccine)
Y5 (Sunset Yellow FCF)
R40 (Allura Red AC)
G3 (Fast Green FCF)
B1 (Brilliant Blue FCF)
R3 (Erythrosine)
R106 (Acid Red)
R104 (Phloxine B)
R105 (Rose Bengal)

■ Settings of Method Scouting Solution

The batch file for the method scouting was created using the dedicated software Method Scoring Solution. Fig. 3 shows the main window of Method Scouting Solution. The batch can be created as follows, (1) Select the mobile phases and columns that have been preregistered in the database. (2) Enter the sample information. (3) Set the analytical conditions such as gradient mode. Isocratic, linear gradient, multilinear gradient and stepwise gradient mode can be selected, this time linear gradient mode was used. (4) Click the Create Batch button to create a batch file. By using the batch file, various analytical conditions can be evaluated automictically.

■ Result of Method Scouting

Fig. 4~9 show the chromatograms obtained from the scouting. Since R3 (Erythrosine) contains an impurity, up to 13 peaks were detected.

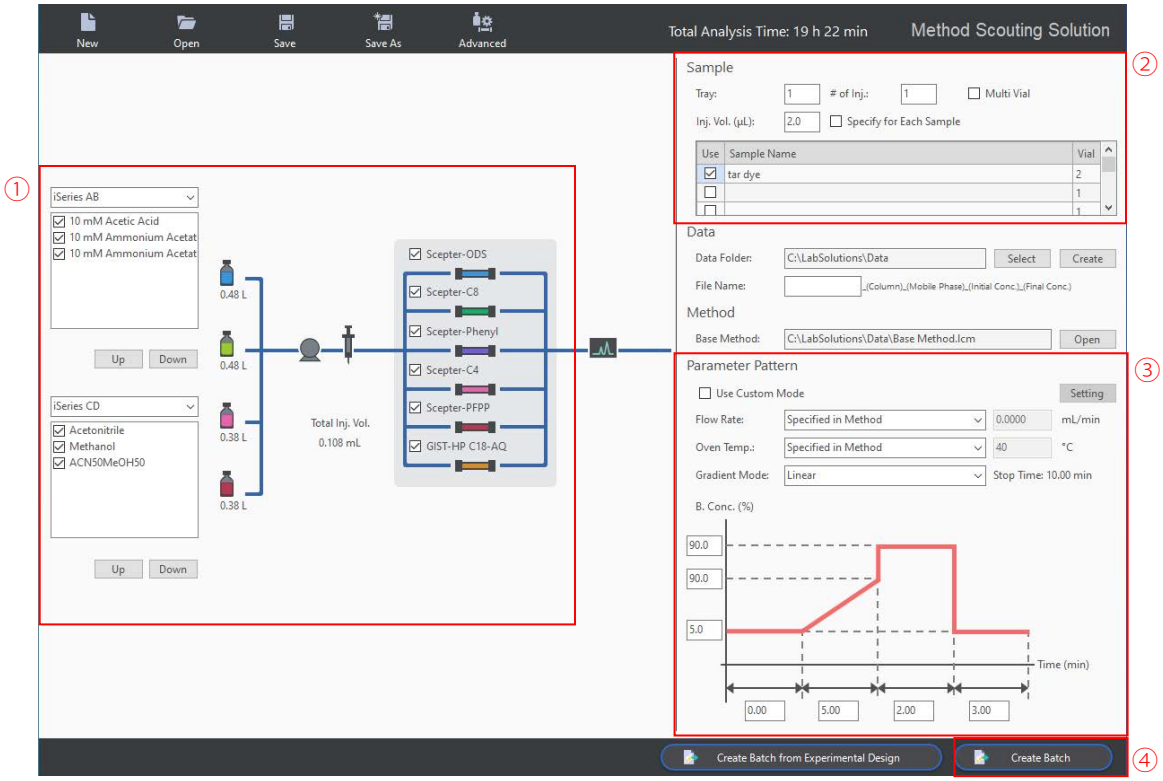

Fig. 3 Main Window of Method Scouting Solution

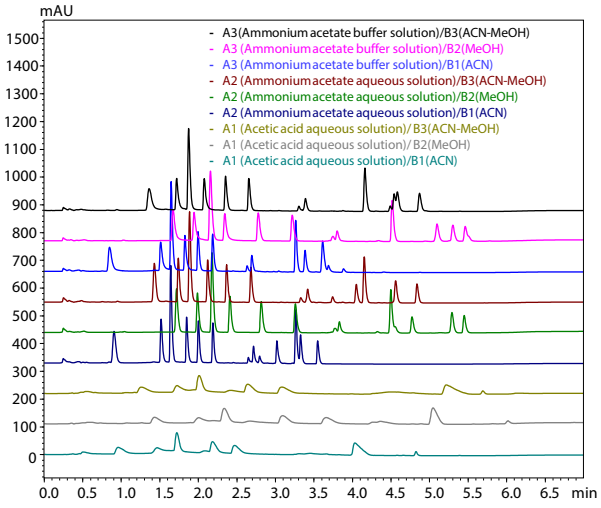

Fig. 4 Chromatograms of Shim-pack Scepter C18-120 (1)

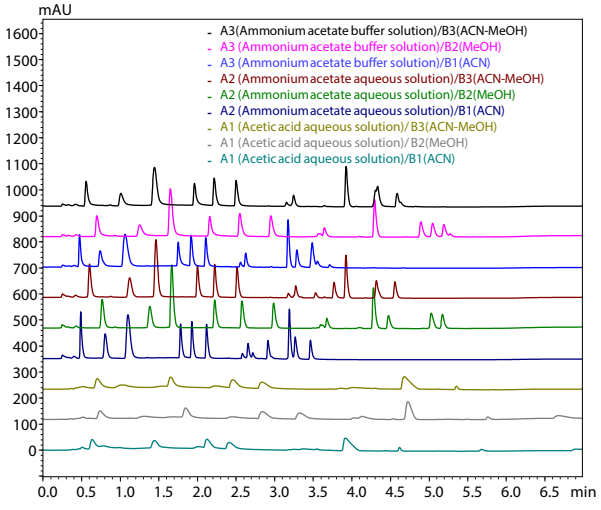

Fig. 5 Chromatograms of Shim-pack Scepter C8-120 (2)

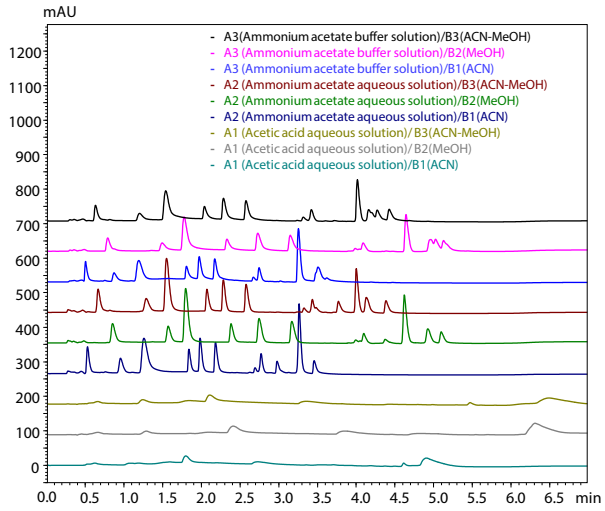

Fig. 6 Chromatograms of Shim-pack Scepter Phenyl-120 (3)

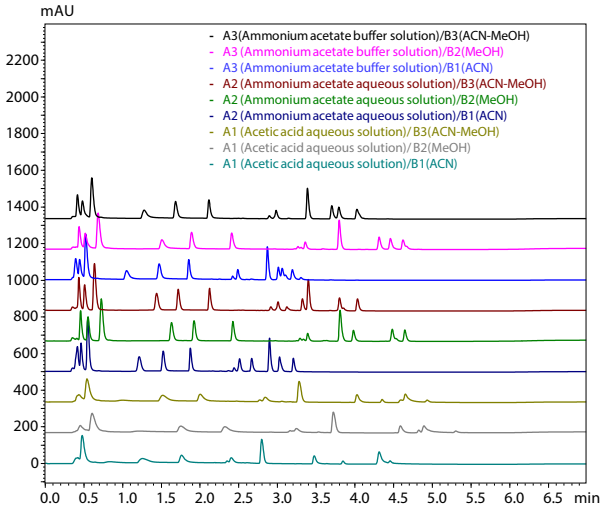

Fig. 7 Chromatograms of Shim-pack Scepter C4-300 (4)

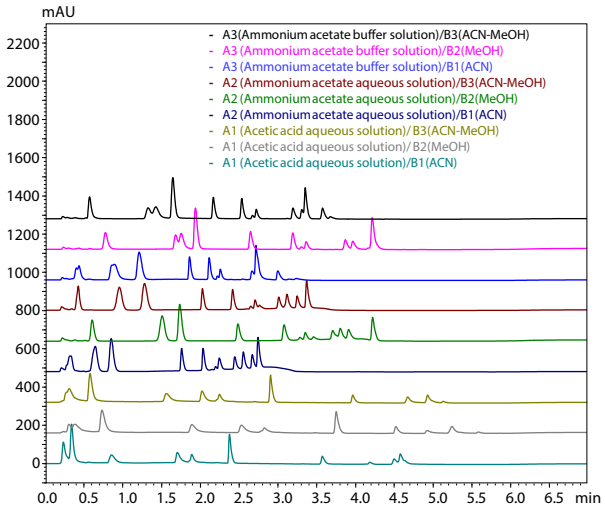

Fig. 8 Chromatograms of Shim-pack Scepter PFPP-120 (5)

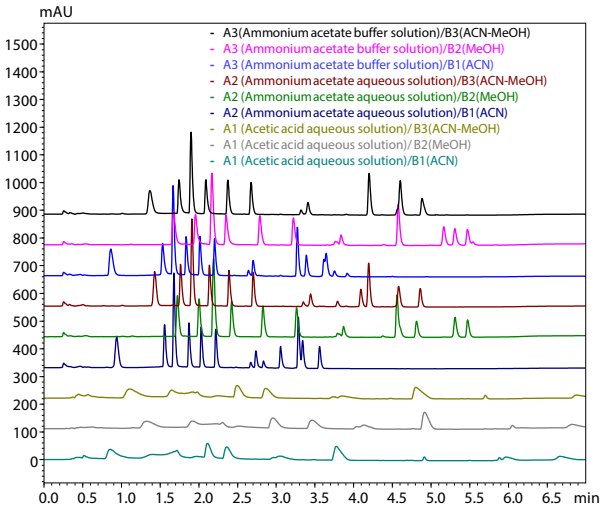

Fig. 9 Chromatograms of Shim-pack GIST C18-AQ HP (6)

Since it is difficult to determine which conditions achieve the best separation from verifying the chromatograms, the multi-data report function* was used to quantitatively evaluate the separation of the target compounds.

The following formula was used for rating, reflecting the resolution status.

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

The evaluation value (E) is calculated using the product of the number of peaks detected (P) and the sum of resolutions (Rs, upper limit: 3.0).

* Multi-data Report is a feature of the LabSolutions™ DB/CS.

Fig. 10 shows the result of the evaluation represented as the bar graphs. High rating provides large bar height, resulting in improved separation. Fig. 11 shows the top ten ratings of the conditions out of the 54 conditions that were evaluated this time. Thus, the optimal combination of mobile phase and column can be easily determined using the multi-data report function.

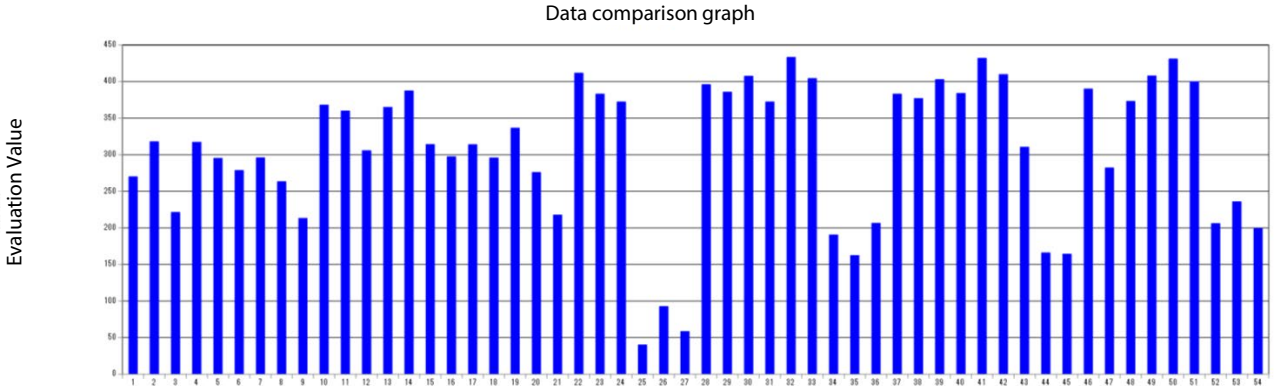

Fig. 10 Result of Evaluation Values obtained using Multi-data Report

Evaluation Report

Rank	Datafile name	Evaluation value	Number of peaks	Number of separated peaks
1	210121_Scepter-ODS_10 mM Ammonium Acetate_ACN50MeOH50_5_90_93.lcd	433.164	13	13
2	210121_Scepter-C8_10 mM Ammonium Acetate_ACN50MeOH50_5_90_96.lcd	431.869	13	12
3	210121_GIST-HP C18-AQ_10 mM Ammonium Acetate_ACN50MeOH50_5_90_108.lcd	430.948	13	13
4	210121_Scepter-Phenyl_10 mM Ammonium Acetate_Methanol_5_90_81.lcd	411.636	13	8
5	210121_Scepter-C8_10 mM Ammonium Acetate_Acetonitrile_5_90_60.lcd	409.751	13	13
6	210121_GIST-HP C18-AQ_10 mM Ammonium Acetate_Methanol_5_90_90.lcd	407.888	13	9
7	210121_Scepter-ODS_10 mM Ammonium_Acetate Buffer_Acetonitrile_5_90_111.lcd	407.288	13	13
8	210121_Scepter-ODS_10 mM Ammonium Acetate_Acetonitrile_5_90_57.lcd	404.318	13	13
9	210121_Scepter-C8_10 mM Ammonium_Acetate Buffer_Acetonitrile_5_90_114.lcd	402.741	13	13
10	210121_GIST-HP C18-AQ_10 mM Ammonium Acetate_Acetonitrile_5_90_72.lcd	399.946	13	13

Fig. 11 Ranking of Evaluation Values Using the Multi-data Report Function (top ten conditions)

■ Optimal Conditions for Simultaneous Analysis of Twelve Tar Dyes

Table 3 shows the analytical conditions that gave the highest rate in the multi-data report, and Fig. 12 shows the chromatogram obtained under those conditions. The retention time, peak area, peak height, and resolution to the previous peak of each compound are shown in Table 4. Since the resolution to the previous peak of each compound was 1.5 or more. The conditions in Table 3 were determined as the optimum.

Table 3 Analytical Conditions with Highest Evaluation Value

Column	: Shim-pack Scepter C18-120 (50 mm x 3.0 mm I.D., 1.9 µm)
Mobile phase	: A: 10 mmol/L Ammonium acetate aqueous solution B: Acetonitrile/methanol=50:50
Flow rate	: 0.7 mL/min
Column temp.	: 40 ˚C
Injection volume	: 2 µL
Detection	: 254 nm
Time program	: B.Conc 5%(0 min)→90%(5 min)→80% (5.01-7 min) →5%(7.01-10 min)

■ Conclusion

The analytical conditions of simultaneous analysis of twelve tar dyes were efficiently developed using the integrated HPLC i-Series.

The method scouting workflow introduced in this article was able to greatly reduce the time and effort required to switch between multiple mobile phases and columns, prepare mobile phase, set conditions, perform analysis, and evaluate results, allowing anyone to develop analytical conditions easily regardless of experience. In addition, since no human operation was required during the scouting batch execution, analytical conditions was able to be developed effectively during the nighttime and holidays.

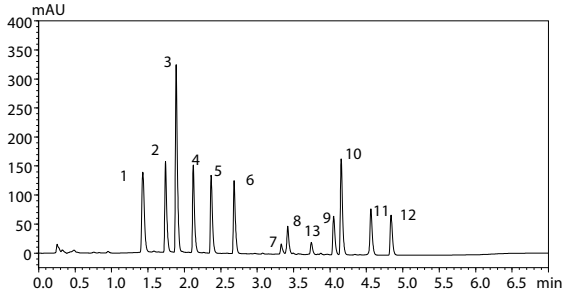

Fig. 12 Chromatogram Obtained with the Optimal Analytical Conditions (50 mg/L each)
See Table 4 for the peak numbers. 13: Impurity of R3 (Erythrosine)

Table 4 Peak Parameters Obtained with Optimal Analytical Conditions

No.	Compound name	Retention time (min)	Area	Height (USP)	Resolution (USP)
1	Y4 (Tartrazine)	1.429	330507	137921	-
2	R2 (Amaranth)	1.741	289011	156399	5.33
3	B2 (Indigo Carmine)	1.888	596078	322442	2.83
4	R102 (New Coccine)	2.123	273436	150807	4.57
5	Y5 (Sunset Yellow FCF)	2.368	249399	134121	4.76
6	R40 (Allura Red AC)	2.683	235599	125620	5.96
7	G3 (Fast Green FCF)	3.331	30969	17154	12.27
8	B1 (Brilliant Blue FCF)	3.419	90527	47121	1.64
9	R3 (Erythrosine)	4.051	133815	66398	5.57
10	R106 (Acid Red)	4.155	330115	165064	1.85
11	R104 (Phloxine B)	4.560	172510	78862	6.99
12	R105 (Rose Bengal)	4.837	145987	68801	4.66

01-00137-EN First Edition: Nov. 2022

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