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C190-E130

Improved R&D Efficiency Through Speedier Method Development (2)

Technical Report vol.34

1. Introduction

Typically, when developing an HPLC analytical method, it is necessary to optimize multiple parameters, including the analytical column phase and eluent type, and the column temperature, flow rate, etc. Regarding the eluent, it is important to determine the organic solvent, the type, concentration, and pH of any buffer modifier, as well as elution conditions based on the properties and structure of the target analytes. In other words, all of the specialized knowledge, technology and experience required in considering these various factors are an indication of the considerable amount of time and labor required for method development. Recently, great advances in analysis throughput have been made in the field of HPLC, significantly improving efficiency and productivity in the analytical operation. In particular, the use of columns with much smaller particle size packing material, which enables ultra-high speed analysis, has been gaining much attention.

In order to achieve improved efficiency in the method development process, Shimadzu has developed the “Prominence UFLC High-Speed Method Development System,” a high-speed system for automated solvent switching specifically designed to enable selection of eluent and optimize separation conditions under ultra-high speed conditions using an ultra-high speed analytical column. The resulting ultra-high speed analytical conditions accommodate conventional analytical conditions with a standard analytical column. This system will prove useful in applications such as the inter-departmental transfer of analytical methods during the drug development process, or when conventional analytical conditions are required at the analysis method application stage of the process.

An overview of this system was introduced in Technical Report vol. 33 “Improved R&D Efficiency Through Speedier Method Development (1).” In this report we introduce an example of the analytical method construction process for batch analysis of non-steroidal anti-inflammatory drugs.

2. Method Development System

Fig. 2 and Fig. 3 show two examples of method development systems that can be constructed using the Prominence system. The eluent switching system of Fig. 2 allows the user to select any of the prepared eluents (A-D on each pump) in any combination. This automated solvent switching enables a sequence to be run with multiple solvent combinations without shutting down the system and exchanging eluent bottles in between each run. The column-switching system of Fig. 3, on the other hand, is equipped with a valve for connecting up to 6 columns, allowing analysis to be conducted using any of those columns with a single mobile phase. With multiple columns on a valve, column switching is automatic and the user is not required to shut down the system, then disconnect and reconnect a different column for each run.

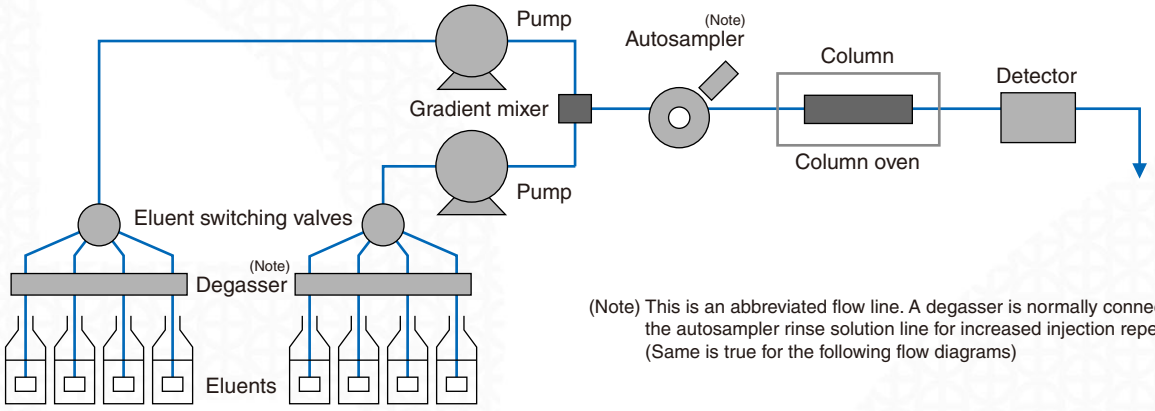
Fig. 1 Prominence x LCMS-2020 System

3. Hybrid Method Development System

The Prominence UFLC High-Speed Method Development System is a hybrid method development system with eluent-switching capability (Fig. 2) inline with both ultra-high speed and conventional HPLC columns (Fig. 5). Fig. 6 shows the flow diagram of this hybrid system, which can be used not only to optimize eluent selection and gradient conditions at ultra-high speed, but also to migrate the ultra-high speed analytical conditions to conventional analysis conditions.
The rationale for supporting the migration to conventional analysis is based on the assumption that when forwarding the analytical method to a process control section or quality management section located either on-site or at a consignment destination site, that section may not possess a system that supports ultra-high speed analysis, in which case the ultra-high speed analysis method must be converted to a conventional analysis method.
In order to combine the functionalities of ultra-high speed analysis with conventional analysis, this system is constructed such that a gradient mixer for ultra-high speed analysis and another gradient mixer for conventional analysis are connected respectively to an ultra-high speed column and a conventional column, and switching between the ultra-high speed analysis flow line and conventional analysis flow line is accomplished using 2 flow-line switching valves.
The ultra-high speed analysis flow line (flow line that includes a gradient mixer and column, both for ultra-high speed analysis) is used for examining the ultra-high speed analysis conditions, and the conventional analysis flow line (flow line that includes a gradient mixer and column, both for conventional analysis) is used for examining the conventional analysis conditions.
Two solvent delivery pumps are used, each with a 4-position switching valve serving as a solvent selector. Four types of aqueous eluents (pump A) and 4 types of organic eluents (pump B) provide 16 possible eluent combinations for continuous examination of analysis conditions. Regarding detectors, the SPD-M20A PDA (photodiode array) detector, which allows simultaneous multi-wavelength measurement and UV spectral measurement, together with an in-line LCMS-2020 mass spectrometer for mass-related measurement of target analytes, provide the improved peak identification accuracy that is desired. Furthermore, the ultra-high speed Shim-pack XR series column is used to ensure that the greatest possible time-savings are achieved in testing a wide range of analysis conditions.

4. Method Development Workflow

When this system is used, method development is accelerated according to the flow chart shown in Fig.4.
In Step 1, the chromatographic pattern is expressed numerically using a unique evaluation formula intended to optimize the eluent, and in Step 2, the gradient conditions are optimized based on the result of that evaluation. In Step 3, peak identification is conducted using the mass spectrometer (LCMS-2020), and with these Steps 1 – 3, the ultra-high speed analysis conditions are established.



(Note) This is an abbreviated flow line. A degasser is normally connected to the autosampler rinse solution line for increased injection repeatability. (Same is true for the following flow diagrams)

Fig. 2 Flow Diagram for Automated Eluent Switching System

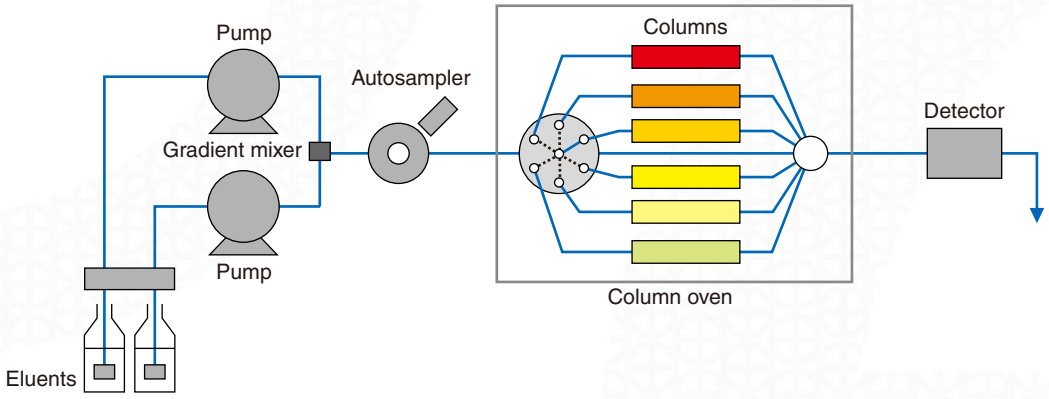


Fig. 3 Flow Diagram for Automated Column Switching System

Continuing in Step 4, the UFLC Method Transfer Program Ver. 2 is used to establish the conventional analysis conditions which will provide the same separation pattern, based on the established ultra-high speed analysis conditions.
In this report, we present the process used to establish the conditions for the simultaneous analysis of these 8 non-steroidal anti-inflammatory drugs: piroxicam, ketoprofen, indomethacin, diflunisal, ampiroxicam, diclofenac, etodolac, and flurbiprofen, as shown in Fig. 7.

5. Step 1: Eluent Optimization

In Step 1, the best combination of eluents under high-speed analysis conditions is examined.
For ultra-high speed analysis, we used the Shim-pack XR-ODS column (particle size 2.2 µm). For the eluents, we prepared 4 types of aqueous eluents and 3 types of organic solvent eluents according to those shown in Table 1, and by combining these in different ways, we examined 12 different combinations of eluents.
Since this step is the stage at which combinations of eluents are examined, we used gradient conditions like those underlined in Table 1 as a universal gradient elution method that would address a wide range of substance characteristics.

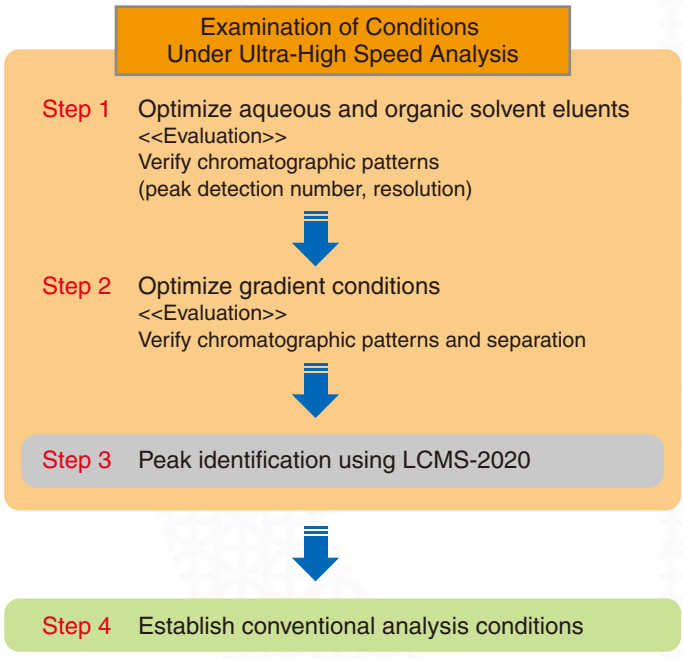


Fig. 4 Flow up to Method Development

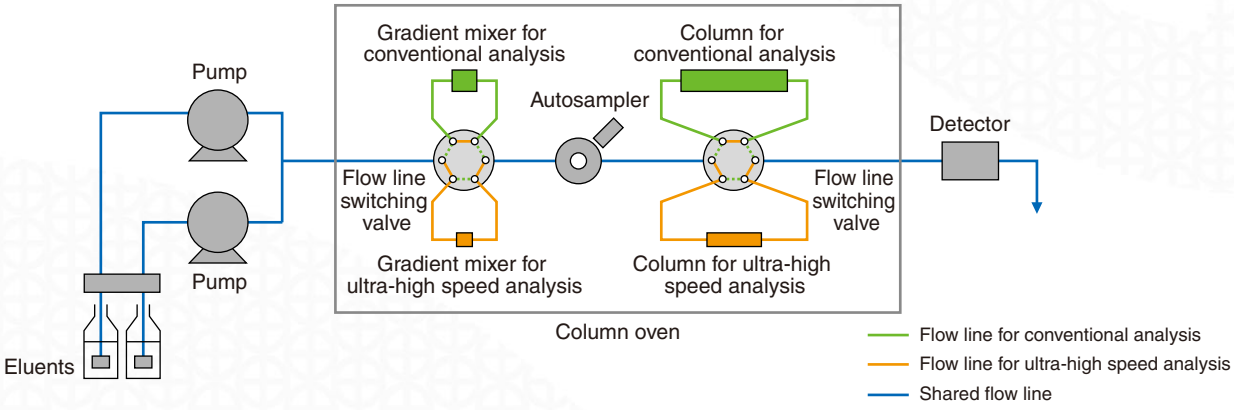


Fig. 5 Flow Diagram of Ultra-High Speed / Conventional Analysis Auto Switching System

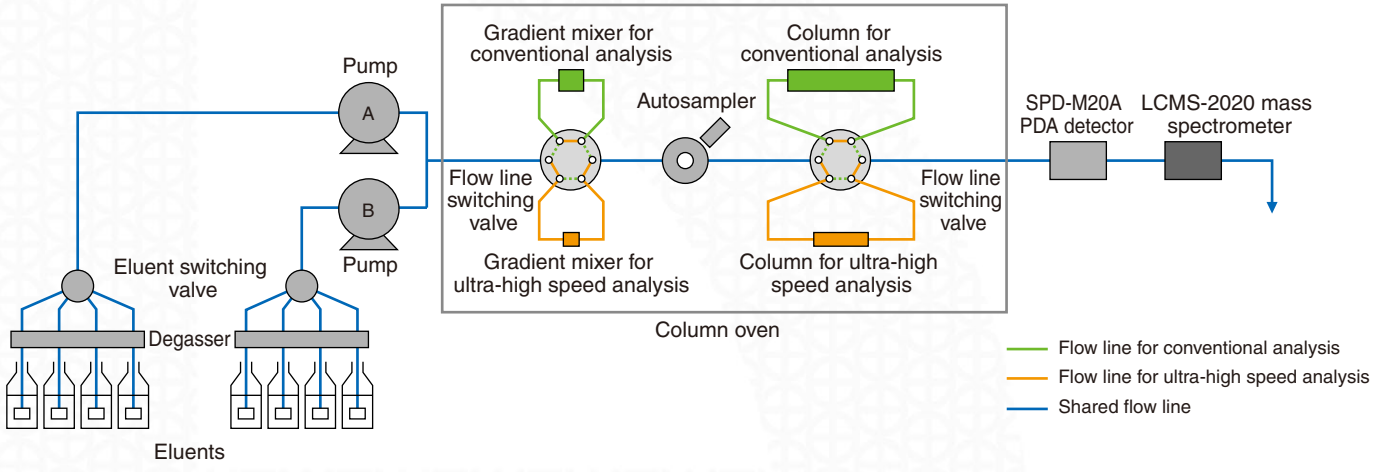


Fig. 6 Flow Diagram of Prominence UFLC High Speed Method Development System
<<Auto Switching System for High Speed / Conventional Analysis>>

5-1. Automated Analysis Functions

Before starting analysis under each set of eluent conditions, it is first necessary to conduct an eluent purge (replacement of eluent) and baseline stabilization. With this system, as shown in Fig. 8, manually opening the drain valve to perform the purge each time the eluent is replaced is unnecessary, because eluent replacement can be conducted automatically using the auto-purge function. Further, using the baseline check function, the baseline status is automatically evaluated, and if it is determined to be acceptable (stable baseline), the analysis is started automatically. The ability to examine multiple eluents without having to perform laborious manual operations allows truly efficient method development for large numbers of unknown samples.

5-2. Continuous Analysis Schedule

Fig. 9 shows the contents of a batch file used for continuous analysis to conduct examination of eluent conditions. After pumping each solvent system for about 15 minutes to allow sufficient time for stabilization to be attained, the baseline check function embedded in the batch program is executed to automatically determine the state of baseline stability. Directly following the baseline check, a non-sample-injection gradient analysis (gradient analysis conducted without sample injection) is conducted, and analyses with sample injection are repeated 3 times (n=3). This series of operations for each combination of eluents is taken as one set, and a total of 12 sets of eluent conditions are evaluated fully automatically as shown in the operation flow of Fig. 8.

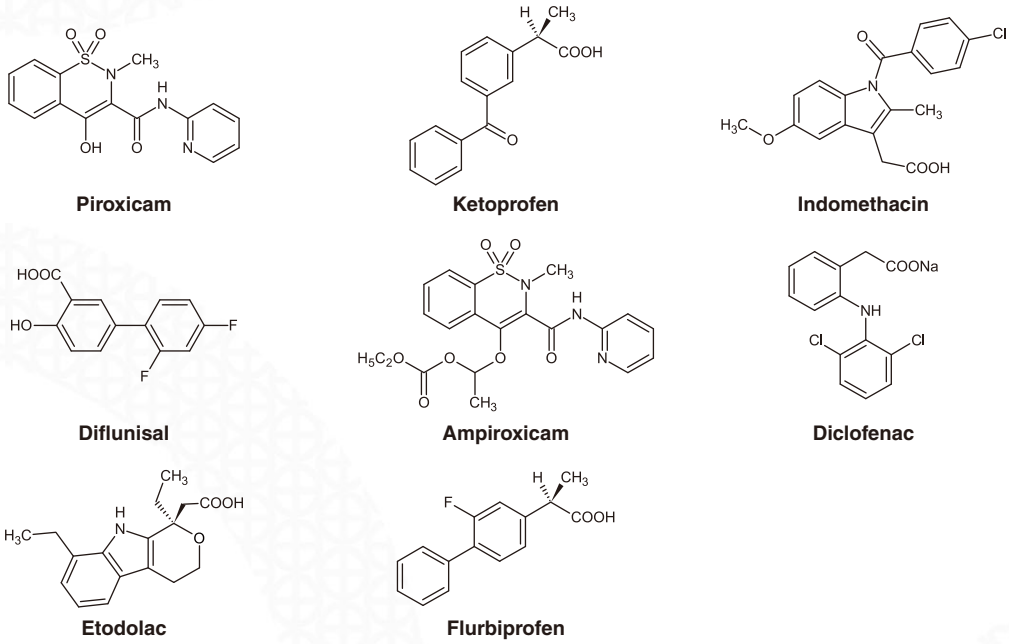


Fig. 7 Eight Non-Steroidal Anti-Inflammatory Drug Substances

Column	: Shim-pack XR-ODS (50 mm L. x 3.0 mm I.D., 2.2 µm)
Mobile Phase	: A a) 0.1 % (vol./vol.) Formic Acid-Water (pH 2.6)
	b) 0.1 % (vol./vol.) Acetic Acid-Water (pH 3.3)
	c) 10 mmol/L Ammonium Acetate Buffer (pH 4.7)
	d) 10 mmol/L Ammonium Acetate Solution (pH 6.6)
	B a) Acetonitrile
	b) Methanol
	c) Acetonitrile/Methanol = 50/50 (vol./vol.)
Time Program	: B.Conc. 10 % (0 min) → 95 % (5 - 8 min) → 10 % (8.01 - 10 min)
Flow Rate	: 1.0 mL/min
Injection Volume	: 2 µL
Temperature	: 40 °C
Detection	:
(1) SPD-M20A	
Absorbance	: 254 nm
Response	: 25 msec
(2) LCMS-2020	
Detection Mode	: ESI(+), ESI(-)
Scan Range	: m/z 100-1000

Aqueous eluents (4 types)

Organic solvent eluents (3 types)

Universal gradient elution method

Table 1 Analytical Conditions for Eluent Optimization

5-3. Chromatogram Evaluation

The analysis results are shown in Fig. 10. Simply displaying the 12 chromatograms side by side like this makes it difficult to judge which is associated with the best conditions, so some sort of indexing system is required for ranking these chromatograms.
The chromatograms could be ranked based on such factors as (1) how well the target analytes are separated, (2) how sufficiently the target analytes are retained, and (3) how good the peak shapes are, but in simultaneous analysis of multiple constituents, priority is generally given to how well the various analytes are separated, and evaluation is conducted using the number of detected peaks and resolution as parameters.
Thus, in this report, the method adopted for evaluating chromatograms using the number of detected peaks and resolution is:

Evaluation value = Number of detected peaks x Sum of peak resolution values

In other words, as is clear from the example of Fig 11, the ranking value for each chromatogram is a value obtained by multiplying “the number of detected analyte peaks” by the “total of the resolution values of those respective peaks.” Further, when the evaluation value is calculated, the resolution is set to the upper value. In Pattern 1 and Pattern 2 of Fig. 11, although the resolution of Pattern 2 is obviously better, the degree of separation between peaks 2 and 3 in Pattern 1 is extremely large, so despite the poor separation between peaks 1 and 2 and between 3 and 4, the calculated evaluation value of Pattern 1 is higher. To control such an overestimation, an upper limit is established for the resolution setting. In this report, the upper limit for resolution is set to “3,” so even if the resolution is 5 or 10, for example, it is treated as “3.”
As a result, a better ranking value will be assigned to the chromatogram that has a greater number of separated peaks.

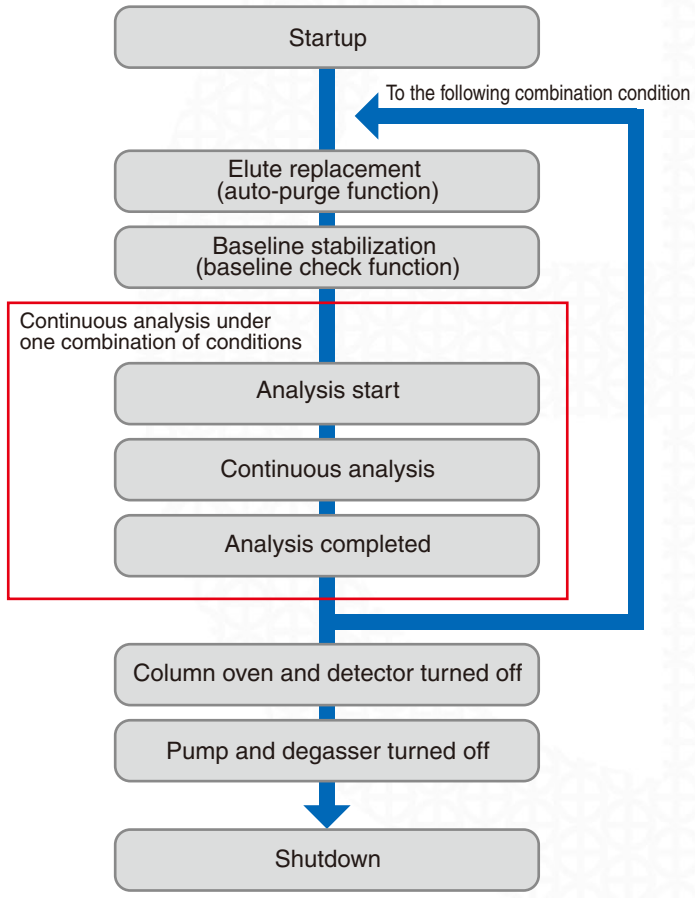


Fig. 8 Flow of Fully Automated Analysis

Folder : D¥tsukamoto¥MDSDemo

Analysis	System check	Auto-purge	Baseline check	Vial	Injection volume	Sample	Method file	Execution mode	Data file	Data comment
1	<COMMON O			-1	4		start_up.lcm	DL	System check .lcd	System check
2		☑		-1	4		autopurge_A-A.lcm	DL	Auto-purge 01.lcd	Auto-purge
3		☑		-1	4		autopurge_B-B.lcm	DL	Auto-purge 02.lcd	Auto-purge
4		☑		-1	4		autopurge_C-C.lcm	DL	Auto-purge 03.lcd	Auto-purge
5		☑		-1	4		autopurge_D-D.lcm	DL	Auto-purge 04.lcd	Auto-purge
6			☑	-1	4		stabilize_A-A.lcm	DL AQ DP	Formic acid aq-ACN_STB.lcd	Stabilization
7				-1	4		stabilize_A-A.lcm	DL AQ DP	Formic acid aq-ACN_00.lcd	Column conditioning
8				1	4	NSAIDs 8mix	analize_A-A.lcm	DL AQ DP	Formic acid aq-ACN_01.lcd	1st data
9				1	4	NSAIDs 8mix	analize_A-A.lcm	DL AQ DP	Formic acid aq-ACN_02.lcd	2nd data
10				1	4	NSAIDs 8mix	analize_A-A.lcm	DL AQ DP	Formic acid aq-ACN_03.lcd	3rd data
11			☑	-1	4		stabilize_A-B.lcm	DL AQ DP	Formic acid aq-MeOH_STB.lcd	Eluents & Stabilization
12				-1	4		stabilize_A-B.lcm	DL AQ DP	Formic acid aq-MeOH_00.lcd	Column conditioning
13				1	4	NSAIDs 8mix	analize_A-B.lcm	DL AQ DP	Formic acid aq-MeOH_01.lcd	1st data
14				1	4	NSAIDs 8mix	analize_A-B.lcm	DL AQ DP	Formic acid aq-MeOH_02.lcd	2nd data
15				1	4	NSAIDs 8mix	analize_A-B.lcm	DL AQ DP	Formic acid aq-MeOH_03.lcd	3rd data
16			☑	-1	4		stabilize_A-C.lcm	DL AQ DP	Acid aq-ACN+MeOH_STB.lcd	Eluents & Stabilization
17				-1	4		stabilize_A-C.lcm	DL AQ DP	Formic acid aq-ACN-MwOH_00.lcd	Column conditioning
18				1	4	NSAIDs 8mix	analize_A-C.lcm	DL AQ DP	Formic acid aq-ACN-MwOH_01.lcd	1st data
19				1	4	NSAIDs 8mix	analize_A-C.lcm	DL AQ DP	Formic acid aq-ACN-MwOH_02.lcd	2nd data
20				1	4	NSAIDs 8mix	analize_A-C.lcm	DL AQ DP	Formic acid aq-ACN-MwOH_03.lcd	3rd data
21			☑	-1	4		stabilize_B-A.lcm	DL AQ DP	Acetic acid aq-ACN_STB.lcd	Eluents & Stabilization
22				-1	4		stabilize_B-A.lcm	DL AQ DP	Acetic acid aq-ACN_00.lcd	Column conditioning
23				1	4	NSAIDs 8mix	analize_B-A.lcm	DL AQ DP	Acetic acid aq-ACN_01.lcd	1st data
24				1	4	NSAIDs 8mix	analize_B-A.lcm	DL AQ DP	Acetic acid aq-ACN_02.lcd	2nd data
25				1	4	NSAIDs 8mix	analize_B-A.lcm	DL AQ DP	Acetic acid aq-ACN_03.lcd	3rd data
26			☑	-1	4		stabilize_B-B.lcm	DL AQ DP	Acetic acid aq-MeOH_STB.lcd	Eluents & Stabilization

	stabilize_A-A.lcm	DL AQ DP	Formic acid aq-ACN_STB.lcd	Stabilization
	stabilize_A-A.lcm	DL AQ DP	Formic acid aq-ACN_00.lcd	Column conditioning
NSAIDs 8mix	analize_A-A.lcm	DL AQ DP	Formic acid aq-ACN_01.lcd	1st data
NSAIDs 8mix	analize_A-A.lcm	DL AQ DP	Formic acid aq-ACN_02.lcd	2nd data
NSAIDs 8mix	analize_A-A.lcm	DL AQ DP	Formic acid aq-ACN_03.lcd	3rd data

Solution pumped for stabilization + baseline check

Gradient without sample injection

Successive repeat analyses

Fig. 9 Continuous Analysis Batch File

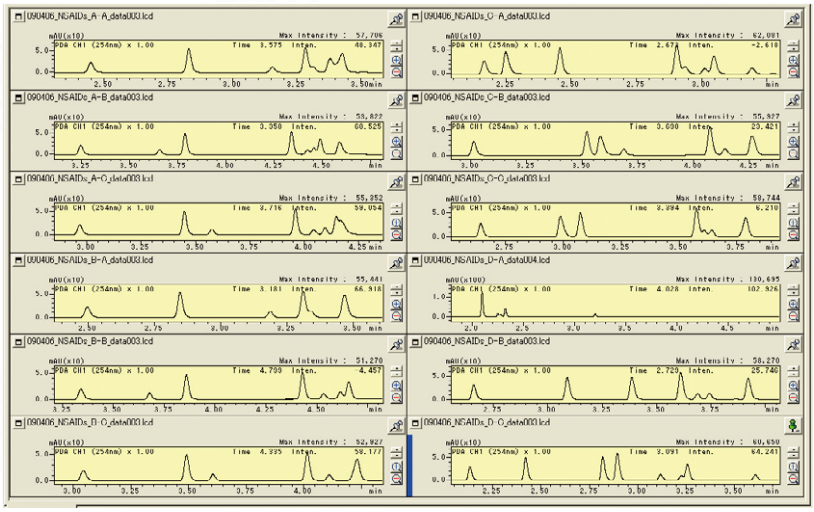
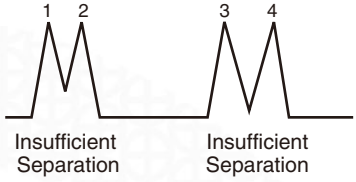


Fig. 10 Measurement Results for Eluent Combination Optimization

Pattern 1



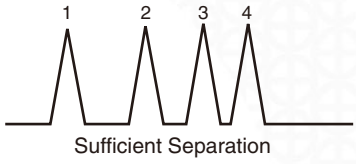
Peak	Resolution
1	–
2	1.1
3	8.0
4	1.4

Evaluation Value 42

Resolution (after setting upper limit)
–
1.1
3.0
1.4

Evaluation Value 22

Pattern 2



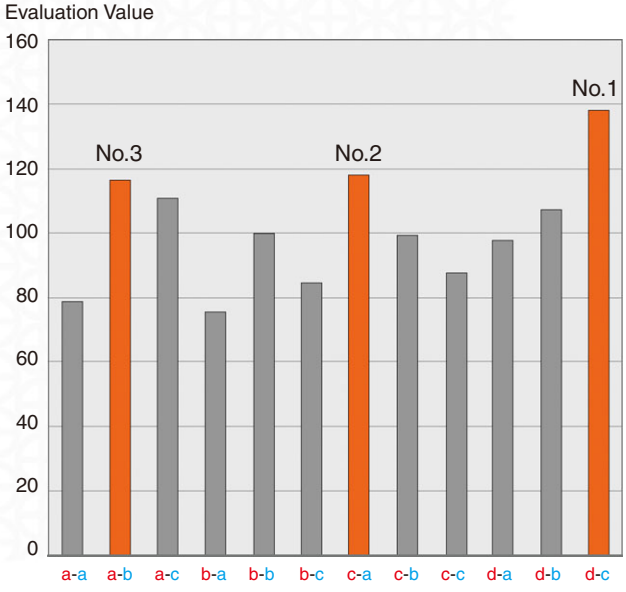
Peak	Resolution
1	–
2	4.0
3	2.5
4	2.2

Evaluation Value 35

Resolution (after setting upper limit)
–
3.0
2.5
2.2

Evaluation Value 31

Fig. 11 Evaluation Method Emphasizing Separation



[a-a] 0.1 % formic acid aq. - ACN
[a-b] 0.1 % formic acid aq. - MeOH
[a-c] 0.1 % formic acid aq. - ACN/MeOH

[b-a] 0.1 % acetic acid aq. - ACN
[b-b] 0.1 % acetic acid aq. - MeOH
[b-c] 0.1 % acetic acid aq. - ACN/MeOH

[c-a] 10 mmol/L acetate (ammonium) buf. - ACN
[c-b] 10 mmol/L acetate (ammonium) buf. - MeOH
[c-c] 10 mmol/L acetate (ammonium) buf. - ACN/MeOH

[d-a] 10 mmol/L ammonium acetate aq. - ACN
[d-b] 10 mmol/L ammonium acetate aq. - MeOH
[d-c] 10 mmol/L ammonium acetate aq. - ACN/MeOH

Organic solvent eluents

Aqueous eluents

Fig. 12 Evaluation of Eluent Combination Optimization

5-4. Measurement Results Examination Using Evaluation Value

Fig. 12 shows the evaluation values of the respective eluent combinations displayed graphically. It is clear from this that of the 12 patterns of eluent combinations, [d-c] has earned the highest evaluation value. The [c-a] and [a-b] combinations follow with relatively high evaluations.

Fig. 13 shows the chromatograms arranged in the order of their evaluation values. Utilizing the evaluation values for selecting the best conditions is an extremely useful and effective technique, as will become clear as the process proceeds.

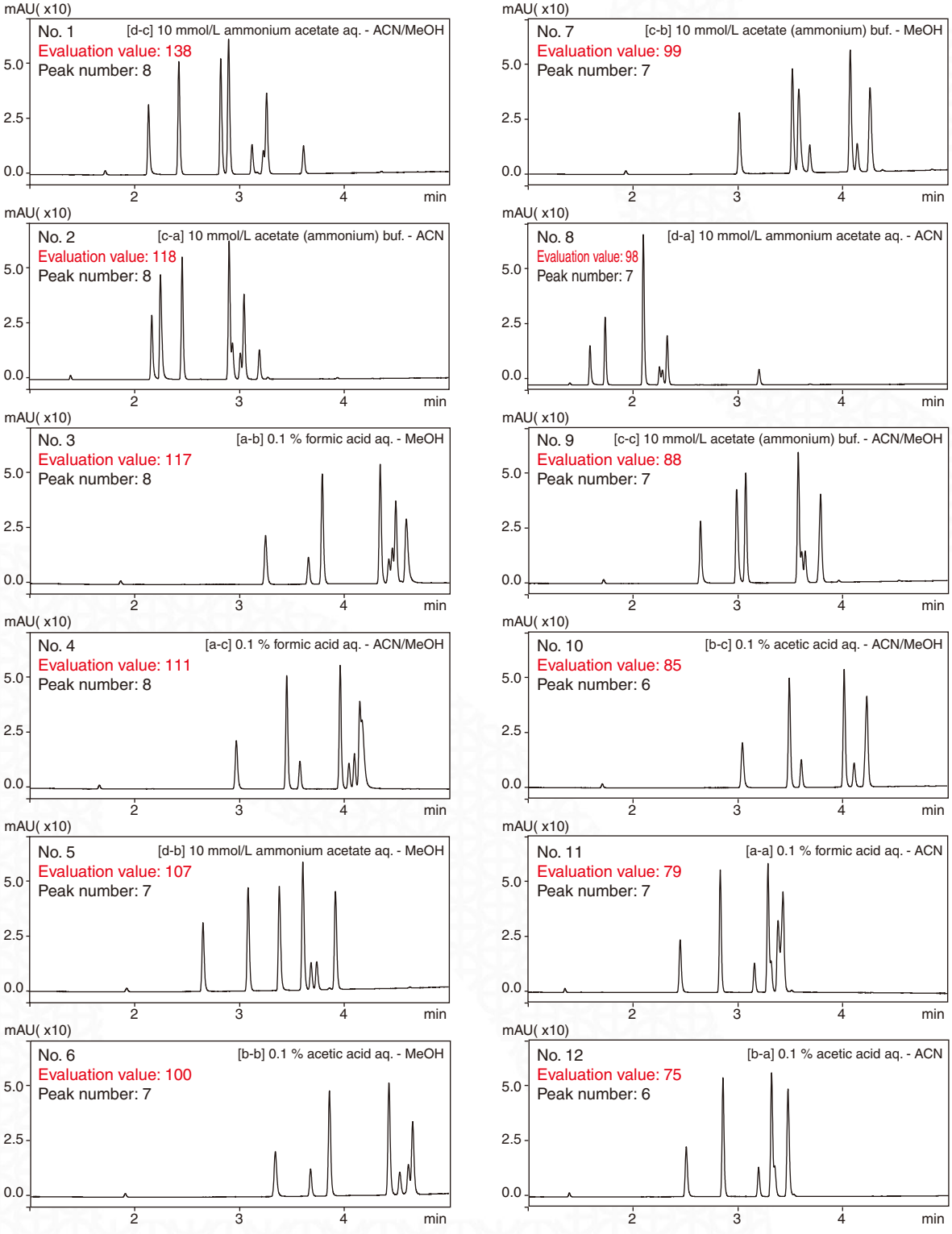


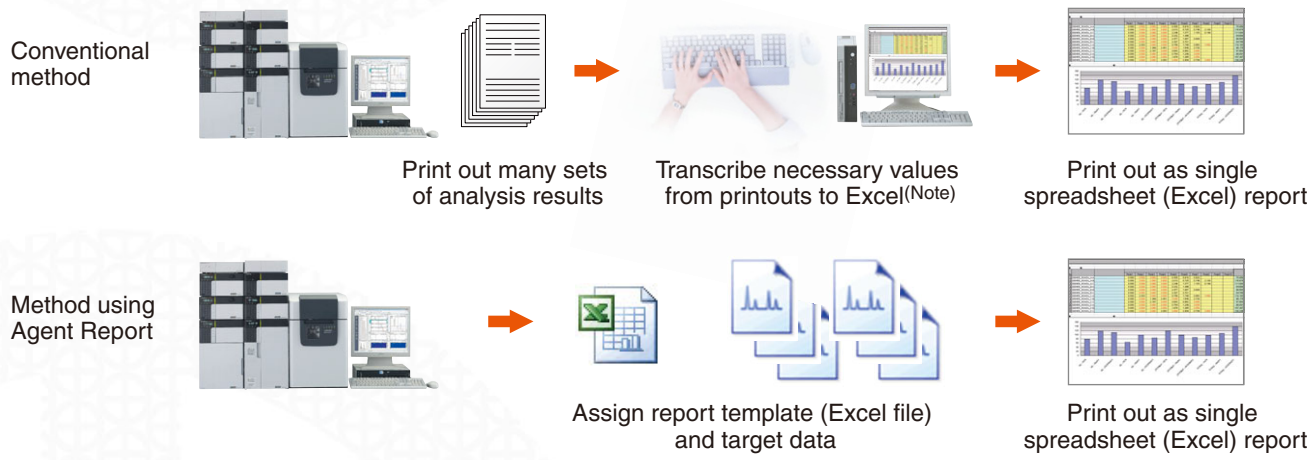
Fig. 13 Chromatograms Lined Up in Order of Evaluation Value

5-5. Output of Evaluation Results Report

Calculating the evaluation values and generating the graph are easily accomplished using the “Agent Report” software. Agent Report automatically extracts the parameters (number of detected peaks, peak resolution) necessary for calculating the evaluation values from the series of measurement results obtained using the respective eluent conditions. Then, after entering these parameters into a template prepared beforehand, Agent Report automatically generates a report.

Using Agent Report, the 12 sets of measurement data obtained in this step are summarized in a single Excel(Note) worksheet, as shown in Fig. 15. Since Agent Report utilizes Microsoft Corporation’s Excel spreadsheet software application, Excel’s mathematical functions and graphing features can be utilized to create reports that are tailored to suit the intended purpose (see Fig. 14).

The process of continuous analysis conducted here for examination of eluent conditions took about 11 hours. If such an operation were executed as an overnight automated analysis, the ability to have an evaluation report in hand the following morning surely represents an improvement in method development efficiency using this system.



(Note) Excel is a trademark or registered trademark of Microsoft Corporation in the United States and other countries.

Fig. 14 Improved Report Generation Efficiency

Report

■ Result ■

No	Data file	Eluent conditions	Resolution										Evaluation value
			Peak1	Peak2	Peak3	Peak4	Peak5	Peak6	Peak7	Peak8	Peak9	Peak10	
1	090406_NSAIDs_A-A	Formic acid aq. -ACN	0.000	3.000	3.000	3.000	0.435	0.910	0.922				78.869
2	090406_NSAIDs_A-B	Formic acid aq. -MeOH	0.000	3.000	3.000	3.000	2.013	0.725	0.746	2.138			116.976
3	090406_NSAIDs_A-C	Formic acid aq. -ACN/MeOH	0.000	3.000	3.000	3.000	2.246	1.377	1.103	0.149			111.000
4	090406_NSAIDs_B-A	Acetic acid aq. -ACN	0.000	3.000	3.000	3.000	0.332	1.446					64.668
5	090406_NSAIDs_B-B	Acetic acid aq. -MeOH	0.000	3.000	3.000	3.000	2.477	1.857	0.908				99.694
6	090406_NSAIDs_B-C	Acetic acid aq. -ACN/MeOH	0.000	3.000	3.000	3.000	2.427	2.701					84.768
7	090406_NSAIDs_C-A	Acetic acid (NH4)buf. - ACN	0.000	2.303	3.000	3.000	0.759	1.748	0.963	3.000			118.184
8	090406_NSAIDs_C-B	Acetic acid (NH4)buf. - MeOH	0.000	3.000	1.399	2.401	3.000	1.648	2.720				99.176
9	090406_NSAIDs_C-C	Acetic acid (NH4)buf. - ACN/MeOH	0.000	3.000	2.187	3.000	0.683	0.652	3.000				87.654
10	090406_NSAIDs_D-A	Acetic acid NH4aq. - ACN	0.000	3.000	3.000	3.000	0.764	1.249	3.000				98.091
11	090406_NSAIDs_D-B	Acetic acid NH4aq. - MeOH	0.000	3.000	3.000	3.000	1.976	1.365	3.000				107.387
12	090406_NSAIDs_D-C	Acetic acid NH4aq. - ACN/MeOH	0.000	3.000	3.000	2.063	3.000	2.509	0.709	3.000			138.248

■ Evaluation results (Eluent optimization) ■

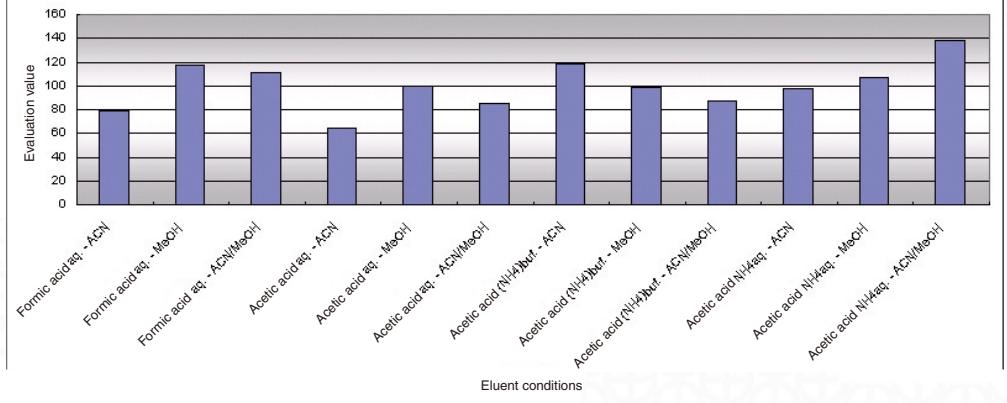


Fig. 15 Evaluation Results Report

6. Optimizing the Gradient Conditions

When checking the chromatogram having the highest evaluation value (Fig. 16), it is clear that there is room for improvement in the analysis time range of 2.7 minutes to 3.4 minutes with respect to the insufficient separation. Further, in the first 2 minutes of the analysis, there is a range of time where no target peaks are detected, so some room for improvement can also be acknowledged with respect to this time period. Accordingly, in this step, the gradient conditions will be optimized with the aim of improving the separation.

6-1. Examination Parameters

The method used for optimizing the gradient conditions utilizes the initial concentration and final concentration of the gradient program as examination parameters, and multiple analyses are conducted using different combinations of values for these parameters. As shown in Fig. 17, we used a linear gradient with 6 different values for the initial concentration (10 – 35 % range at 5 % intervals) and 6 values for the final concentration (70 – 95 % range at 5 % intervals), which provided a total of 36 gradient combinations.
Now, the gradient combination that was determined to be the best in Step 1 is used as the eluent in this step.

6-2. Measurement Results (Gradient Conditions Examination)

In this step, just as in Step 1, we created a continuous analysis batch file and conducted automated analysis. Some of the chromatograms obtained from this automated analysis are shown in Fig. 18.
Fig. 19 shows a graph of the evaluation values calculated from the measurement results by Agent Report. This clearly indicates that No. 1, with 30 % initial and 70 % final concentration gradient conditions, has received the best evaluation value. Further, when comparing the evaluation values obtained here to that of the gradient conditions associated with the left-most bar in this bar graph, corresponding to the conditions used in Step 1 (eluent selection), that is, the “universal gradient conditions,” the suitability of the gradient conditions obtained in this step is clearly seen.

6-3. Gradient Conditions Determination

Fig. 21 is confirmation of how the actual separation was improved by optimizing the gradient conditions. Here (a) is the chromatogram that received the best evaluation value in Step 1, while (b) is the chromatogram that was obtained following optimization of those gradient conditions.

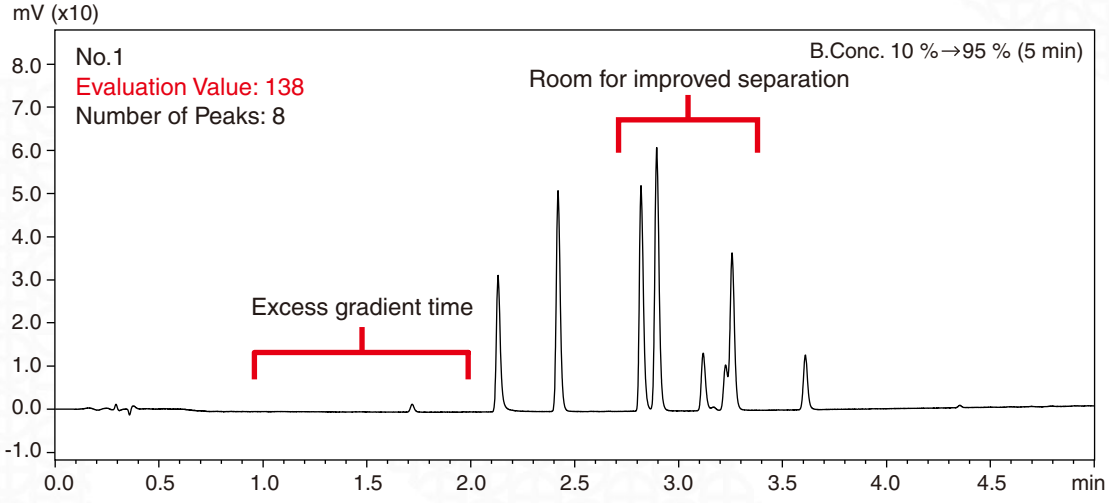
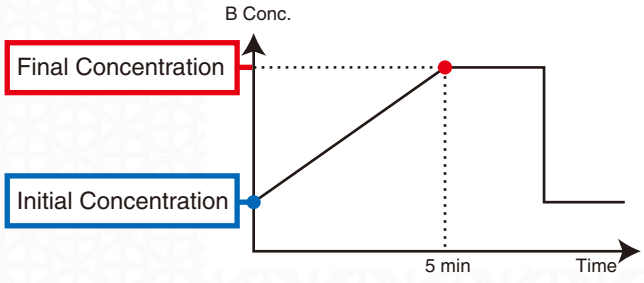


Fig. 16 Gradient Conditions Examination



Initial Concentration (%)
10
15
20
25
30
35

×

Final Concentration (%)
70
75
80
85
90
95

= 36 combinations of gradient conditions

Fig. 17 Content of Gradient Program

Regarding both of the places in (a) which were considered to have room for improvement, that is, the analysis time range from 2.7 to 3.4 minutes in which separation was incomplete, and the analysis time prior to 2 minutes during which no target peaks appeared, the changes to the program as described in Fig. 17, resulted in dramatic improvement between chromatograms (a) and (b).
Now, in this particular analysis, the automated analysis of Step 2 took about 24 hours (Step 1 took about 11 hours). When considered in terms of man-hours, if the automated analysis of Step 1 is started in the evening, and a determination is made the following morning as to which eluent combination is best based on the results obtained from Step 1, and then, if the automated continuous analysis for examination of the gradient conditions according to Step 2 is started that morning, the ultra-high speed analysis method would be completed during the morning of the following day.

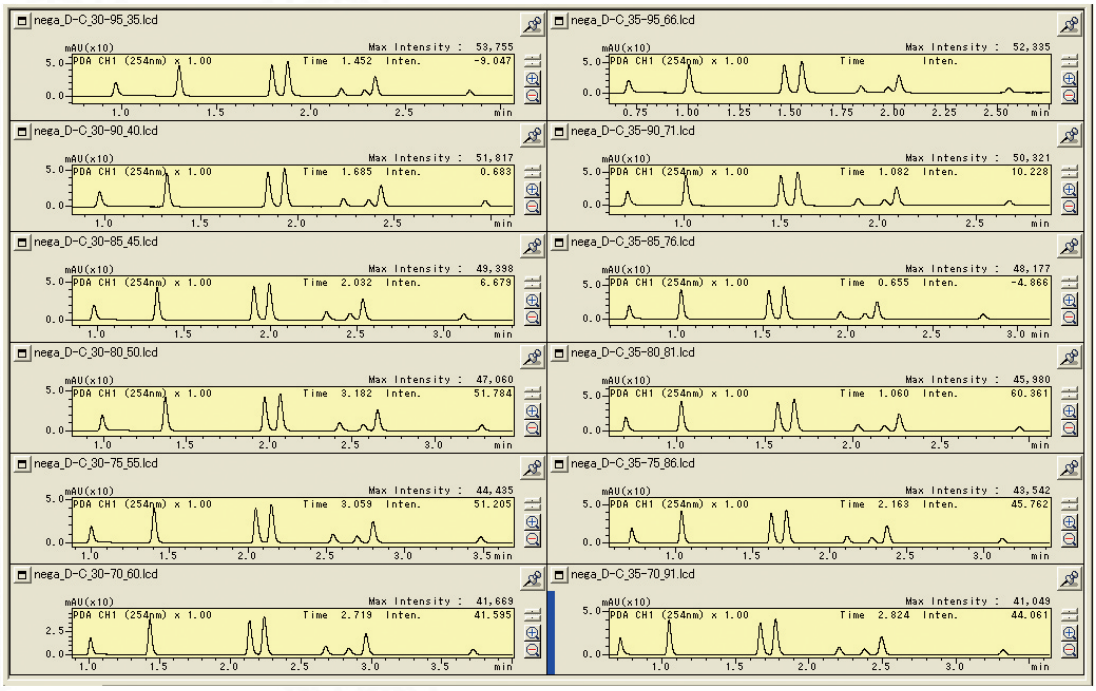


Fig. 18 Measurement Results for Optimization of Gradient Conditions

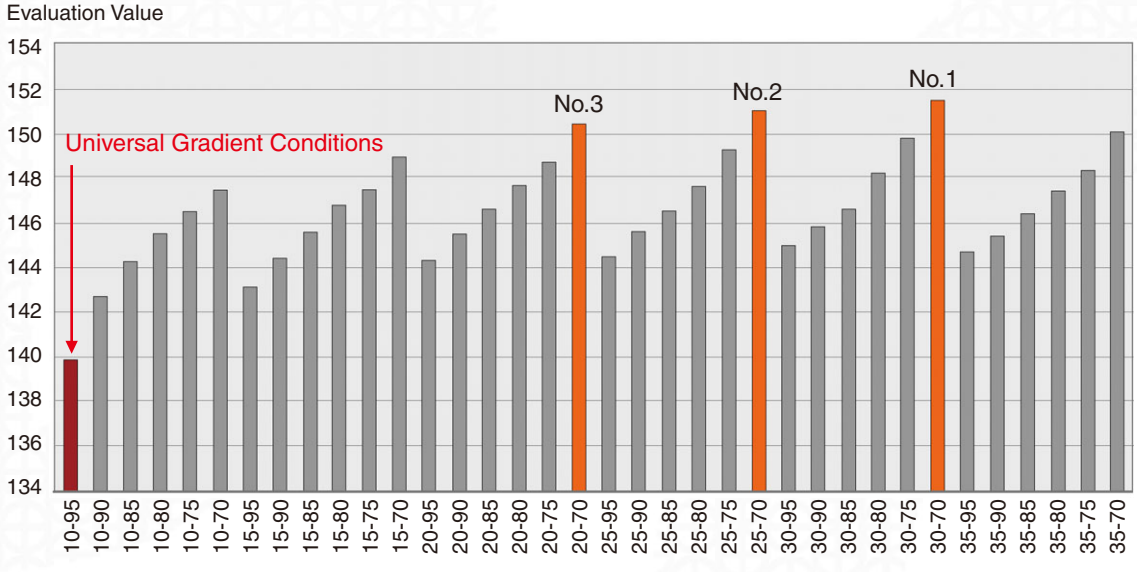


Fig. 19 Evaluation of Gradient Conditions Optimization

7. Step 3: Peak Identification Using LCMS-2020

Continuing from Steps 1 and 2, the process moves to the job of identifying each of the peaks. In identifying each peak, mass information is used to achieve high-accuracy peak identification through effective utilization of the mass spectrometer, as shown in Fig. 22.

The LCMS-2020, with its ultra-high speed performance features of 15 msec positive / negative polarity switching and a scan speed of 15,000 u/sec, achieves reliable measurement even for these peaks generated at such ultra-high speed.

Fig. 20 LCMS-2020

8. Step 4: Establishing Conventional Analysis Conditions

In Step 4, the final step, conventional analysis conditions are established so as to provide the same separation pattern as that using the ultra-high speed analysis conditions established through Step 3.

8-1. UFLC Method Transfer Program Ver. 2

When migrating to conventional analysis, the UFLC Method Transfer Program Ver. 2 is used (see Fig. 23). In the original version of this program, the parameters necessary for transferring conventional analysis conditions to ultra-high speed analysis conditions of flow rate, sample injection volume, gradient conditions and the like, are automatically calculated (simulated). When migrating from high-speed to conventional analysis, the necessary parameters (flow rate, injection volume, etc.) can also be automatically calculated based on the optimized conditions from the high-speed runs.

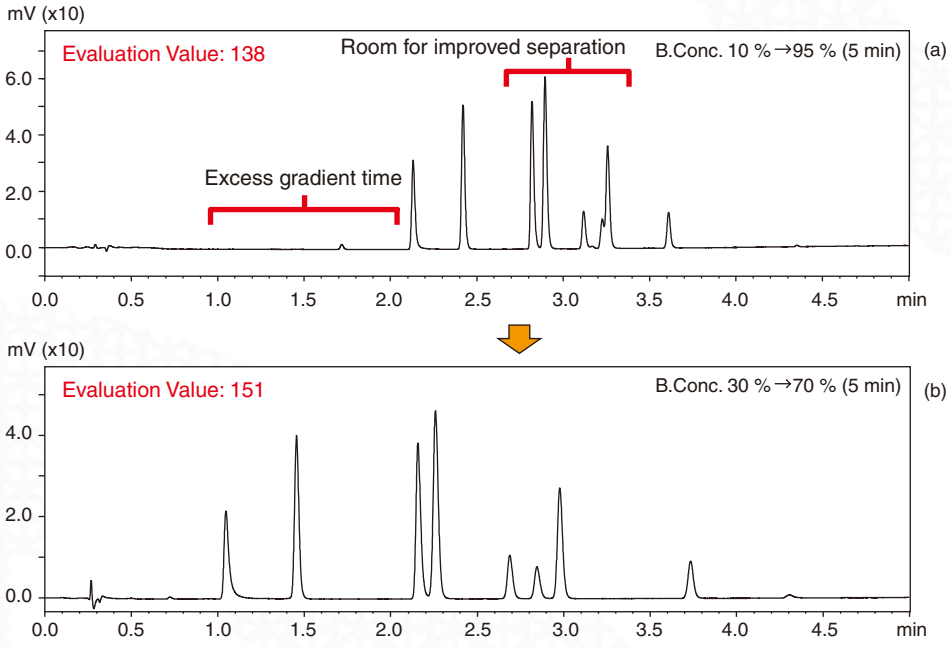


Fig. 21 Effect of Gradient Conditions Improvement

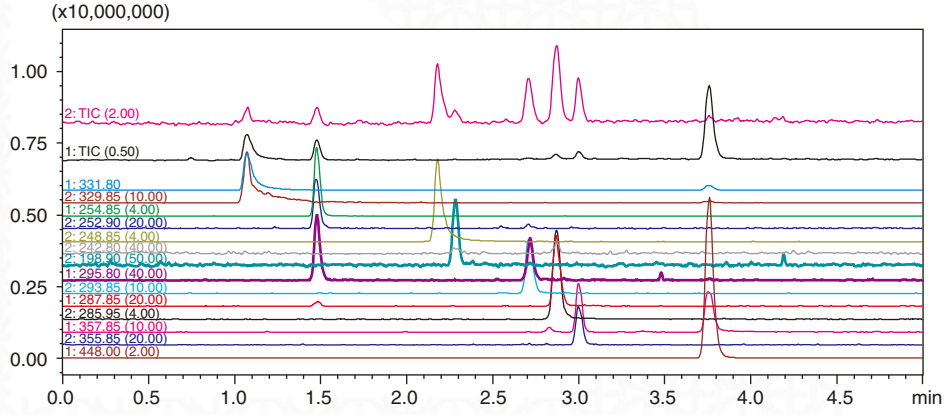


Fig. 22 Peak Identification by Mass Spectrometer (LCMS-2020)

The conventional analysis conditions calculated using this program are shown in Table 2, and the actual analysis results obtained using these analysis conditions are shown in Fig. 24 (b). Before conducting this conventional analysis, the flow line of this method development system was switched from the flow line (mixer and column) for ultra-high speed analysis to that for conventional analysis.

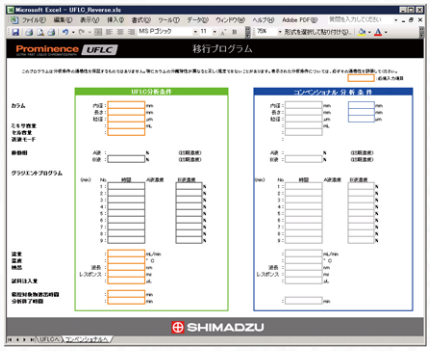


Fig. 23 Method Transfer Program Ver. 2

8-2. Comparison of UFLC and Conventional Analysis Results

As seen in Fig. 24, the 5-minute analysis time of the ultra-high speed analysis took 7 times as long using conventional analysis, but the separation patterns in both were about the same.
Here, column selection is important for maintaining the separation pattern. Similar separation patterns were obtained by using the Shim-pack XR-ODS (2.2 µm particle size) ultra-high speed analysis column and the Shim-pack VP-ODS (4.6 µm particle size) conventional analysis column. The similarity is due to the packing material having comparable properties, even though they have different particle sizes.

Column	: Shim-pack VP-ODS (150 mm L. x 4.6 mm I.D., 4.6 µm)
Mobile Phase	: A; 10 mmol/L Ammonium Acetate Solution (pH 6.6)
	B; Acetonitrile/Methanol = 1/1 (vol./vol.)
Time Program	: B.Conc. 30 % (0 min) → 70 % (35 - 56 min) → 30% (56.01 - 70min)
Flow Rate	: 1.0 mL/min
Injection Volume	: 5 µL
Temperature	: 40 °C
Detection	: SPD-M20A at 254 nm
Response	: 640 msec

Table 2 Calculated Conventional Analysis Conditions Using Method Transfer Program Ver. 2

9. Conclusion

This system not only enables optimization of eluent selection and separation using ultra-high speed analysis, but also facilitates the transfer of conditions used for ultra-high speed analysis to those that provide comparable separation in conventional analysis. It is a powerful system that provides the means to improve R&D efficiency through greatly accelerated method development.

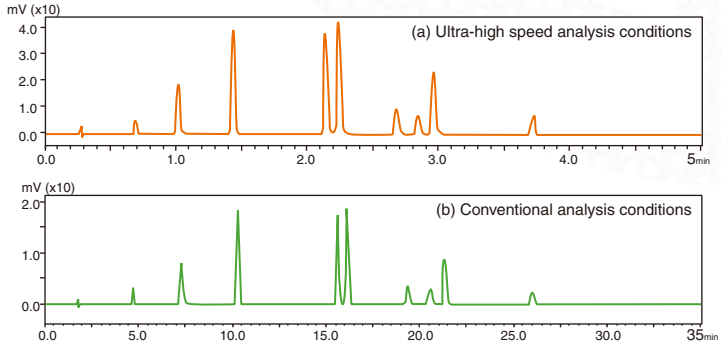


Fig. 24 Migration from Ultra-High Speed Analysis to Conventional Analysis

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