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C10G-E069

Pharmaceuticals

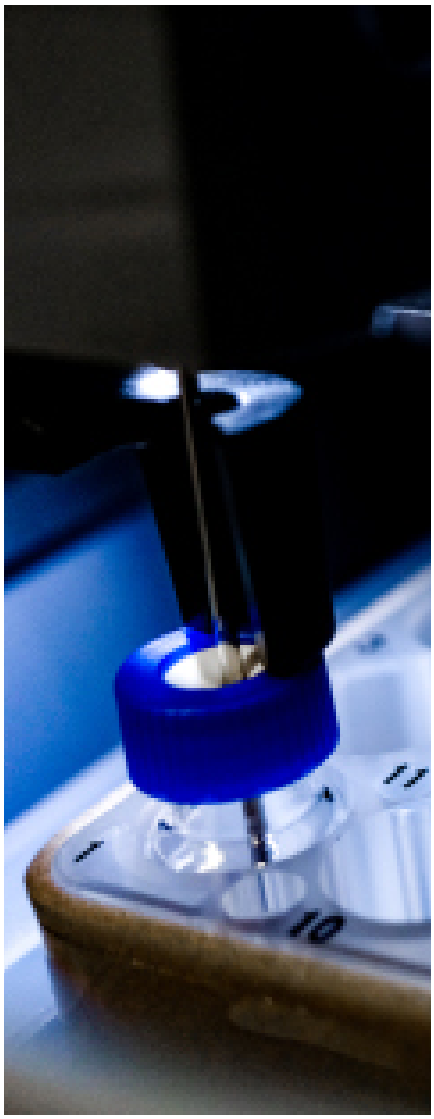
Technologies for Enhanced LC and SFC Workflow

Introduction

With the rapid growth of global population, the increased incidence and prevalence of diseases, and the continuous aging society in major economies, the global demand for pharmaceuticals can only increase. In order to meet these demands and stand out from the fiercely competitive industry, pharmaceutical companies need to invest heavily on drug research, development and production. Furthermore, they have to ensure drug safety, quality and compliance to global pharmaceutical regulations.

As a global leader in analytical instruments and laboratory services, Shimadzu Corporation has always been a reliable partner for the pharmaceutical industry. We provide comprehensive analytical solutions for all drug research analysis and productions, and help companies to increase efficiency, reduce cost and ensure drug safety and quality.

This brochure introduces the various technologies to enhance pharmaceutical workflows and processes. It demonstrates how liquid chromatography (LC) and supercritical fluid chromatography (SFC) is applied to accelerate drug development, monitor production processes and ensure drug quality. Together with the use of Shimadzu's laboratory informatics products, such as LabSolutions CS and Laboratory Information Management System (LIMS), it builds a strict compliance network system and protect the overall data integrity and management of the laboratory.



Contents

Enhanced Pharmaceutical LC & SFC Workflows

SOLUTION 1: LC METHOD DEVELOPMENT

- Method Scouting System automatically screens for the best combination of mobile phases and column
- Minimize the time needed for method development without compromising the quality

SOLUTION 2: LC METHOD TRANSFER

- New UHPLC/HPLC dual-flow LC system
- Easy implementation and one-click transfer of chromatographic methods between different LC instruments

SOLUTION 3: SAMPLE PREPARATION & PURIFICATION

- A range of configuration options for preparative and purification chromatography
- Obtain higher compound purity and faster purification efficiency than traditional methods

DATA INTEGRITY

- A unified platform that fully meets regulatory requirement
- In addition to chromatography, it also covers a wide range of instruments including mass spectrometry, spectroscopy, and balances

SOLUTION 4: IMPURITY DETECTION & PURITY CONFIRMATION

- Shimadzu PDA detector provides the unique deconvolution function
- Ensure that impurity peaks are never missed

SOLUTION 5: IMPURITY IDENTIFICATION

- Trap-free 2D LC/MS is a proven platform for integrating conventional LC and LC-MS/MS
- Spend no more time on fractionation or development of a separate method

WORKFLOW MANAGEMENT WITH LIMS

- LabSolutions provides an open data interface to different vendors
- Compatible with a wider range of LIMS systems than other manufacturers

SOLUTION 6: CHIRAL SEPARATION

- SFC provides orthogonal chemistry for chiral separation
- Likely to achieve better separation than normal phase chromatography

SOLUTION 7: DISSOLUTION TESTING

- Shimadzu's new LC connects online with various dissolution testers
- One-step complete dissolution and analysis with automatic evaluation of results

SOLUTION 8: QUANTITATIVE DATA PROCESSING

- LabSolutions provides new algorithms on peak identification and quantification
- Identify peaks in complex matrices over a wider dynamic range for accurate quantitation of both active ingredient and impurities

Solution 1: LC Method Development

For drug research and analysis, LC method development is often the time-limiting factor.

The manual optimization of chromatographic parameters such as mobile phases, columns and gradient elution program, is tedious, time and labor intensive. The parameters may not even be optimal and could likely affect the evaluation of drug quality.

Shimadzu offers a smarter solution for LC method development for a more cost-effective workflow for your drug research and analysis.

Nexera Method Scouting System

1) Define your analytical conditions

Mobile Phases | Columns | Gradient Conditions

2) Automatic method screening with different conditions

No. of Mobile Phases X No. of Columns

= No. of Screening Combinations

3) Evaluate and identify the method with the optimal resolution value

	Resolution value	Rank
Method 1	140.012	4
Method 2	120.213	5
Method 3	290.057	1
Method 4	289.281	2
Method 5	100.901	6
Method 6	250.783	3

Improved features and fully compatible with more systems

- The Nexera Method Scouting System supports all Nexera and i-Series products and configurations. Omni-directional screening is performed by a combination of automatic switching and permutation of more columns and mobile phases.
- The new software with an effective user interface enables a precise assessment of the methods by additional parameters and evaluation options.

Establish a LC method quickly and accurately

- Instrument runtime, manual operation time and data processing time are greatly reduced thanks to the automated system and easy-to-use operating software.
- The innovative scientific evaluation software helps you to select the best separation conditions automatically and accurately from the massive analysis results.

Suitable for all type of LC systems

Nexera X2
- Automatically verify 192 sets of conditions @ 130 MPa
- pH simulation in the method for given mobile phase compositions

i-Series
- Save space and cost

Nexera UC
- Method scouting for chiral compounds
- LC/SFC switching, improved efficiency for method development

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Technical Report

Improved R&D Efficiency Through Speedier Method Development (3)

Tadayuki Yamaguchi

Abstract:

Method scouting is the search for analytical conditions (column, mobile phase, and gradient settings) that optimize an LC separation. When used with a UHPLC (ultra high performance LC) system, the time to produce a robust, validated method is greatly shortened, which leads to an increased interest in and application of UHPLC systems for method development. In this report, we introduce an example of such scouting using the Nexera Method Scouting System together with its specialized Method Scouting Solution software to conduct a search for simultaneous analytical conditions to be used in analysis of 13 cephem antibiotic substances.

Keywords: UHPLC, Nexera Method Scouting, Method Scouting Solution, Cephem Antibiotics Simultaneous Analysis

1. Introduction

The process of method development in HPLC can be categorized into four steps: 1) Offline simulation to predict retention behavior, 2) Method scouting to search for the optimum column and mobile phase, 3) Method optimization to further improve various parameters including flow rate, column temperature, and gradient conditions, and 4) Method validation to verify the robustness of the method. Due to the necessity of addressing the significant work effort, time, and level of skill required to develop an effective method, the method scouting process is used to determine the method that uses the most suitable among many mobile phases (pH, salt concentration, organic solvent ratio) and columns (ODS, C8, Phenyl, etc.).

There is increasing interest in high throughput systems with the objectives of improved business efficiency and productivity, and even in HPLC, the prevalence of ultra high performance liquid chromatography (UHPLC) is growing, mainly due to the use of ultra-small particle size columns. And, in response to this trend, the introduction of UHPLC in method scouting is also underway.

This report introduces the Nexera Method Scouting System and the special Method Scouting Solution software, newly developed with the aim of improving efficiency in the method scouting process. Also introduced is the application of the Nexera Method Scouting System in determining the optimum analytical conditions for analysis of cephem antibiotics.

2. Nexera Method Scouting System

Fig. 1 and Fig. 2 show the Shimadzu Ultra High Performance Liquid Chromatograph Nexera Method Scouting System and a flow-line diagram of the overall process. This is a method scouting system with the Nexera ultra high performance liquid chromatograph as its base. It is a system that not only features a high 130 MPa pressure capacity, superb performance, reliability and extendibility, it is ideal for the fully automated examination of mobile phases and columns.

Regarding the examination of mobile phases, a combination of up to 16 types of mobile phases can be examined by installing a reservoir switching valve in each pump. In addition, up to 6 different columns can be investigated automatically by installing a 100 MPa pressure capacity flow line switching valve (FCV-34AH) in the column oven. Simply put, it permits the fully automated investigation of up to 96 combinations of mobile phases and columns, thereby enabling exhaustive scouting of analytical conditions in a fraction of the time required by traditional methods.

Fig. 1 Nexera Method Scouting System

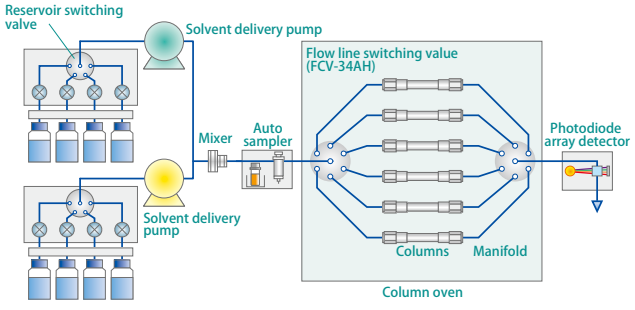


Fig. 2 Nexera Method Scouting System Flow Line Diagram

3. Method Scouting Solution

Fig. 3 shows a screen capture from Method Scouting Solution, the special software used with the Nexera Method Scouting System.
Method Scouting Solution is a software application specially designed to support automation of the method scouting process, while providing a user-friendly graphical interface to facilitate the scouting setup operations. Settings for the columns, mobile phases, and gradient conditions are made in the main window (Fig. 3), and by merely entering the necessary parameters according to the provided on-screen guidance, setup can be easily completed through to creation of the batch table. With the built-in database management functions, entering and storing the names of columns and mobile phases not only improves management efficiency, it can help to ensure much fewer operational mistakes, especially when multiple operators share the system. Furthermore, since Method Scouting Solution integrates seamlessly with Shimadzu's LabSolutions analysis software, a series of analysis schedules for method scouting, from analysis preparation at instrument startup to analysis completion, can be created automatically. This achieves a significant reduction in the time and effort spent on setting method scouting conditions and creating the batch table. For details regarding Method Scouting Solution, also refer to the Technical Report "Ultra Fast Method Scouting."

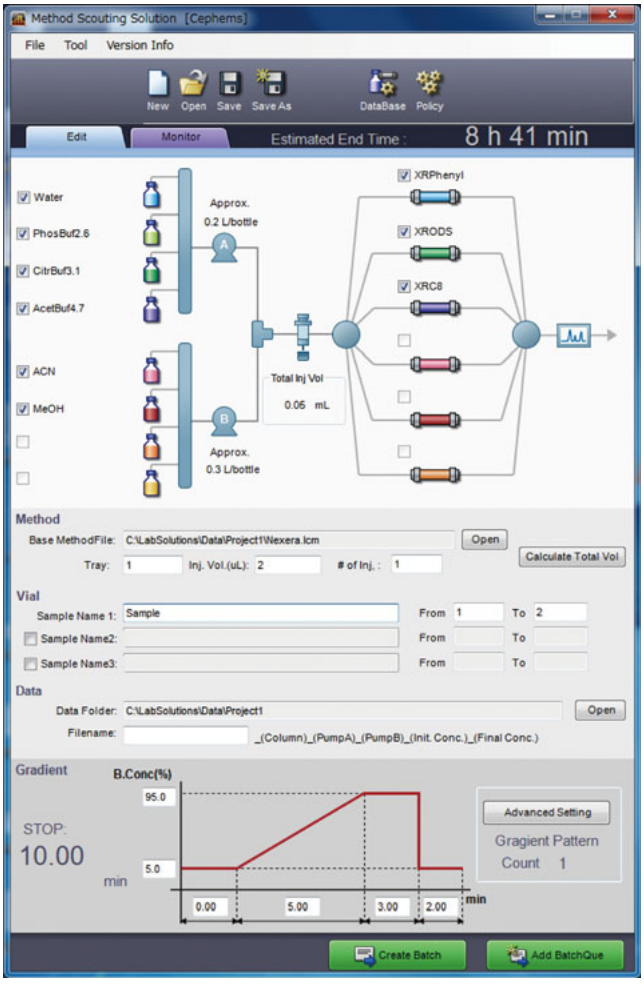


Fig. 3 Method Scouting Solution

4. Building Analytical Conditions for Simultaneous Analysis of Cephem Antibiotics

Here we introduce an application example using the Nexera Method Scouting System. Thirteen types of Cephem antibiotics were used as analytes, including cephradine, cephalexin, cephapirin, cefazolin, cefadroxil, cefaclor, cefuroxime, cephalothin, cefotaxime, cefoperazone, cefoxitin, cefsulodin and cefmetazole. Cephem antibiotics are a type of β-lactam antibiotic, and because they are broad-spectrum antibiotics possessing strong antibacterial activity, they are commonly used as injectable and oral medicines. However, since these compounds possess similar structures, their complete separation by reversed-phase chromatography is notoriously difficult. Fig. 5 shows the workflow used for building the analytical conditions for simultaneous analysis of the cephem antibiotics.

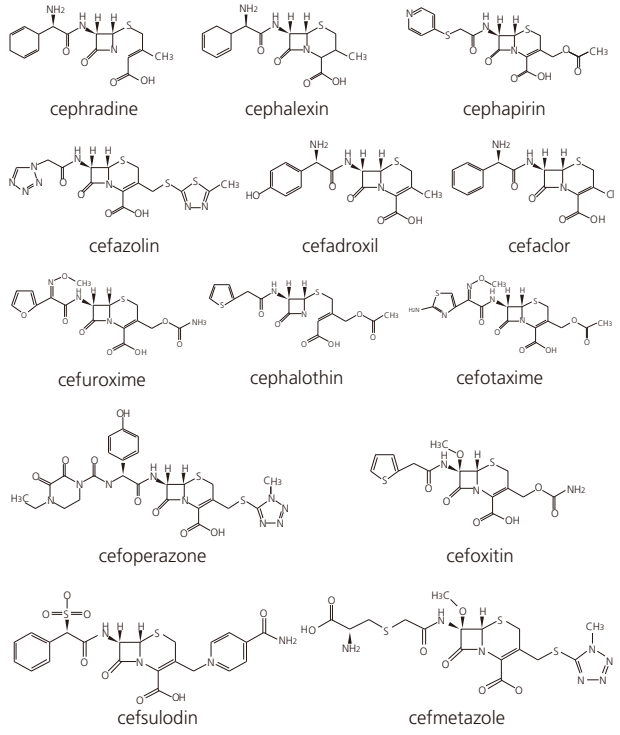


Fig. 4 Cephem Antibiotic Substances

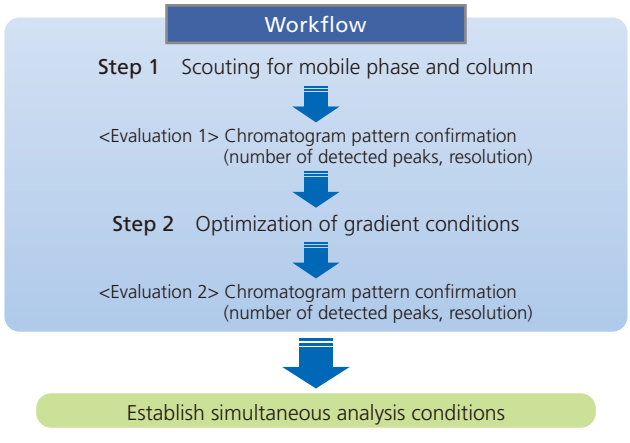


Fig. 5 Method Scouting Workflow

4-1. Step 1: Scouting for Mobile Phases and Column

The objective in Step 1 is to scout for the optimum mobile phase and column. For the mobile phases, 4 aqueous mobile phases with different pH values (phosphate, citrate, ammonium acetate buffer solutions, etc.) and 3 organic mobile phases (acetonitrile, methanol, etc.) were used, and for the column, 6 types of columns were used, including 3 of the Shimpack XR series HPLC columns with 2.2 µm particle size packing material, and 3 of the Core-shell type columns with 2.6 µm particle size packing material. The column size was specified as 50 mm length and 3 mm inner diameter in all cases. In order to elute compounds having a wide range of polarity, for the time program we used universal gradient conditions in which the organic solvent ratio was set to 5% to 90% in linear fashion. A UV-VIS photodiode array detector (detection wavelength set to 260 nm) was used for detection, and comprehensive investigation was conducted using a total of 72 conditions. Details of the analytical conditions are as shown in Table 1.

Table 1 Step 1 Analytical Conditions

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 aqueous solution pH 6.7	
(B)	(a) Acetonitrile	
	(b) Methanol	
	(c) Acetonitrile / methanol = 50/50 (v/v)	
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)
Time program	: B Conc. 5% (0 min)→90% (5.01-7 min)→5% (7.01-9 min)	
Flow rate	: 1.0 mL/min	
Injection volume	: 5 µL	
Column temperature	: 40°C	
Detection wavelength	: 260 nm (SPD-M20A)	

4-2. Chromatogram Verification (Step 1)

Using the LabSolutions browser feature, the 16 representative chromatograms studied in Step 1 are shown in Fig. 6. Due to the difficulty in judging the quality of some of the data by visual comparison and assessment of the chromatograms, quantitative evaluation (4-3) was conducted in addition to the visual evaluation of the chromatograms.

4-3. Quantitative Assessment of Chromatograms

In this report, to quantitatively assess the state of separation in the chromatogram obtained by method scouting, an equation for conducting the assessment was devised (Equation 1), in which the assessment value (E) is calculated using the number of detected peaks (P) and resolution (Rs, with upper limit value set to 3). This was applied in the investigation of the analytical conditions for simultaneous analysis of the cephem antibiotics.

$$E = P\left(R_{s1} + R_{s2} + \ldots + R_{sP}\right) \cdots \text{Equation 1}$$

This evaluation equation was devised to emphasize peak resolution, and therefore utilizes the "number of peaks" in the chromatogram and the "sum of the resolution values". Following is a detailed explanation of this process, using the 2 chromatographic patterns of Fig. 7.

Pattern 1 shows a chromatogram consisting of 4 unevenly separated components, and Pattern 2 shows a chromatogram in which the 2 peaks are completely separated. At first glance, pattern 2 appears to show good separation, but when applying the calculation method using Equation 1 (based on the number of detected peaks and sum of resolution values) to assess the chromatograms quantitatively, the assessment value for pattern 1, with the apparently poor separation, is the higher of the two. This is due to the overly large separation between the 2nd and 3rd peak of Pattern 1, thereby resulting in a higher calculated resolution than that of pattern 2. Therefore, to correct the discrepancy between the visual assessment and the calculated assessment, an upper limit of "3" was set for the resolution upper limit (the resolution will be taken as "3" even if a value of "5" or "10" is entered).

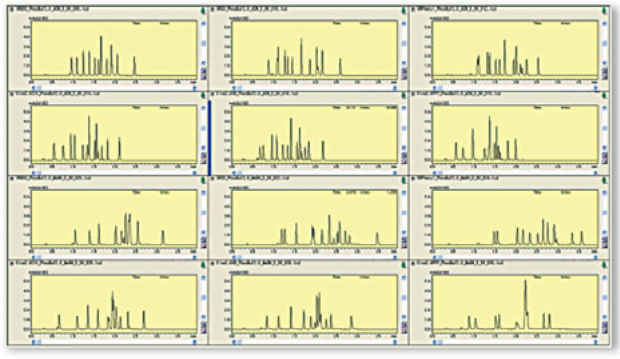
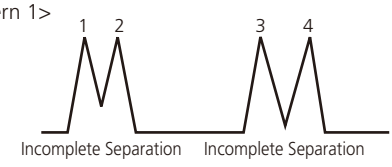


Fig. 6 Typical Chromatograms of Step 1

<Pattern 1>



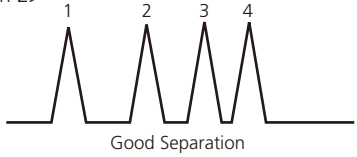
Peak	Resolution
1	–
2	1.1
3	8.0
4	1.4

Assessment Value 42

Resolution (after setting upper limit)
–
1.1
3.0
1.4

Assessment Value (22)

<Pattern 2>



Peak	Resolution
1	–
2	4.0
3	2.5
4	2.2

Assessment Value 35

Resolution (after setting upper limit)
–
3.0
2.5
2.2

Assessment Value (31)

Fig. 7 Separation-Emphasized Assessment of Chromatograms

4-4. Chromatogram Assessment Results (Step 1)

The assessment results for the chromatograms of Step 1 using the quantitative assessment method of Section 4-3 are shown in Fig. 8 (Upper tier: Shim-pack Series, Lower tier: Kinetex Series), and the results in the order of higher-assessed chromatograms are shown in Fig. 9. Also, the higher-assessed combinations of separation columns and mobile phases are shown in Table 2. In this investigation, the Kinetex XB-C18 Core-Shell column received the highest assessment among the columns when used in combination with a phosphate buffer solution (pH 2.6) and acetonitrile as mobile phases. In addition, from the results of Fig. 8 and Fig. 9, good correlation was clearly obtained between the assessment values and the chromatogram's visual assessments.

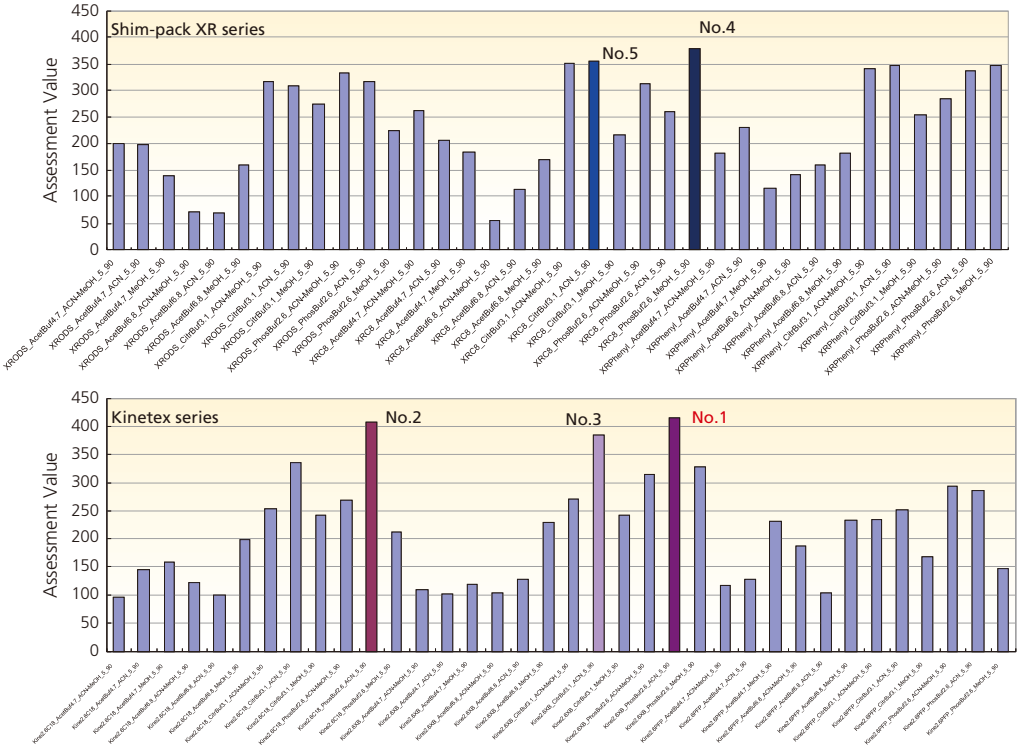


Fig. 8 Chromatogram Quantitative Assessment Results (Step 1)

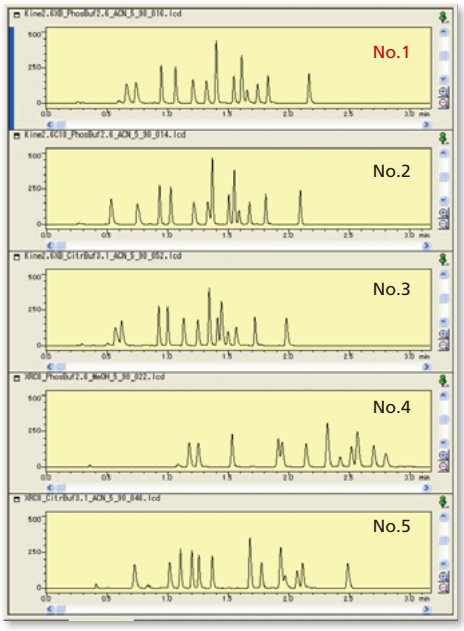


Fig. 9 Quantitative Assessment Higher Ranked Chromatograms (Step 1)

Table 2 Chromatogram Quantitative Assessment Results (Step 1)

Assessment Value		Separation Column	Mobile Phase A	Mobile Phase B
No.1	415	Kinetex XB-C18	Phosphate buffer solution (pH 2.6)	Acetonitrile
No.2	407	Kinetex C18	Phosphate buffer solution (pH 2.6)	Acetonitrile
No.3	382	Kinetex XB-C18	Citrate buffer solution (pH 3.1)	Acetonitrile
No.4	380	Sim-pack XR-C8	Phosphate buffer solution (pH 2.6)	Methanol
No.5	356	Sim-pack XR-C8	Citrate buffer solution (pH 3.1)	Acetonitrile

4-5. Step 2: Optimization of Gradient Conditions

After establishing the highest evaluated mobile phase (combination of phosphate buffer solution and acetonitrile) and column (Kinetex XB-C18), next we focused on determining the optimum gradient conditions. Based on a total of 9 combinations of initial concentration (3 types, including 5%, 10%, 15%) and final concentration (3 types, including 40%, 65%, 90%), we investigated 5-minute gradient programs from initial to final concentration. The other conditions were the same as those shown in Table 1. The analytical conditions are shown in Table 3.

Table 3 Step 2 Analytical Conditions

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	:(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)
Flow rate	:1.0 mL/min
Injection volume	:5 µL
Column temperature	:40°C
Detection wavelength	:260 nm (SPD-M20A)

4-6. Chromatogram Verification (Step 2)

The chromatograms that were studied in Step 2 are shown in Fig. 10. Also, the results of quantitative assessment of the chromatograms using Equation 1 as in Step 1 are shown in Fig. 11. From the results of Fig. 10 and Fig. 11, the gradient program providing the best separation was determined to be an initial concentration of 5% and a final concentration of 65%.

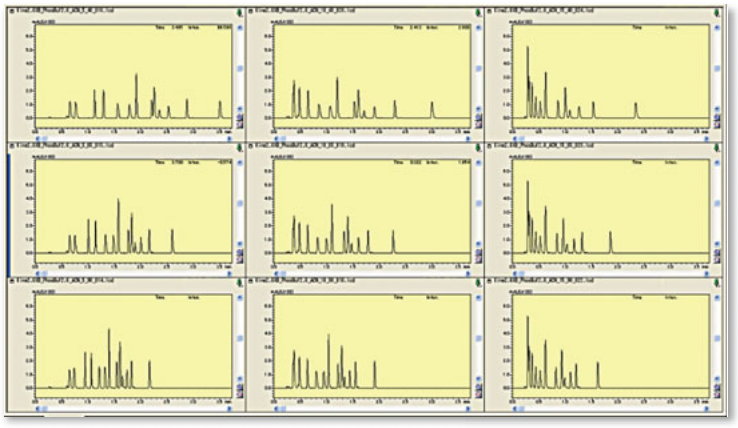


Fig. 10 Chromatograms of Step 2

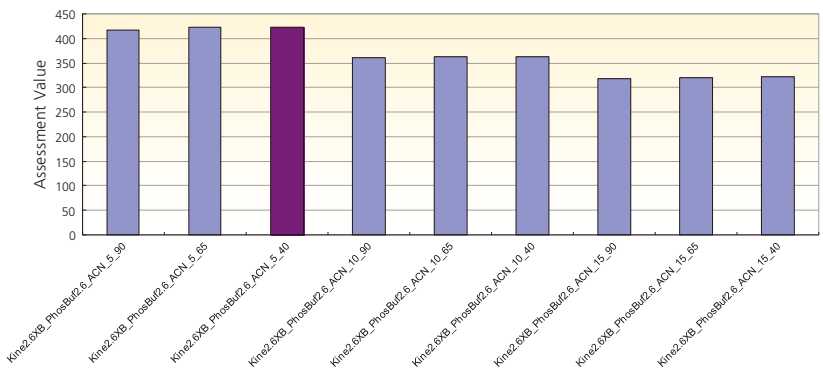


Fig. 11 Chromatogram Quantitative Assessment Results (Step 2)

4-7. Determining Analytical Conditions for Simultaneous Analysis

The analytical conditions optimized in Step 2 for the ultra high performance LC simultaneous analysis of the 13 cephem antibiotic substances are shown in Table 4, and their respective chromatograms are shown in Fig. 12. Due to optimization of the mobile phase, column, and gradient program described in Step 1 and Step 2, we were able to establish analytical conditions that permitted the completion of one cycle of simultaneous analysis of 13 cephem antibiotics within 9 minutes.

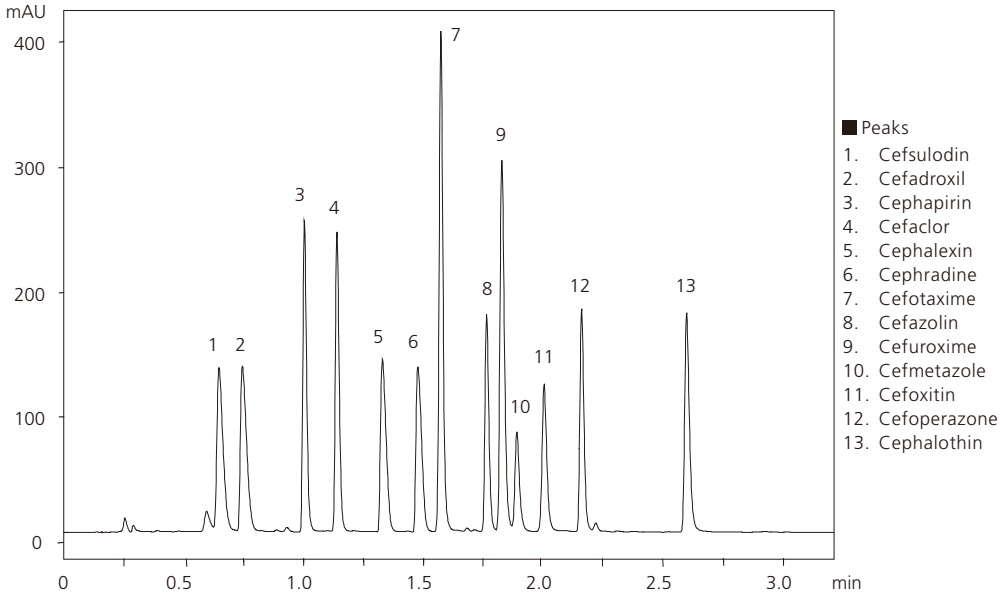


Fig. 12 Simultaneous Analysis of 13 Cephem Antibiotic Substances

Table 4 Analytical Conditions for Simultaneous Analysis of 13 Cephem Antibiotics

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	:B 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)

(Note) Kinetex is a trademark or registered trademark of Phenomenex, Inc. in the U.S. and other countries.
For details regarding this product, please contact the Shimadzu GLC. – Phenomenex Support Center (e-mail psc@glc.shimadzu.co.jp) or your Shimadzu representative

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Technical Report

Rapid Method Scouting of Chiral Compounds

Tadayuki Yamaguchi[1], Hidetoshi Terada[1], Takeo Sakai[1], Sadahiro Hayakawa[1]

Abstract:

Enantiomer resolution by chiral column chromatography is a variation of column chromatography that is actively being researched in the pharmaceutical field of drug discovery. One drawback of this method, however, is the extensive time and effort required to determine the optimum mobile phase conditions and the most suitable column for separation of the analyte among a wide variety of available chiral columns. This has spurred the demand for faster scouting of chiral separation conditions.
Here, using the Nexera Method Scouting system with the *i*Chiral-6 polysaccharide-based columns (Daicel Corporation), we report an example in which high-resolution column conditions are constructed for analysis of chiral compounds.

Keywords: UHPLC, Nexera Method Scouting, chiral column, *i*Chiral-6, chiral compounds

1. Introduction

The safe and secure use of chiral compounds in their pure form (enantiomers) is essential for direct biological applications, specifically in the field of pharmaceuticals. It is often the case where one enantiomer has detrimental or toxic effects while the other enantiomer has curative properties, so it is critical that enantiomers be fully separated. Optical separation (chiral separation) by HPLC is one method typically used to obtain chiral compounds. This HPLC method normally involves a labor-intensive, time-consuming search for an appropriate mobile phase and column suitable for a particular chiral compound, a process referred to as method scouting. Much effort has been directed recently at accelerating such method scouting to speed up drug synthesis and the production of pharmaceutical intermediates.

As an associated technology, ultra high performance liquid chromatography (UHPLC) has been attracting much attention as an HPLC technique capable of increasing throughput in commercial analytical enterprises by improving productivity and efficiency. Introduction of UHPLC is also being promoted for method scouting as a means of increasing overall throughput while minimizing solvent consumption.

Here we introduce the results obtained in the analysis of three chiral compounds: bromacil, α-methyl-α-acethyl-γ-butylrolactone, and methylclothiazide using the Nexera Method Scouting system with the high-resolution conditions that are possible with the *i*Chiral-6 polysaccharide-based columns.

2. Experiment

2-1. System

Fig. 1 shows the flow diagram depicting the Nexera Method Scouting system that was constructed for this application. The system is configured by installing a column switching valve inside the oven and a solvent switching valve within each of the Nexera ultra high performance pump, thereby permitting comprehensive data collection while continuously switching through a maximum of 96 unique combinations of columns and mobile phases.

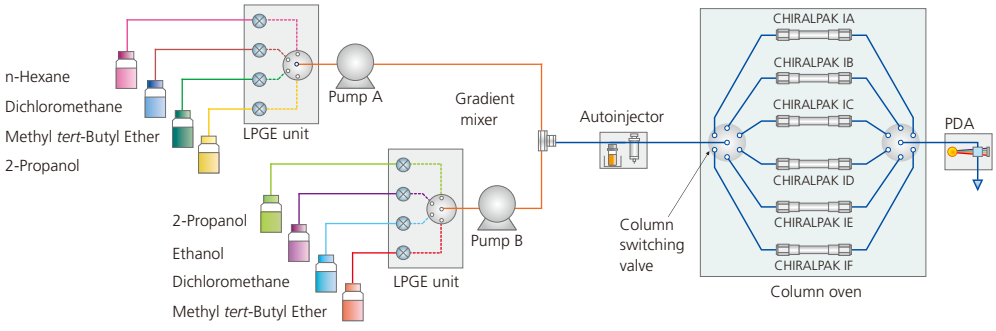


Fig. 1 Flow Diagram of the Nexera Method Scouting System

1 Analytical & Measuring Instruments Division

2-2. Sample Information

Three standards of chiral compounds (bromacil, α-methyl-α-acethyl-γ-butylrolactone, and methylclothiazide) were analyzed, as shown in Fig. 2. These standards were prepared by dissolving in hexane and MTBE as needed at 1.0 mg/mL.

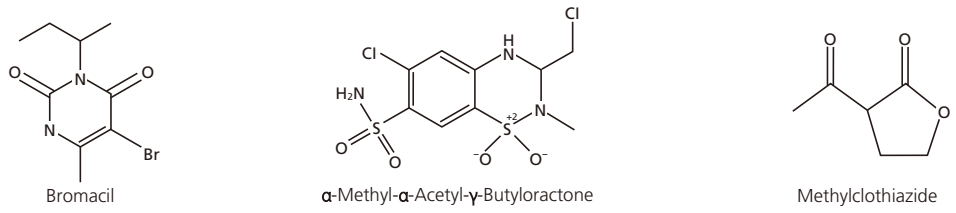


Fig. 2 Structural Formulas of Analyte Chiral Compounds

2-3. Chiral Columns

The *i*Chiral-6 high-resolution polysaccharide-based columns (CHIRALPAK® IA/IB/IC/ID/IE/IF) available from Daicel Corporation were utilized to provide the high-resolution conditions for the displayed chiral compounds. Fig. 3 shows the respective column functional groups. Since the *i*Chiral-6 columns are compatible with the range of organic solvents specified here, these columns are applicable to method scouting analysis of chiral compounds.

(A) CHIRALPAK® IA (150 mmL. × 4.6 mmI.D., 5 µm)
Amylose tris(3,5-dimethylphenylcarbamate)

(B) CHIRALPAK® IB (150 mmL. × 4.6 mmI.D., 5 µm)
Cellulose tris(3,5-dimethylphenylcarbamate)

(C) CHIRALPAK® IC (150 mmL. × 4.6 mmI.D., 5 µm)
Cellulose tris(3,5-dichlorophenylcarbamate)

(D) CHIRALPAK® ID (150 mmL. × 4.6 mmI.D., 5 µm)
Amylose tris(3-chlorophenylcarbamate)

(E) CHIRALPAK® IE (150 mmL. × 4.6 mmI.D., 5 µm)
Amylose tris(3,5-dichlorophenylcarbamate)

(F) CHIRALPAK® IF (150 mmL. × 4.6 mmI.D., 5 µm)
Amylose tris(3-chloro-4-methylphenylcarbamate)

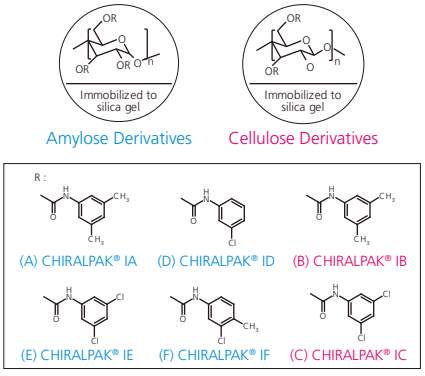


Fig. 3 Chiral Selector

2-4. Separation Conditions

For the mobile phases, eight different solvent mixtures were used, consisting of various combinations of hexane, 2-propanol, ethanol, dichloromethane, and methyl-t-butyl ether. Details of the separation conditions are shown in Table 1. Using a total of 8 mobile phase combinations and 6 different columns, the 48 unique separation conditions yielded an exhaustive search for the conditions suitable for the separation of each of the chiral compounds.

Table 1 Analytical Conditions

Separation Conditions No.	Mobile Phase	Flow Rate	Analysis Time	Other
1	Hexane / 2-Propanol = 9 / 1(v/v)	3 mL/min	9 min	Column temperature: 40°C Injection volume: 10 µL Detection: 230 nm
2	Hexane / 2-Propanol = 6 / 4(v/v)	3 mL/min	9 min	
3	Hexane / Ethanol = 8 / 2 (v/v)	3 mL/min	14 min	
4	Ethanol	1 mL/min	18 min	
5	Hexane / Dichloromethane = 9 / 1(v/v)	3 mL/min	4 min	
6	Dichloromethane / Ethanol = 100 / 2(v/v)	3 mL/min	4 min	
7	Hexane / Methyl *tert*-Butyl Ether = 9 / 1(v/v)	3 mL/min	4 min	
8	Methyl *tert*-Butyl Ether / Ethanol = 9 / 1(v/v)	3 mL/min	4 min	

Note: For analysis of methylclothiozide, 0.1% diethylamine was added to each mobile phase.

3. Results

The chromatograms obtained for bromacil using all 48 sets of conditions are shown in Fig. 4, and the optimum conditions for each of the chiral compounds are shown in Fig. 5.

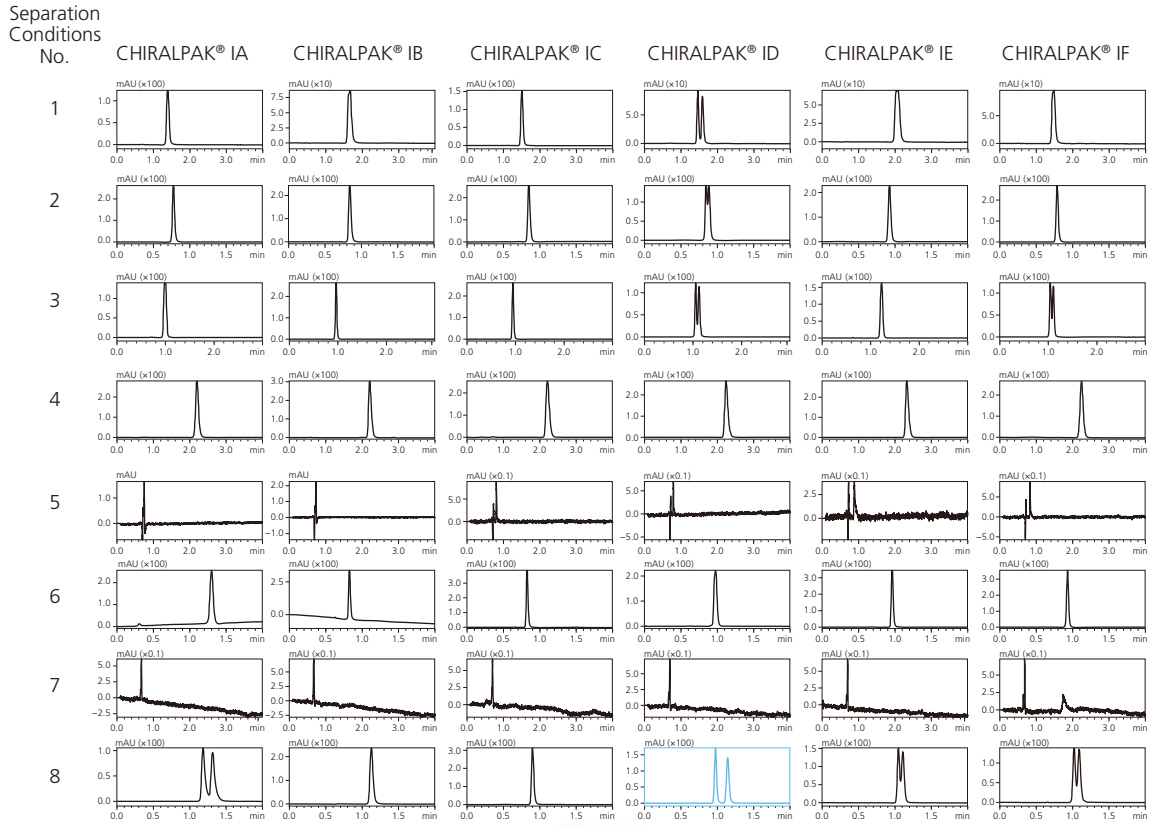
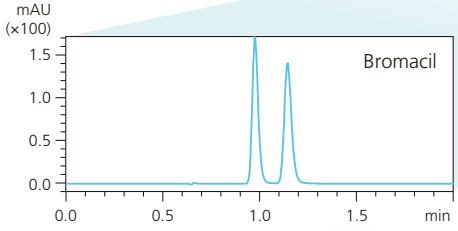
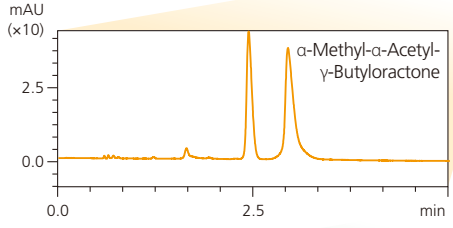


Fig. 4 Method Scouting Results for Bromacil



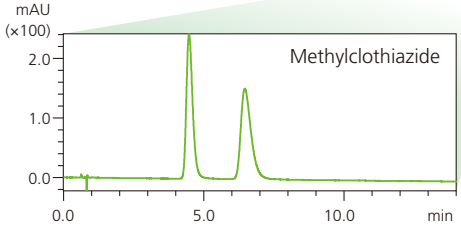
Separation Conditions 8 CHIRALPAK® ID

Peak	Retention Time t_n	Retention Factor k	Resolution Rs	Separation Factor α
1	0.974	0.522	—	—
2	1.142	0.784	2.643	1.504



Separation Conditions 3 CHIRALPAK® IF

Peak	Retention Time t_n	Retention Factor k	Resolution Rs	Separation Factor α
1	2.434	3.125	—	—
2	2.936	3.977	3.167	1.273



Separation Conditions 3 CHIRALPAK® ID

Peak	Retention Time t_n	Retention Factor k	Resolution Rs	Separation Factor α
1	4.465	6.202	—	—
2	6.449	9.401	3.724	1.516

Fig. 5 Chromatograms of Bromacil, α-Methyl-α-Acetyl-γ-Butyloractone and Methylclothiazide

4. Analysis

CLASS-Agent Report data processing software (Shimadzu Corp.) can quickly select the best separation conditions by comparing the data trace along with chromatographic parameters such as resolution, number of detected peaks, etc. With this software, it is possible to compare data both visually and quantitatively, thus making data processing more efficient.

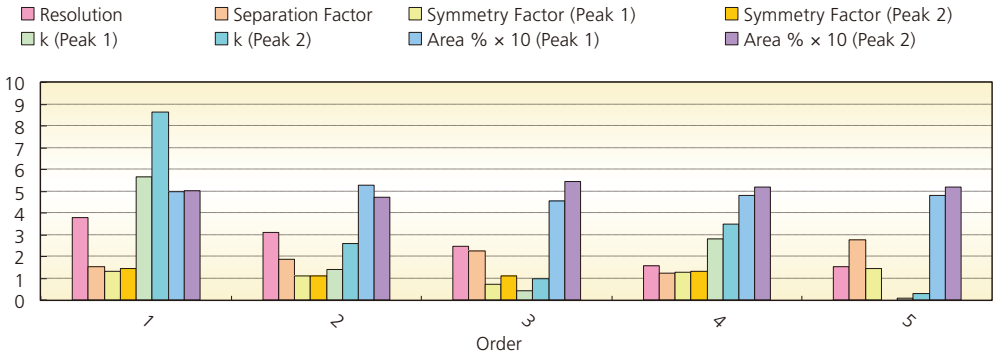


Fig. 6 Quantitative Comparison of Chromatograms Using CLASS-Agent Report

Table 2 Analysis Results for Methylclothiazide

Order	Run No.	Analytical Conditions	Resolution	Separation Factor	Symmetry Factor		k		Area %		No. of Peaks Detected
					Peak 1	Peak 2	Peak 1	Peak 2	Peak 1	Peak 2	
1	18	Methylclothiazide_ID_n-Hex_EtOH_3_analysis_B20%_14 min	3.785	1.523	1.31	1.463	5.665	8.626	49.777	50.223	2
2	27	Methylclothiazide_IF_MC_EtOH_6_analysis_B2%_4 min	3.086	1.858	1.127	1.094	1.39	2.583	52.748	47.252	2
3	6	Methylclothiazide_IB_MC_EtOH_6_analysis_B2%_4 min	2.456	2.248	0.715	1.094	0.443	0.995	45.633	54.367	2
4	13	Methylclothiazide_IC_n-Hex_EtOH_3_analysis_B20%_14 min	1.577	1.238	1.264	1.3	2.821	3.493	47.96	52.04	2
5	30	Methylclothiazide_IF_n-Hex_EtOH_4_analysis_B100%_18 min	1.515	2.759	1.465	0	0.102	0.282	48.153	51.847	2

5. Conclusion

Using the Nexera Method Scouting system and the *i*Chiral-6 high-resolution polysaccharide-based columns, it was possible to quickly determine through exhaustive analysis the best column and mobile phase for each of three types of chiral compounds. Further, by comparing such numerical values representing the resolution and symmetry index of each chromatogram using CLASS-Agent Report, it was possible to conduct a comparative evaluation of the chromatograms as well as a visual comparison, permitting greatly improved efficiency of data analysis.

We believe that the system and columns used in this research will be useful in a variety of markets including the synthetic drug discovery sector and pharmaceutical intermediates sector responsible for chiral analysis, new method development in pharmaceutical CMC departments, and chemical and food R&D.

References

1) Technical Report "Ultra Fast Method Scouting" (C190-E158)
2) Technical Report "Improved R&D Efficiency Through Speedier Method Development (3)" (C190-E159)

Acknowledgment

We wish to express our deep appreciation to Daicel Corporation for their guidance and cooperation for us to proceed with this study.

Note: CHIRALPAK is a registered trademark of Daicel Corporation.

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Technical Report

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

Akihiro Kunisawa[1], Yusuke Osaka[1], Daiki Fujimura[1], Satoru Watanabe[1], Shinichi Kawano[1]

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 on line 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

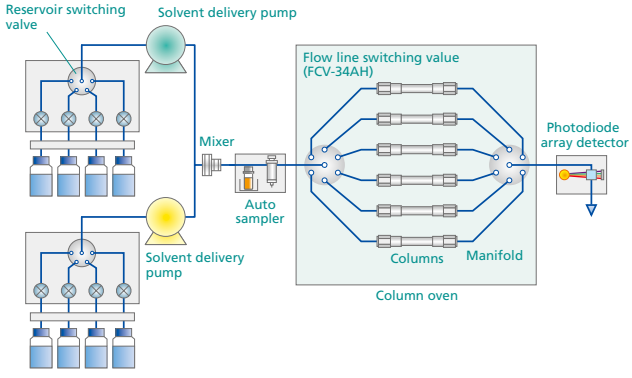


Fig. 2 Flow Line Diagram of Nexera Method Scouting System (Shown with Six Types of Columns)

1 Analytical & Measuring Instruments Division

2. Simultaneous Analysis of Sulfa Drugs Using Method Scouting Solution

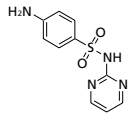
This section describes an example of scouting conditions for simultaneous analysis of sulfa drugs, which are used in synthetic antibacterial agents, anticancer agents, and other pharmaceuticals. The initial conditions considered for the sulfa drugs are shown in Figure 3. In this case, a total of four 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

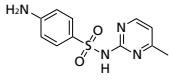
The main window displayed in Method Scouting Solution is shown in Figure 4. It shows the various parameters for columns, mobile phases, sample information, gradient conditions, and so on, and corresponding icons that can be clicked to easily change the respective parameters.

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 ÿnished (shutdown). Consequently, the software can signiÿcantly reduce the amount of time and effort required to specify conditions and execute analyses for method scouting.

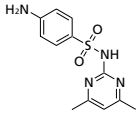
Analytes: Nine Sulfa Drug Compounds



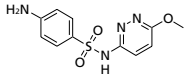
1. Sulfadiazine



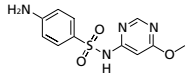
2. Sulfamerazine



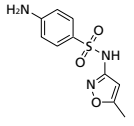
3. Sulfadimidine



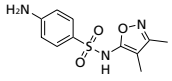
4. Sulfamethoxypyridazine



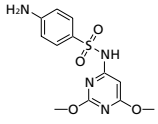
5. Sulfamonomethoxine



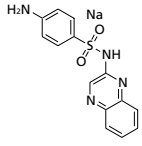
6. Sulfamethoxazole



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.

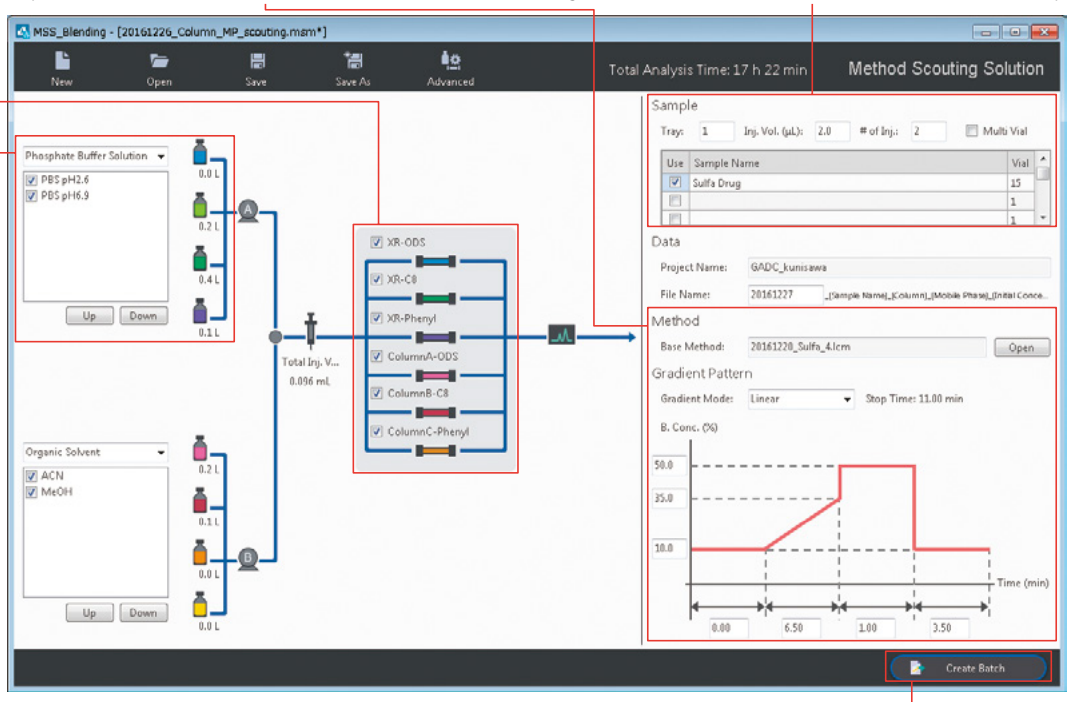


Fig. 4 Method Scouting Solution (Main Window)

(4) Create batch and start scouting.

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

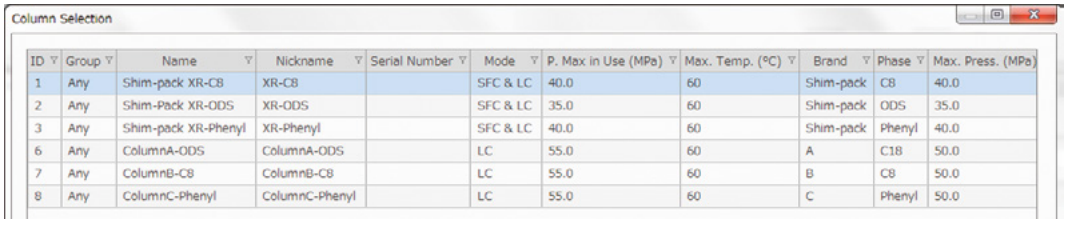
Clicking on a mobile phase or column icon in Method Scouting Solution displays a list of columns or mobile phases registered in the database (Fig.‘5). This window is used to select the mobile phases or columns used to scout for analytical conditions.

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 speciÿed for automatically blending solvents B and C at a 50:50 ratio*. Conditions were also speciÿed 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 speciÿed 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 speciÿed 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

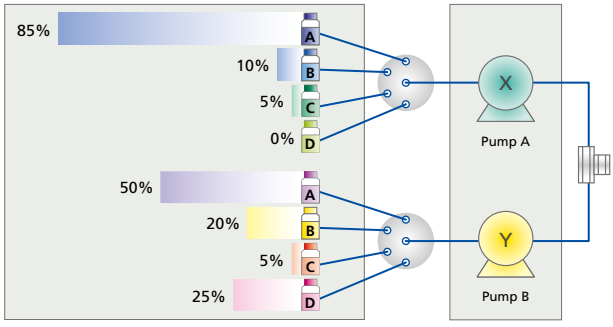


Fig. 6 Mobile Phase Blending Function

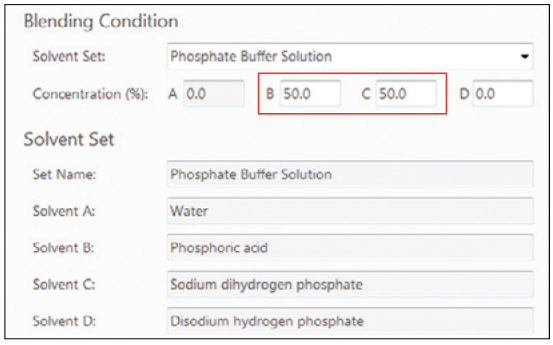


Fig. 7 Buffer Solution Prepared Using the Mobile Phase Blending Function (example of settings for a pH 2.6 (sodium) phosphate buffer solution)

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 proÿle 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 …owrate, oven temperature, and detection wavelength). Therefore, analytical conditions can also be scouted using the gradient conditions speciÿed in the basic method.

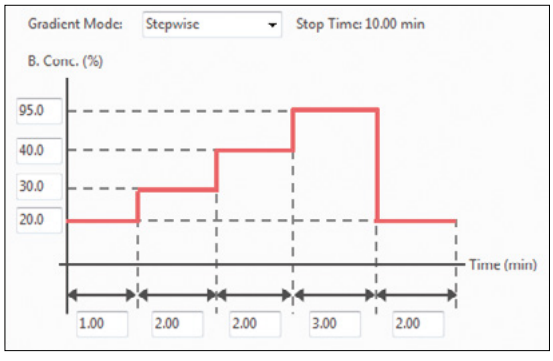


Fig. 8 Stepwise Mode

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.

Click [Create Batch] to display the [Preview] window.

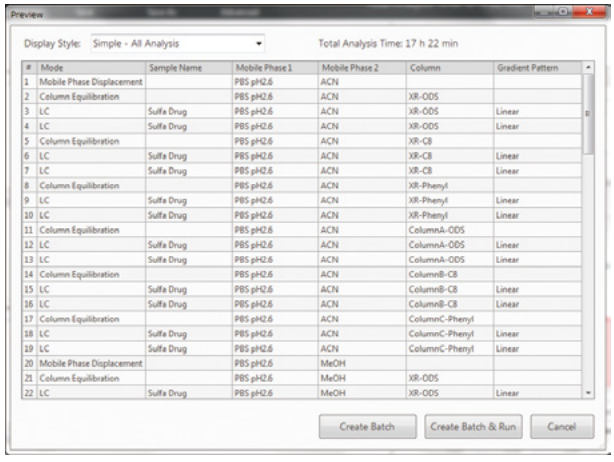


Fig. 9 Batch Creation Preview Window

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 Efÿciency Through Speedier Method Development (3)).

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

Equation 1 was conÿgured 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 ÿle 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 identiÿed 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.

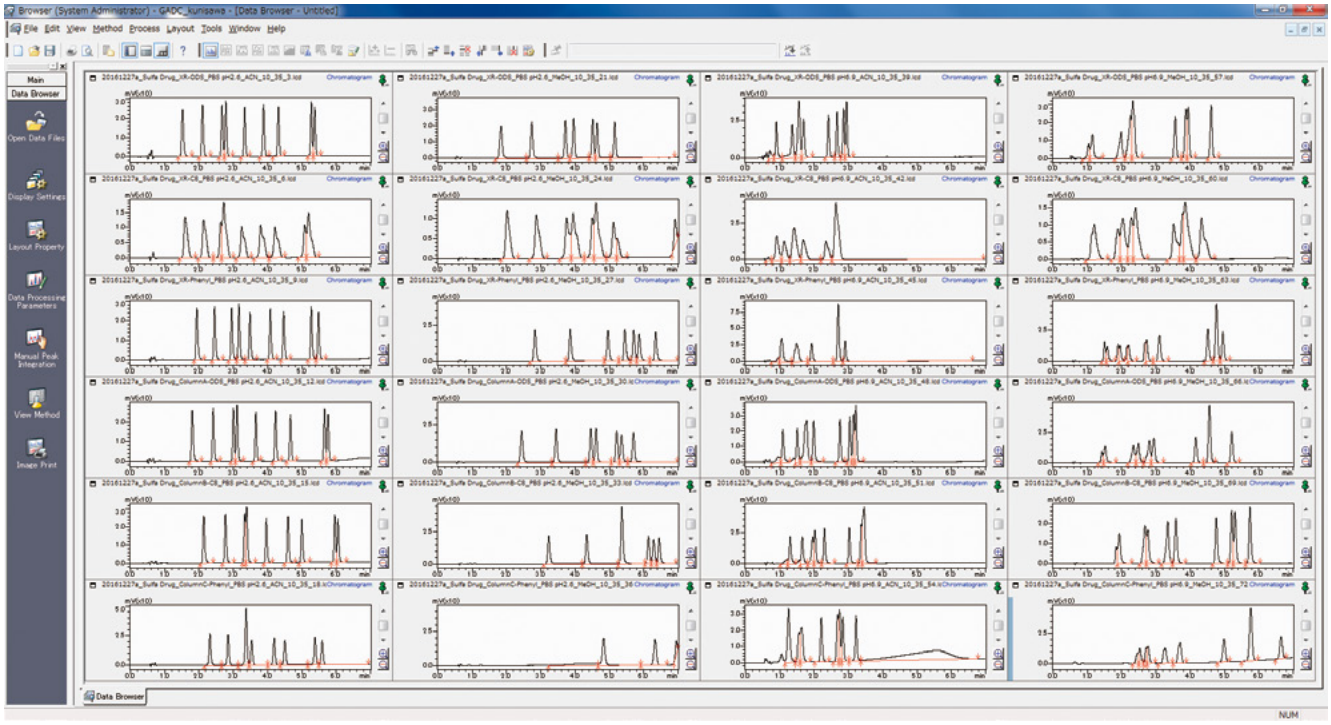
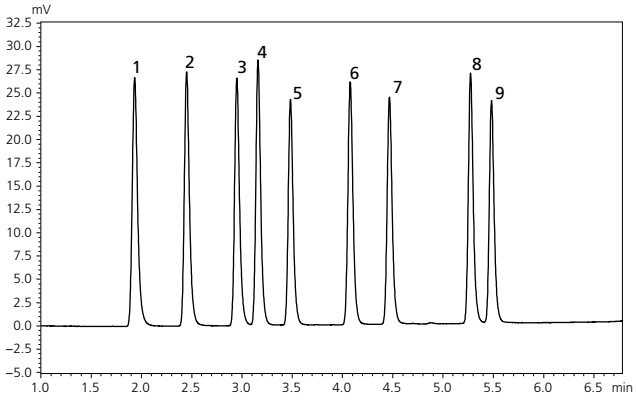


Fig. 11 Comparison of Chromatograms Using the Data Browser Function



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

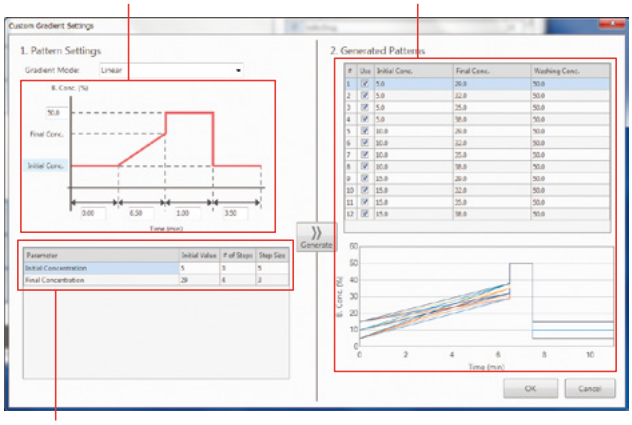
4. Optimizing Gradient Conditions

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, ÿnal concentration, washing concentration, and time program settings. In addition, multiple concentration patterns can be speciÿed for the initial and ÿnal concentration settings. In this example, the initial concentration was set to 5, 10, and 15 % and the ÿnal concentration was set to 29, 32, 35, and 38 %. Using all of these patterns for analysis results in 12 gradient condition conÿgurations. Evaluation results obtained using these 12 gradient condition conÿgurations are shown in Table 1. The resulting optimal analytical conditions and chromatogram are shown in Figure 14. Optimizing the initial and ÿnal 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.

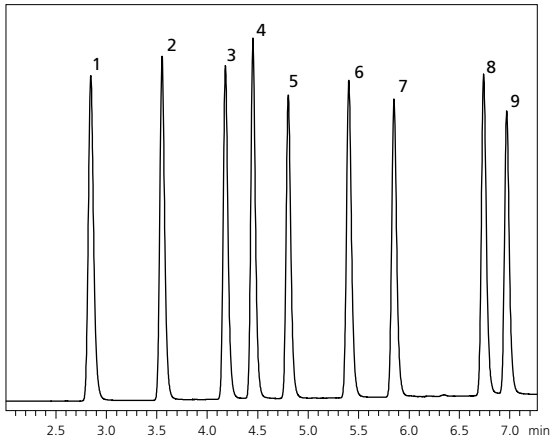
Fig. 13 Gradient Condition Settings Window

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 conÿgured 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 conÿgured 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 ÿle, by specifying the created template ÿle in advance.



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	: 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

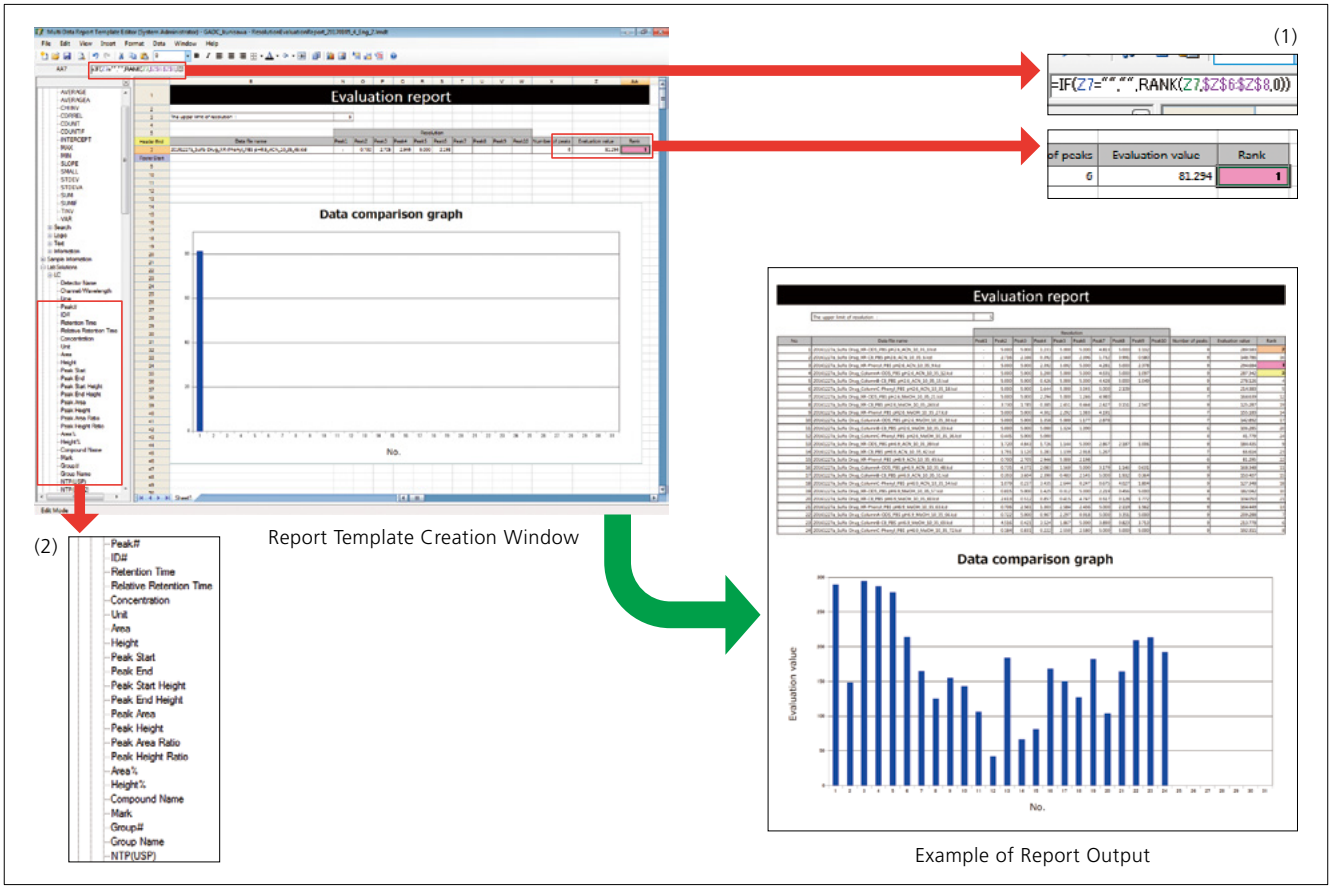


Fig. 15 Creating Multi-Data Reports

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 signiÿcantly 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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Solution 2: LC Method Transfer

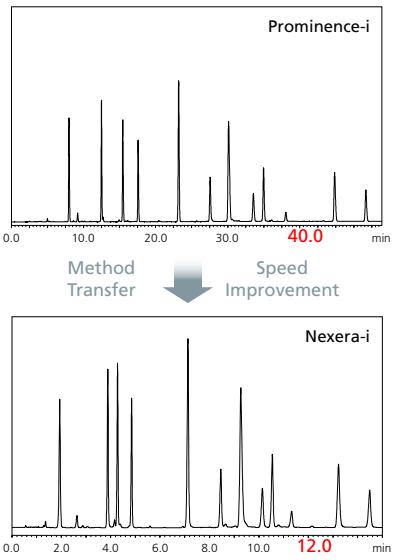
When the R&D department completes the development and verification of the LC method, it needs to be transferred to other laboratories such as QA/QC and CMO.

Due to the use of different analytical instruments and configurations, pharmaceutical companies may encounter difficulties with LC method transfer in their drug development and research processes. Issues such as irreproducibility of resolution and retention time may be faced.

Shimadzu understands this problem and developed the Nexera-iMT method transfer system. Its Analytical Condition Transfer and Optimization (ACTO) function achieves seamless transfer of LC analytical methods.

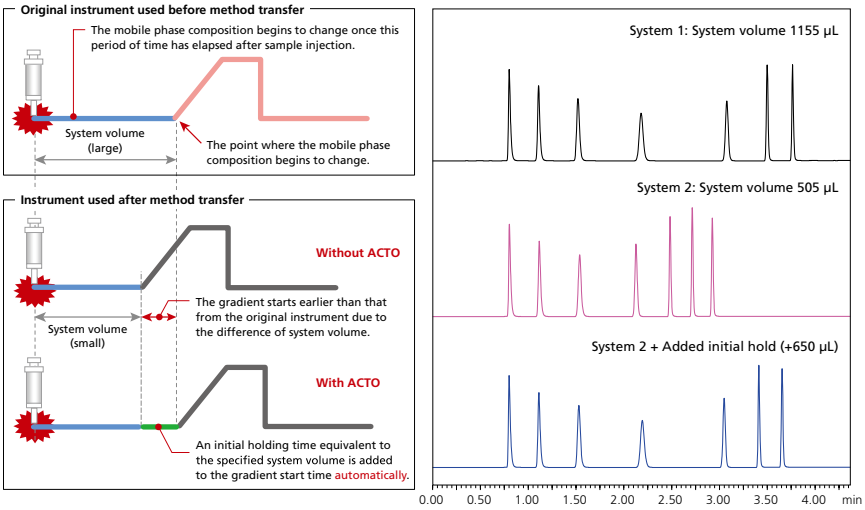
Nexera-iMT

i) Method transfer between HPLC and UHPLC



Method transfer of Cephem antibiotics

ii) Method transfer between systems with different delay volume



Retention time correction by adding an initial holding time

Excellent dual-flow method transfer system

- The new method transfer system based on the outstanding i-Series ensures excellent intersystem reproducibility. Unique dual-flow system is designed for both HPLC and UHPLC analysis and transfer.
- The new ACTO function can automatically adjust the delay volume to fit different systems. Within a few clicks, the new Nexera-iMT method can be generated.

Transfer methods easily

- Using Nexera-iMT, analytical methods can be freely converted to fit HPLC and UHPLC systems. This greatly simplifies the method transfer process and maximize laboratory efficiency.
- The chromatograms of the original method were perfectly reproduced, and the separation effect and retention time of the original condition were maintained, regardless of system differences such as instrument model and delay volume.

Nexera-iMT

C190-E202

SHIMADZU
Excellence in Science

Technical Report

Effective Analysis Management Achieved by Method Transfer between HPLC and UHPLC

Akihiro Kunisawa[1], Daiki Fujimura[1], Yusuke Osaka[1]

Abstract:

High performance liquid chromatography (HPLC) is widely used for the qualitative and quantitative analysis. In recent years, ultra-high performance liquid chromatography (UHPLC) has been developed signiÿcantly. Facilities that have both UHPLC and HPLC systems often apply newly established UHPLC method to ordinary HPLC as well as applying existing HPLC method to UHPLC. During such inter-system method application, the method must be modiÿed properly to meet the requirements from another system. This modiÿcation is called "method transfer". Method transfer requires to modify existing parameters such as °ow rate and/or time pr ogram.
Here, we describe the relationship between column dimensions including particle size of packing material and analytical conditions, then method transfer from HPLC to UHPLC for high speed analysis and that from UHPLC to HPLC for generalization of analytical conditions as well as that from Shimadzu's system to other vendor's. We also describe the Shimadzu integrated LC system "Nexera-i MT" that supports method transfer and ACTO (Analytical Conditions Transfer and Optimization) Function equipped in the latest LabSolutions LC software.

Keywords: HPLC, UHPLC, method transfer, ACTO, Nexera-i MT

1. Background

In pharmaceutical, food and various industrial fields, HPLC is widely used for the analysis of target compounds and related impurities. In recent years, UHPLC system that has more than 100 MPa of pressure tolerance affords more efficient analysis at ultra-high speed. Due to these features, UHPLC systems have been introduced in a variety of facilities where R&D departments often use UHPLC systems to develop an efficient analytical method then modify it to match HPLC analysis. The method transferred are then used by the QC department. Conversely, an existing HPLC method can be transferred into a UHPLC method to improve the speed and efficiency of analyses. In this report, we define method transfer as both directions of method modifications from HPLC to UHPLC and from UHPLC to HPLC.

We often face inadequate separation in gradient elution after a method transfer from HPLC where the separation is completed, to UHPLC (Fig.1). Furthermore, even though a method may have been created successfully using UHPLC analysis, the method transfer from UHPLC to HPLC gives different selectivity of separation, resulting in a degradation of efficiency for the entire laboratory.

Generally, a method transfer accompanied by employing different column dimensions including particle size must be required to modify analytical conditions such as flow rate and time program. However, to optimize conditions is not easy and method transfer can result in poor separation compared to those obtained prior to the method transfer. Keeping a consistent separation pattern before and after method transfer in terms of parameters, such as resolution and relative retention times, requires complex calculations based on pre- and post-transfer data, which include column dimensions (length, internal diameter, and particle size).

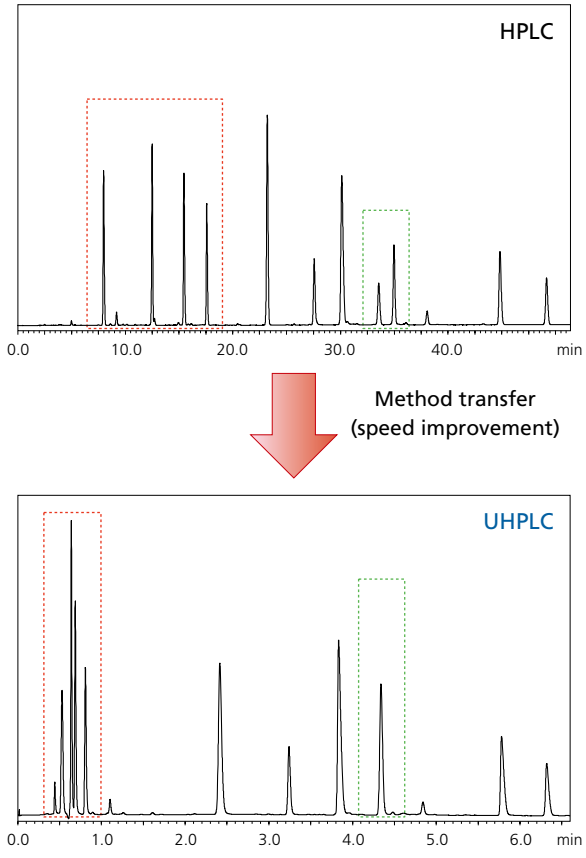


Fig. 1 Problems That Arise During Method Transfer (Analysis of cephem antibiotics)

1 Analytical & Measuring Instruments Division

2. Column Dimensions and Analytical Conditions

UHPLC analysis generally requires a column that is shorter, has smaller internal diameter, and smaller packing material size. However, once the column dimensions are modified, the existing analytical method must also be altered. This is because analytical conditions optimized for the HPLC analysis and the corresponding HPLC columns will not be optimal for the UHPLC analysis. The same is true when transferring a method from UHPLC to HPLC.

When a method is transferred, the main items that need to be changed are as follows.

- Flow rate
- **Time program**
- System delay volume
- Sampling rate
- Detector response
- Sample injection volume
- Upper pressure limit

Among these parameters, the time program is particularly important for gradient analyses. If an inappropriate gradient program is configured, it will result in an inferior separation after method transfer (*see* Fig. 1). Further, if the relative retention times and resolution are specified in testing regulations, the time program must be modified to keep the separation pattern. Due to these complications, it is much more efficient to modify a gradient program using theoretical calculations based on flow rate, column length, column internal diameter and system delay volume.

3. Example of Method Transfer

We have discussed method transfers from HPLC to UHPLC and UHPLC to HPLC. We will now describe examples of method transfer among multiple systems including Shimadzu's systems and other vendor's.

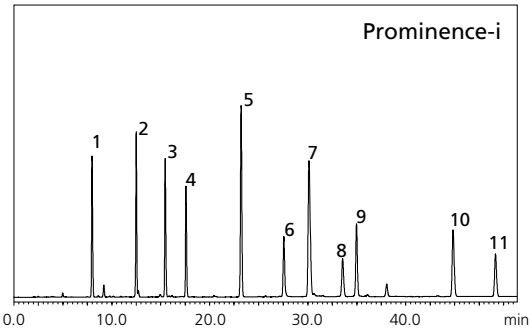
3-1. Improvement in the Speed of Analysis of Cephem Antibiotics

Cephem antibiotics are beta-lactam antibiotics that can be administered as an oral or injectable formulation. Here, we describe improving the analysis speed using a mixture of 11 cephem antibiotics as samples.

We used a Shimadzu Prominence-i system to analyze cephem antibiotics under HPLC conditions (Fig. 2a). Based on the column dimensions, we then modified the HPLC method such as the flowrate, sample injection volume, and time program for UHPLC analysis (*see* Fig. 2 for details). We used the UHPLC method to perform an analysis with a Shimadzu Nexera-i system. As seen from the results shown in Fig. 2b, we succeeded in improving the speed of the analysis of the cephem antibiotic mixture while keeping the separation pattern almost identical to that obtained from the HPLC analysis. The example shown in Fig. 1, which was a failed attempt at improving speed of analysis, also used cephem antibiotics as an analysis sample. These results show that appropriate analytical conditions must be determined during method transfer.

Improving the speed of an existing analysis leads to higher overall efficiency. For example, although quality control tests may be performed according to the test methods registered with regulatory authorities, process control tests may not be under the influence of such regulatory controls. In such cases, improving the speed of analysis during process control facilitates higher overall efficiency.

(a)



■ Peaks
1. Cefadroxil, 2. Cefapirin, 3. Cefalexin, 4. Cefradine, 5. Cefotaxime, 6. Cefazolin, 7. Cefuroxime, 8. Cefoxitin, 9. Cefoperazone, 10. Cefalothin, 11. Cefamandole.

■ Analytical Conditions

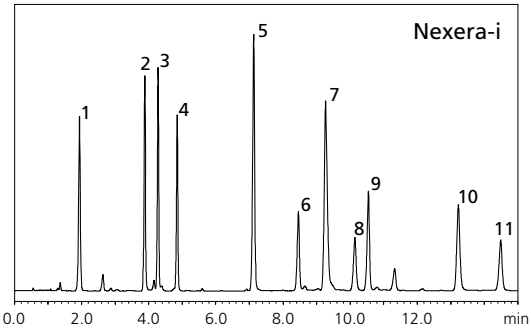
Column	: Shim-pack VP-ODS (250 mm L. × 4.6 mm I.D., 5.0 µm)
Mobile phase A	: 0.1 % formic acid
Mobile phase B	: Acetonitrile
Initial concentration	: 5% B
Flowrate	: 1.0 mL/min
Temp.	: 45 °C
Detection	: Ultraviolet-visible absorbance detector (260 nm)
Injection volume	: 10 µL

Time program

Time	Unit	Func.	Value
2.00	Pump	B.Conc	5
50.00	Pump	B.Conc	35
55.00	Pump	B.Conc	35
55.10	Pump	B.Conc	5
65.00	Controller		Stop

Method transfer
(speed improvement)

(b)



■ Analytical Conditions

Column	: Shim-pack XR-ODS II (100 mm L. × 3.0 mm I.D., 2.2 µm)
Flowrate	: 1.00 mL/min
Injection volume	: 4 µL

Time program

Time	Unit	Func.	Value
0.51	Pump	B.Conc	5
12.76	Pump	B.Conc	35
14.04	Pump	B.Conc	35
14.06	Pump	B.Conc	5
17.00	Controller		Stop

Note: Mobile phases A and B and initial concentrations are identical to those before method transfer

Fig. 2 An Example of Method Transfer (Cephem antibiotics)

3-2. Converting a Method for Sulfa Drug Analysis for General Use

Sulfa drugs (sulfonamides) is a generic term given to synthetic antibacterial drugs and chemotherapy drugs that contain a 4-aminobenzenesulfonamide moiety. These drugs are used to treat infections and for other purposes. Here, we describe an example of developing an efficient UHPLC analysis method for a sample containing nine sulfa drugs and transferring this UHPLC method to HPLC conditions. Using this HPLC method, we then performed an analysis using a Shimadzu Prominence-i system and another vendor's system and verified the compatibility of the method with these systems.

UHPLC analysis was performed using a Shimadzu Nexera-i MT system. As seen from the chromatogram in Fig. 3a, separation of the nine sulfa drugs was achieved in around 5 min.

In order to transfer this method from UHPLC to HPLC, the column was changed from Shim-pack XR-ODS II (75 mm L. × 3.0 mm I.D., 2.2 µm) to Shim-pack VP-ODS (150 mm L. × 4.6 mm I.D., 5.0 µm). Based on this column information, we then modified the time program, flow rate, sampling rate, and other parameters (for method transfer to HPLC) by using the Analytical Conditions Transfer and Optimization (ACTO) function (described later).

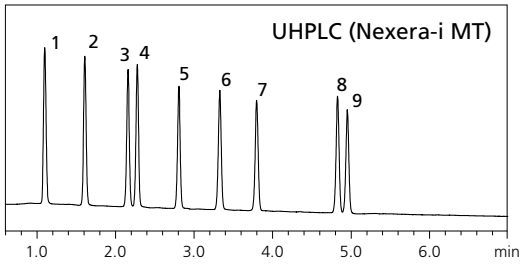
We then performed the HPLC analysis on the sulfa drugs using the new method and using the same Nexera-i MT system. We successfully separated the sulfa drugs in around 20 min. As shown in the chromatogram in Fig. 3b, an appropriate method transfer enabled us to achieve a separation almost equivalent to that obtained with UHPLC.

Due to compatibility of Nexera-i MT system with a variety of other systems, we also performed the same analyses on other systems. Almost identical chromatograms were obtained when the analyses were performed on a Prominence-i system and another vendor's system using the same method (Fig. 3c). The table at the bottom left of Fig. 3 shows the retention times and percentage errors for each compound compared to the results obtained using the Nexera-i MT system. The retention time percentage error is within 2 % for almost all compounds.

The examples presented here suppose that methods created for UHPLC by R&D departments are transferred to methods for HPLC. In addition, they also suppose that those HPLC methods will be used in systems in other departments. As can be seen from the results, transfer can be done without any differences in the separation patterns.

Furthermore, if there is compatibility among systems, then comparable analytical results can be obtained even using other systems. For this reason, the same analysis can also be performed by departments that have different systems from R&D department.

(a)



■ Peaks

1. Sulfadiamine, 2. Sulfamerazine, 3. Sulfadimidine, 4. Sulfamethoxypyridazin,
5. Sulfamonomethoxine, 6. Sulfamethoxazole, 7. Sulÿsoxazole,
8. Sulfadimethoxine, 9. Sulfaquinoxaline sodium

■ Analytical Conditions

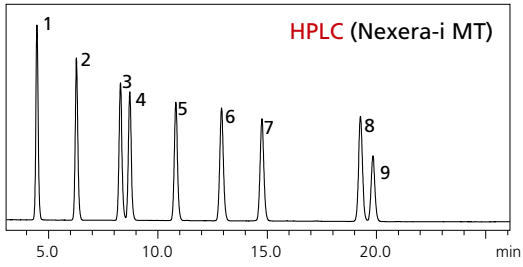
Column	: Shim-pack XR-ODS II (75 mm L. × 3.0 mm I.D., 2.2 µm)
Mobile phase A	: 0.5 % acetic acid
Mobile phase B	: Acetonitrile
Initial concentration	: 35% B
Flowrate	: 1.2 mL/min
Temp.	: 40 °C
Detection	: Photodiode array ultraviolet-visible absorbance detector
Injection volume	: 2 µL

Time program

Time	Unit	Func.	Value
7.00	Pump	B.Conc	35
7.01	Pump	B.Conc	10
10.00	Controller		Stop

Method transfer
(for general use)

(b)



■ Analytical Conditions (changes only)

Column	: Shim-pack VP-ODS (150 mm L. × 4.6 mm I.D., 5.0 µm)
Flowrate	: 1.24 mL/min
Injection volume	: 5 µL
Internal volume adjustment	: +1364 µL

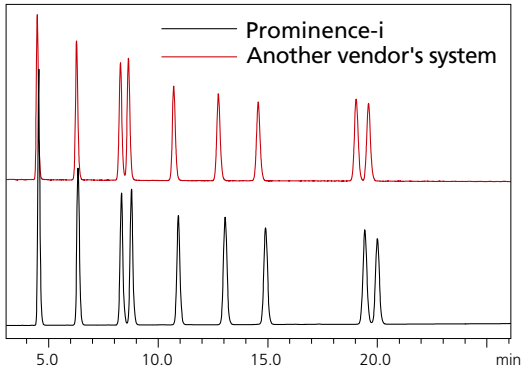
Time program

Time	Unit	Func.	Value
31.85	Pump	B.Conc	35
31.90	Pump	B.Conc	10
45.00	Controller		Stop

Note: Other conditions are identical to conditions before transfer

Analysis with
other systems

(c)



Component	Nexera-i MT	Prominence-i		Another vendor's system	
	RT (min)	RT (min)	Error	RT (min)	Error
1	4.649	4.747	2.12%	4.617	–0.68%
2	6.628	6.716	1.33%	6.590	–0.58%
3	8.817	8.878	0.68%	8.769	–0.55%
4	9.306	9.397	0.97%	9.196	–1.19%
5	11.521	11.647	1.09%	11.370	–1.31%
6	13.648	13.812	1.20%	13.439	–1.53%
7	15.586	15.747	1.03%	15.344	–1.56%
8	20.239	20.413	0.86%	19.951	–1.43%
9	20.834	21.007	0.83%	20.533	–1.45%

Fig. 3 Method Transfer and Compatibility with Another Vendor's System

4. Nexera-i MT and ACTO Function

We describe the Shimadzu Nexera-i MT integrated liquid chromatograph system and the ACTO function included in the Shimadzu LabSolutions workstation software, which are products that support method transfer.

4-1. Nexera-i MT Integrated Liquid Chromatography System

Nexera-i MT is an excellent method transfer system from existing LC systems. Nexera-i MT has dual flow lines, one is for HPLC and the other for UHPLC, and can switch these flow lines during a method. This system offers following two advantages:

- The ability to switch HPLC flow line and UHPLC flow line enables the transfer of UHPLC methods to general use (HPLC) methods and to transfer general use methods to UHPLC methods within a single system.
- Attaching an optional flow line to the standard HPLC flow line allows for method compatibility with liquid chromatography (LC) systems that have different system volumes.

Combining the Nexera-i system with the ACTO function described below facilitates smooth method transfer in a variety of applications.

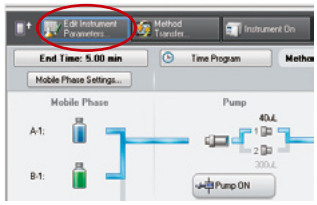
Fig. 4 Nexera-i MT

4-2. ACTO Function (Method Transfer)

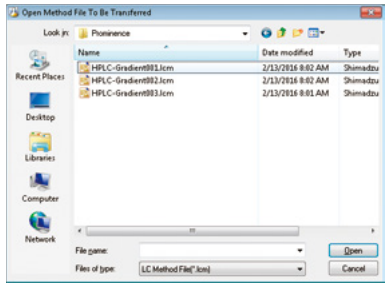
ACTO, which is included in the latest version of LabSolutions, is an efficient method transfer tool provided by Shimadzu. Here we describe one of ACTO's functions called "Method Transfer."

For improving the speed of an existing analysis performed on a HPLC system by converting it for use on a UHPLC system or for converting an analysis performed on an UHPLC system for general use with HPLC, modifications need to be made to the existing analysis methods.

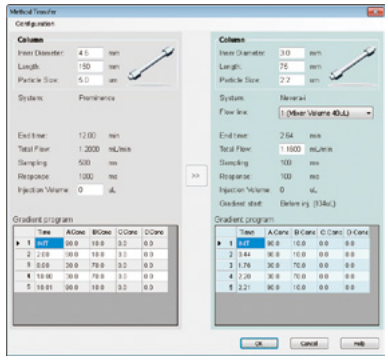
However, transferring a method requires complex calculations based on the internal diameter of the column, column length, particle diameter, and other parameters for determining an appropriate post-transfer flow rate and time program. This task can be simplified by using the "Method Transfer" tool included in LabSolutions. When this tool is used, a method is created automatically by just selecting an existing method, and entering the column information and some other parameters. Automatically created methods can be incorporated in LabSolutions software with a single click of a button; this streamlines the work flow from method conversion to analysis. Method Transfer is a powerful tool and can be utilized in a variety of situations to carry out efficient method transfers.



Step 1: Tool Startup



Step 2: Method File Selection



Step 3: Column Specification

Step 4: Save as Method File

Fig. 5 Method Transfer Work Flow

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Technical Report

Improving Method Transfer by Adjustment of Gradient Delay

Akihiro Kunisawa[1], Daiki Fujimura[1], Yusuke Osaka[1]

Abstract:

In facilities that have number of high performance liquid chromatography (HPLC) systems, an existing method that gives proper result by one HPLC system is often applied to other HPLC systems (method transfer). However, due to the difference in system volume, pump characteristics, and liquid delivery mechanisms among systems, method transfer can yield different results even though the same method is used.
In this report, we ÿrst explain the gradient delay due to the system volume difference and the effect of the delay on separation. We then show an example of method transfer with adjustment of gradient delay using alkylphenones and UV absorbents as samples. We also describe the integrated liquid chromatograph (LC) system "i-Series" that supports to optimize method transfer and the newly designed function of "Analytical Conditions Transfer and Optimization (ACTO) " equipped in the latest version of LabSolutions software.

Keywords: HPLC, method transfer, ACTO, i-Series

1. Background

HPLC is used for the analysis of target compounds and their related impurities in a variety of applications including pharmaceutical and food products. Facilities that use HPLC systems create methods using their own original analytical conditions and/or specified testing regulations. The validated methods are then used with a number of other HPLC systems in many cases. In such situations, reproducibility (compatibility) among systems is an important factor as well as repeatability of measurements. Even when using the same method, different HPLC systems can give different chromatograms (Fig. 1). Particularly in gradient elution, retention times, resolution and other factors will be largely affected as a result of method transfer. For example, while an existing method may succeed in separating a target compound from co-existing impurities in one system, the same method may not succeed in separating these compounds in other systems. So it is often required to optimize analytical conditions for each individual system, which is an extremely time-consuming process. Such variations in retention time and separation are caused by difference in system volume and pump performance among systems (*see* section 2 for details). Especially in ultrahigh-speed analysis, even small difference in system volume can cause great difference in analysis results due to small volume of dedicated column.

Further, in pharmaceuticals, food and other fields where the test methods are specified by regulations, changes in analytical conditions are not permitted, which may be an issue.

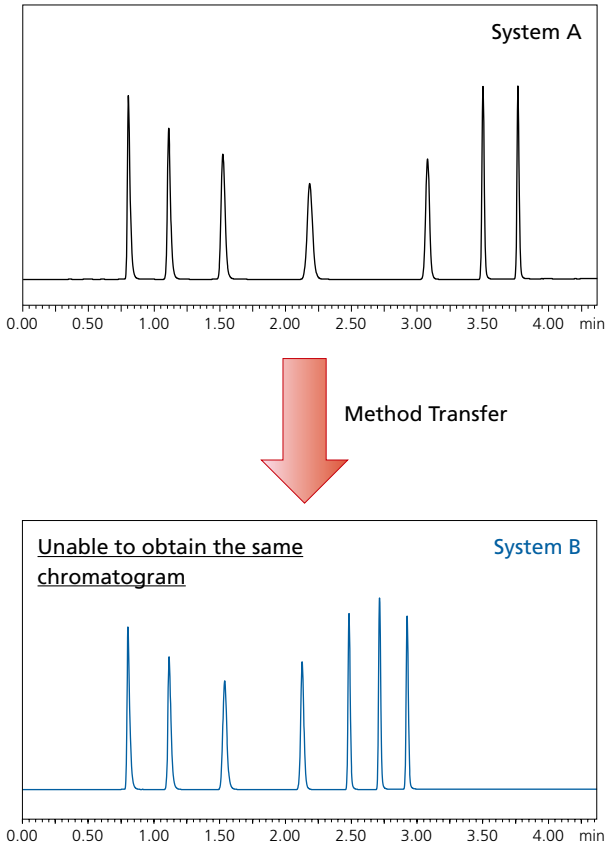


Fig. 1 Problems in Method Transfer

1 Analytical & Measuring Instruments Division

2. System Volume and Gradient Delay

System volume differences must be considered when transferring a method from one system to other systems.

Fig. 2 shows the flow line from the mobile phase reservoir to the column of LC system. Gradient delay volume means the system volume between the point where two or more eluents are mixed and the column inlet. As shown in Fig. 2, the gradient delay volumes are different for low-pressure gradient and high-pressure gradient systems. Even for the same type of gradient system, different lengths and/or internal diameters of piping can provide different gradient delay volumes.

Fig. 3 shows how gradient delay affects separation. In general, even if gradient has already started on the time program, the actual gradient start time (time to increase an organic solvent concentration) is delayed. If the result obtained from a system with a large system volume (Fig. 3, right) is compared to that from a system with a small system volume (Fig. 3, left), we can see the gradient start time is delayed more. This can cause different separation patterns on different systems.

Consequently, system volume difference must be considered when transferring a method and the gradient program must be modified by making an adjustment to the initial hold time (gradient start time). Nevertheless, gradient programs cannot be modified when the analytical conditions are strictly defined by the testing regulations.

3. Correction of Gradient Delay

We have discussed differences in chromatograms caused by method transfer and the origin of these differences. Next, we describe an example analysis and method transfer using multiple LC systems that have different system volumes.

3-1. Alkylphenone Analysis

Alkylphenones are aromatic ketones that can be analyzed by reversed phase chromatography. We analyzed alkylphenones using two systems of different system volumes and compared the chromatograms obtained from each system. We then adjusted the difference in retention time between the two systems by adding an initial hold time.

The results are shown in Fig. 4. A comparison of the two chromatograms (Fig. 4, top and middle) shows differences between the peaks in the latter half of the chromatograms. These chromatograms were obtained from analyses performed under identical conditions but different systems. The system volumes of system 1 and 2 are 1155 µL, 505 µL, respectively. Therefore, there is a difference of 650 µL between the two systems. This difference in the system volumes leads to different separation. The chromatogram at the bottom of Fig. 4 was obtained via an analysis using system 2, after adding an initial hold time equivalent to 650 µL. This chromatogram is almost identical to that obtained from system 1; this confirms that equivalent results can be obtained from different systems by correcting for system volume difference.

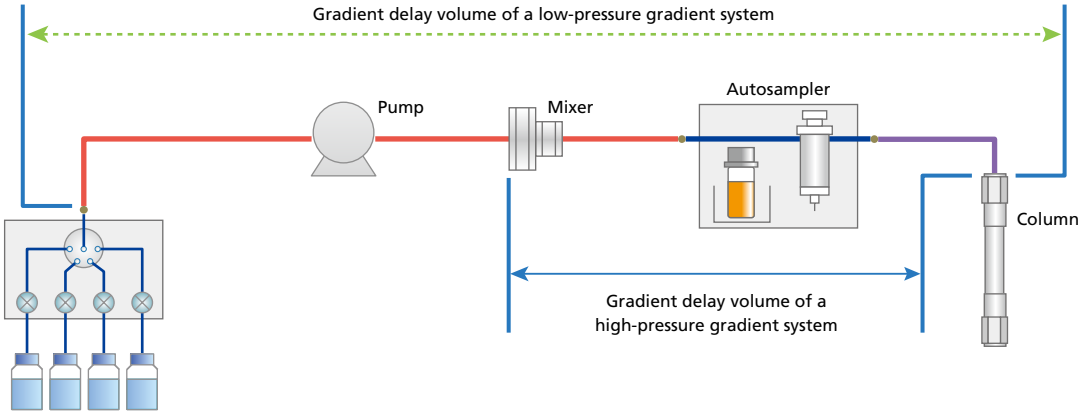


Fig. 2 Gradient Delay Volumes (System Volumes)

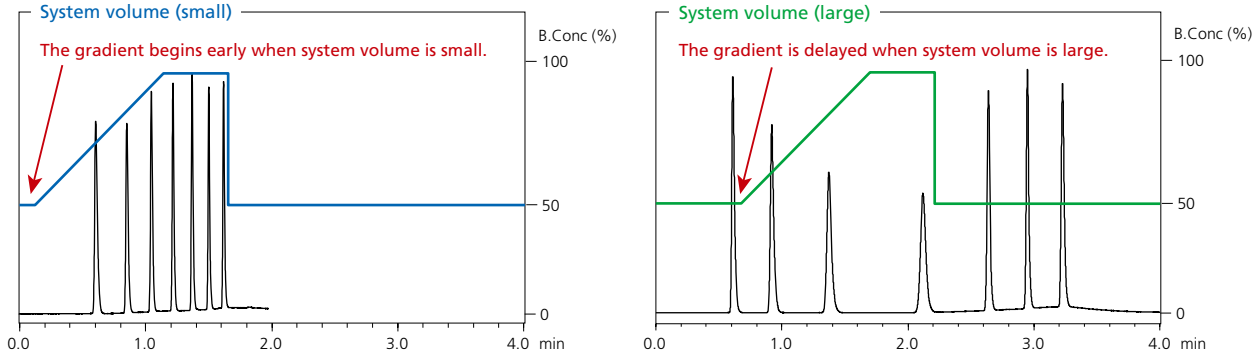
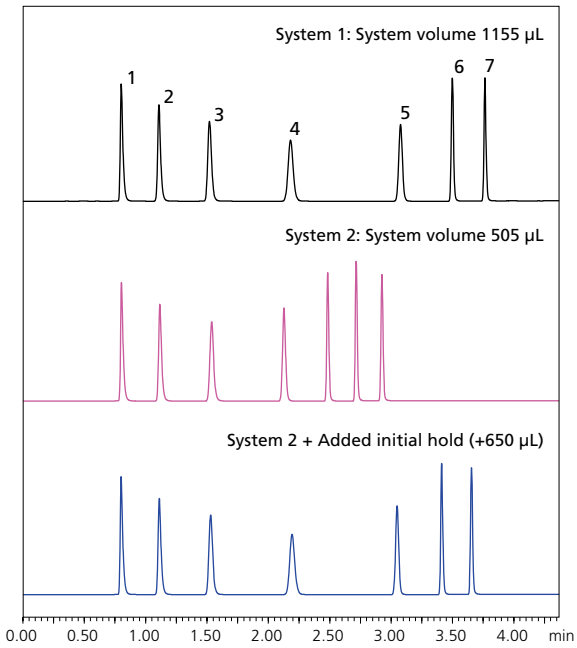


Fig. 3 System Volume and Gradient Delay



■ Peaks
1. Acetophenone, 2. Propiophenone, 3. Butyrophenone, 4. Valerophenone, 5. Hexanophenone, 6. Heptanophenone, 7. Octanophenone

■ Analytical Conditions

Column	: Shim-pack XR-ODS II (75 mm L. × 3.0 mm I.D., 2.2 µm)
Mobile phase A	: Water
Mobile phase B	: Acetonitrile
Initial concentration	: 60% B
Flow rate	: 0.8 mL/min
Temp.	: 40 °C
Detection	: Photodiode array absorbance detector
Injection volume	: 10 µL

Time program

Time	Unit	Func.	Value
0.93	Pump	B.Conc	60
1.59	Pump	B.Conc	95
3.50	Pump	B.Conc	95
3.51	Pump	B.Conc	60
6.00	Controller		Stop

Fig. 4 Different Retention Times Due to Different System Volumes and Their Correction by Addition of an Initial Hold Time (Alkylphenones)

3-2. Analysis of UV Absorbents

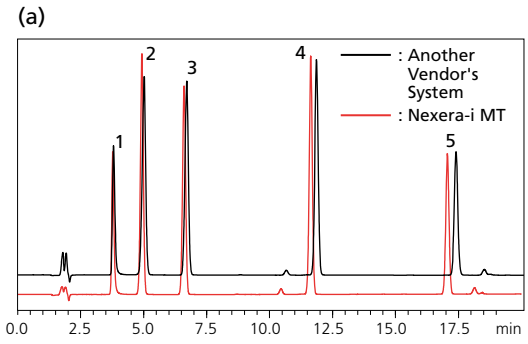
In this section, we describe an example method transfer to a Shimadzu system from another vendor's HPLC system using a sunscreen mixture.

Fig. 5a shows the chromatogram obtained from the analysis of a sunscreen mixture using Shimadzu's Nexera-i MT and another vendor's system. Although these two analyses were performed using identical methods, a huge difference in the peaks after 10 min is observed. This difference is caused by the difference in system volumes of the two systems and is similar to the analysis described earlier in this report.

Using ACTO's gradient start time adjustment function equipped in the Shimadzu LabSolutions workstation software, we adjusted the gradient start time correct the difference in system volumes and performed the analysis. As seen from Fig. 5b and the inset table in Fig. 5, the retention times were almost identical for all peaks.

Using this approach, compatibility between Shimadzu system and other vendors' system can be achieved by adjusting the gradient start time. This means that an adjustment in the gradient start time enables smoother method transfer.

The United States Pharmacopeia (USP) provides the following description to correct for errors between systems:2) "If adjustments are necessary, change in column packing (maintaining the same chemistry), the duration of an initial isocratic hold (when prescribed), and/or dwell volume adjustments are allowed." In other words, the adjustment of initial hold time (or gradient start time adjustment) does not fall under method change, and therefore, does not require revalidation.



■ Peaks
1 to 5: Sunscreen mixture

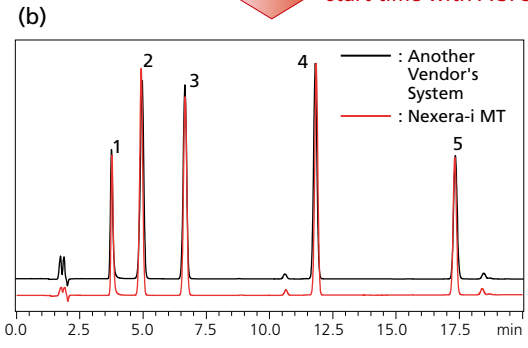
■ Analytical Conditions

Column	: Shim-pack VP-ODS (150 mm L. × 4.6 mm I.D., 5.0 µm)
Mobile phase A	: 20 mmol/L (Sodium) phosphate buffer solution (pH 2.5)
Mobile phase B	: Acetonitrile
Initial concentration	: 30% B
Flow rate	: 1.0 mL/min
Temp.	: 60 °C
Detection	: Photodiode array absorbance detector
Injection volume	: 10 µL
Flow line used	: HPLC Łow line (Nexera-i MT)

Time program

Time	Unit	Func.	Value
2.00	Pump	B.Conc	30
25.00	Pump	B.Conc	70
25.10	Pump	B.Conc	30
32.00	Pump	B.Conc	30
35.00	Controller		Stop

Adjustment of gradient start time with ACTO



■ Analytical Conditions (changes only)
Gradient adjustment : +340 µL (Nexera-i MT)

Retention Time Error (%) Compared to Another Vendor's System

Component	Before gradient adjustment	After gradient adjustment
1	0.29	0.32
2	1.16	1.26
3	1.03	0.16
4	1.38	–0.38
5	1.46	–0.05

Fig. 5 Example Method Transfer with Gradient Adjustment (Sunscreen mixture)

4. i-Series Integrated Liquid Chromatograph System and the ACTO Function

This report has described examples of retention time differences caused by different system volume and adjustment of the initial hold time (adjusting the gradient start time). We now describe the Shimadzu i-Series integrated liquid chromatograph system and the ACTO function equipped in the Shimadzu LabSolutions workstation software that supports a variety of method transfers.

4-1. i-Series Integrated Liquid Chromatograph System

The i-Series is Shimadzu's product line of integrated liquid chromatographs that contain all the functions required for LC analysis in a compact unit. These functions have been optimized for ease of operation and maintenance. Using standard piping or attaching the optional compatibility kit enables the use of i-Series systems with system volumes compatible with other Shimadzu systems and other vendors' systems. This provides good reproducibility between systems when performing analyses using existing methods.

Shimadzu's workstation software also includes the ACTO function, as mentioned earlier, which is designed specifically for the i-Series and enables smooth method transfer.

Fig. 6 i-Series Integrated LC System (Nexera-i)

4-2. ACTO Function

ACTO, which is equipped in the latest version of LabSolutions, is an efficient method transfer tool provided by Shimadzu. Here we describe one of ACTO's functions called "gradient start time adjustment function."

Transferring an analytical method from an existing LC system to another system can cause differences in retention times because of the differences in system volume and specifications of solvent delivery unit. This problem can be resolved using ACTO's gradient start time adjustment function. The gradient adjustment function is configured during method creation. If a user simply enters the difference in system volume, then the corrected initial hold time is automatically added or subtracted during analysis. This enables the acquisition of identical chromatograms before and after method transfer. The function can also correct subtle errors that cannot be considered by the compatibility kit (e.g., pump characteristics and solvent delivery mechanism) and can achieve optimal compatibility. This adjustment is configured in a method separately from the time program. Thus, reconfiguration of an existing time program is unnecessary.

Consequently, using Shimadzu's i-Series instruments and the ACTO function can provide higher efficiency and reliability during method transfer in a variety of applications.

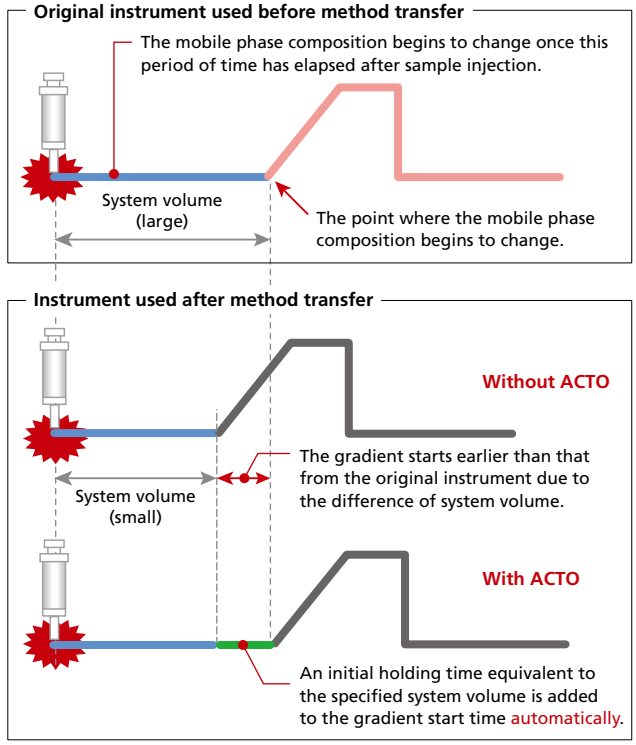


Fig. 7 Adjusting the Gradient Start Time

First Edition: August, 2016

Shimadzu Corporation
www.shimadzu.com/an/

Solution 3: Sample Preparation and Purification

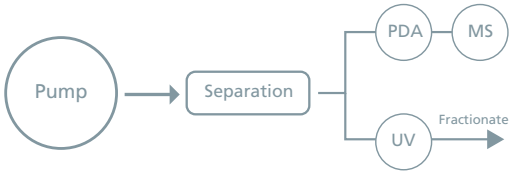
The preparation and purification of drugs is an indispensable part of the pharmaceutical industry. In the process of drug synthesis, both principal components and impurities need to be prepared to obtain sufficient amounts of a single compound for research and other purposes.

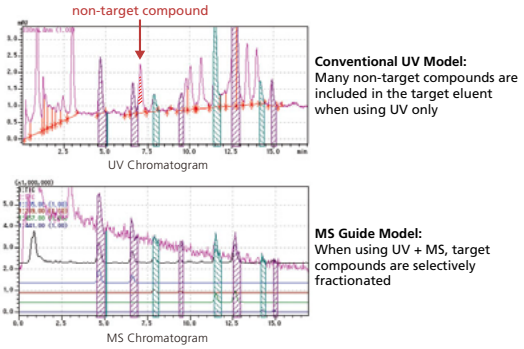
The purity, speed and cost of preparation are important factors that pharmaceutical companies need to consider when conducting separation and preparation.

Shimadzu offers flexible and comprehensive preparation and purification system solutions to meet the individual needs of different users.

Preparative HPLC System

Preparative LCMS System

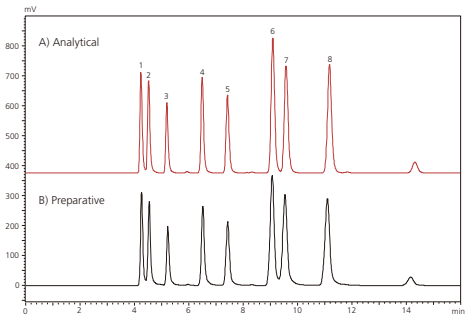




Conventional UV Model:
Many non-target compounds are included in the target eluent when using UV only

MS Guide Model:
When using UV + MS, target compounds are selectively fractionated

Analytical/Preparative System

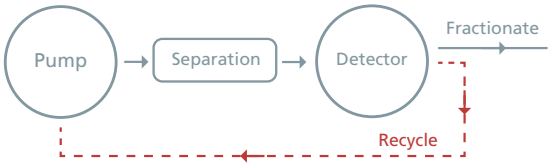


Example of scale-up with the same column resins

Customize prep-LC system for your dedicated needs

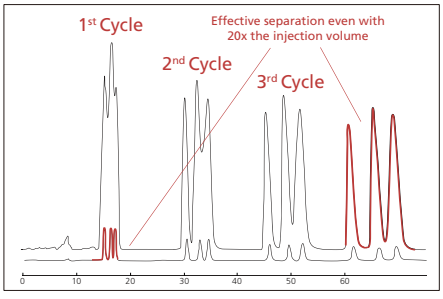
- Based on the expandable Prominence series, Shimadzu's preparative LC system covers a wide range of configurations from analytical to preparative, making it easy to prepare different grades of products.
- Customization to preparative LCMS system, analytical/preparative system or recycle preparative system can greatly improve laboratory productivity while satisfying more uses.

Recycle Preparative System



Achieve higher preparative efficiency

- These versatile configurations and freely selected detector types ensure that you don't miss any impurities.
- Analytical/preparative system makes the preparation scale-up more convenient by supporting efficient transfer of analytical condition to preparative condition with the dual-flow system.
- Through the recycle preparative system, the column length and column capacity can be expanded, and the resolution can be greatly improved under limited conditions, while avoiding a large consumption of mobile phase



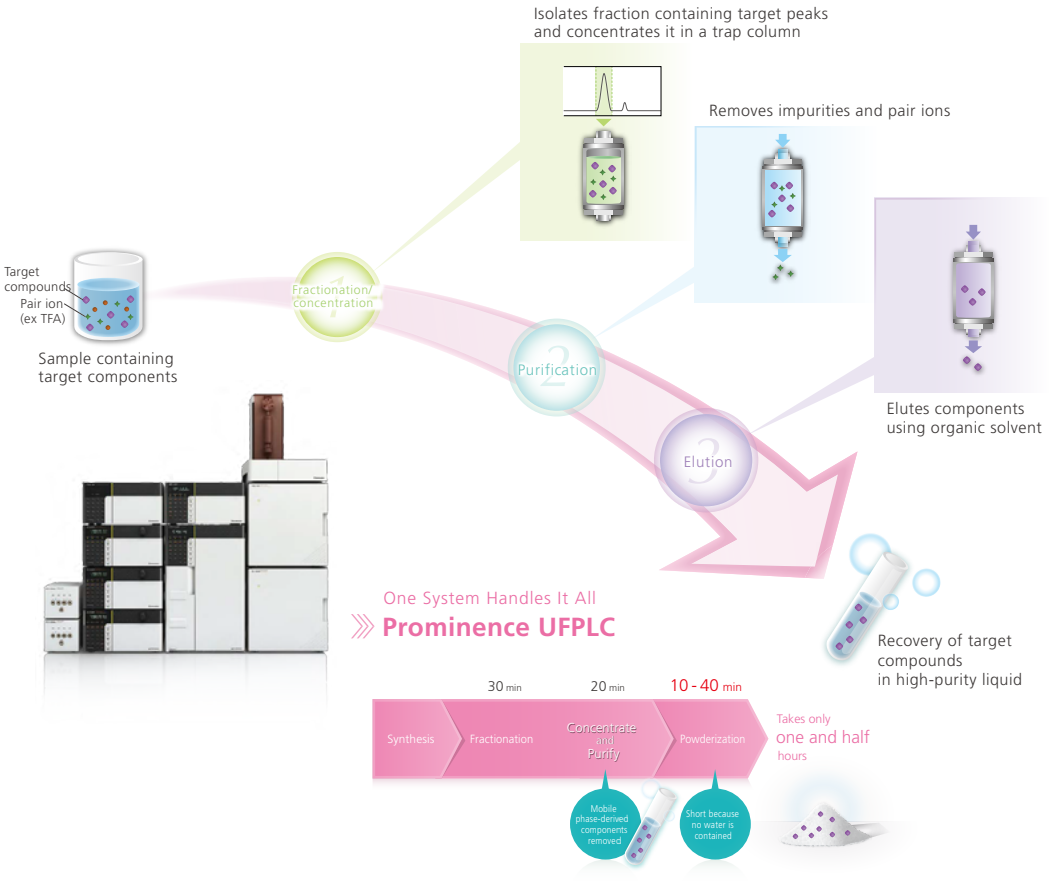
Prominence UFPLC

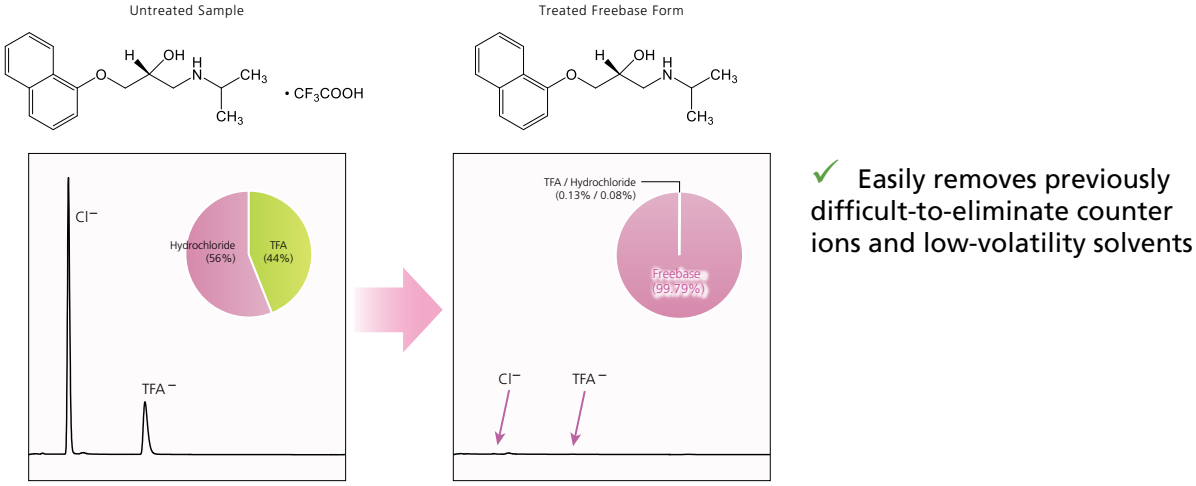
A true complete purification system: from mixtures to high-purity compounds

- The brand new Prominence UFPLC is the ready-to-use system package for online coupling of preparation and purification. All preparative processes can be performed online, from fractionation to concentration, purification, and recovery.
- The flexibility of modular configuration allows online purification of up to 5 components detected in the fractionation step, facilitating the evaluation of sub-components and impurities.

Collect high purity products effortlessly

- Easily eliminate counter ions and low volatility solvents in the prepared distillate to recover high purity and stable powdered samples.
- This exclusive technology ensures that the water carried in the distillate is sufficiently removed in a short period of time, greatly reducing the time required for solvent removal, purification and powdering.



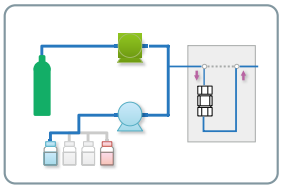


✓ Easily removes previously difficult-to-eliminate counter ions and low-volatility solvents

Nexera UC SFE Pretreatment System

Extraction Vessel

Rack Changer



Fully Automated Extraction Pretreatment

↑↑↑ Reproducibility
↑↑↑ Loading Capacity

↓↓↓ Human Error
↓↓↓ Runtime

New alternative for pretreatment and preparation

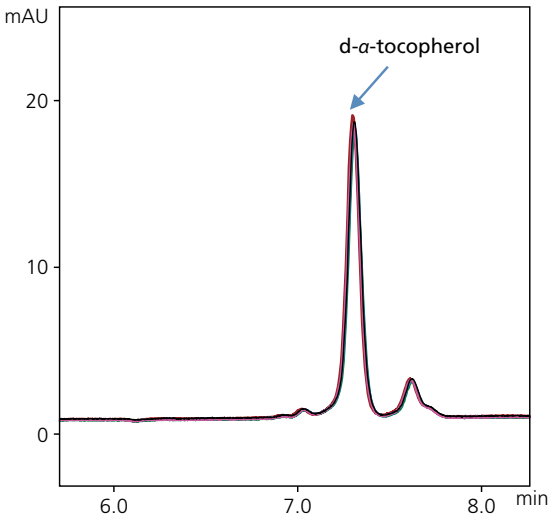
- Shimadzu's unique combination of Nexera UC supercritical fluid technology and preparative chromatography provides more options for your sample preparation.
- Rack Changer offers more sample loading capacity for higher automated sample throughput.

Safer and more efficient sample pretreatment

- The high solubility and high permeability of supercritical fluid allow the efficient elution of target compounds from solid samples.
- Get rid of the cumbersome operation of traditional extraction methods. Automated equipment and non-toxic carbon dioxide make your lab safer.

Nexera UC SFE Pretreatment system

No.	Conc. (mg/mL)	Recovery (%)
1	0.776	104.46
2	0.780	105.00
3	0.772	103.92
4	0.790	106.35
5	0.761	102.44
6	0.758	102.04
Average	0.773	
RSD (%)	**1.549**	



Results for SFE extraction liquid analyses of Vitamin E. The automated consecutive pretreatment of solid sample by the Nexera UC SFE pretreatment system showed both high repeatability and high recovery.

C190-E199

SHIMADZU
Excellence in Science

Technical Report

Improved Sample Pretreatment Using Offline Supercritical Fluid Extraction

Hidetoshi Terada[1], Takato Uchikata[1], Takanari Hattori[1], Keiko Matsumoto[1], Yoshiyuki Watanabe[1], Tadayuki Yamaguchi[1], Yasuhiro Funada[1]

Abstract:

Separation analysis using HPLC, SFC or GC requires a pretreatment step to efficiently extract a target constituent from the sample in various forms (e.g., solid). Constituents are usually extracted from solid samples using dissolution or solid-liquid extraction methods. Dissolution methods can only be used when the sample is soluble in a given solvent, and they are difficult to optimize depending on the analytical conditions. Solid-liquid extraction methods (e.g., Soxhlet extraction) are not suited to the pretreatment of multiple samples as they require considerable time for extraction and non-extraction (e.g., cleaning, preparation) operations. However, extraction methods involving supercritical fluids can utilize the characteristics of supercritical fluids (e.g., high solubility, permeability) to achieve the elution of a target constituent from a solid sample with high efficiency and also allow automation during the extraction process. This article describes the utilization of the Nexera UC SFE pretreatment system, which increases the efficiency of sample pretreatment for analysis.

Keywords: supercritical fluid extraction, SFE, offline SFE

1. What Is Supercritical Fluid Extraction?

Supercritical fluid refers to the state of any substance at temperature and pressure conditions above its critical point. Supercritical fluids combine the ability of liquids to dissolve materials with high diffusivity and low viscosity properties of gases. Supercritical fluid extraction (SFE) with carbon dioxide is widely employed as a pretreatment method for analysis owing to its low critical points (critical temperature: 31.1 °C, critical pressure: 7.38 MPa), which makes it easy to handle, along with its incombustibility, inertness, and low cost. Some advantages of SFE are shown below:

- Supercritical fluids have high permeability and diffusion coefficients and can therefore be used for highly efficient extraction.
- Supercritical fluids achieve extraction at mild temperatures at which target constituents are unlikely to oxidize.
- Carbon dioxide evaporates, which simplifies sample treatment after extraction.
- Solvent costs are low compared with solvent extraction and other methods.
- The extraction process can be automated.

While supercritical carbon dioxide is as hydrophobic as hexane and is suited for the extraction of fat-soluble compounds, it can also be used for the extraction of compounds with a wide range of polarities by adding modifier substances such as methanol and ethanol.

Fig. 1 The Nexera UC SFE Pretreatment System

2. The Nexera UC SFE Pretreatment System

The Nexera UC SFE pretreatment system (Fig. 1) is a dedicated pretreatment system that performs offline SFE. Solid samples are placed in dedicated extraction vessels (Fig. 2) and introduced into the system, after which the system automatically performs the extraction. The extraction vessels are available in volumes of 0.2 mL and 5 mL such that they can be selected based on the sample to be analyzed. The system allows accommodation of a maximum of 48 extraction vessels, which are mounted into a rack changer (Fig. 3). This setup enables extraction pretreatment of multiple samples via automated sample transfer and cycling. The automation of the multi-sample extraction pretreatment using the Nexera UC SFE pretreatment system substantially reduces the time and labor required for the pretreatment operations while also preventing human error.

Fig. 2 Extraction Vessels

Fig. 3 Rack Changer

1 Analytical & Measuring Instruments Division

The extraction conditions can be conÿgured such that the pretreatment operations can be run from the same LabSolutions workstation used for the analysis. Thus, the extraction conditions and pretreatment can be intuitively controlled in the same way as sample analysis.

The material extracted by the supercritical fluid is collected in a trap column, subsequently eluted by an organic solvent, and ÿnally recovered using a fraction collector before performing the analysis by LC (LC/MS), GC (GC/MS), or NMR. Comprehensive and complementary sample analysis can be achieved by combining the results from several of these analysis methods.

Compared with Soxhlet extraction, SFE uses a much lower quantity of organic solvent during the pretreatment, thereby reducing costs and allowing a more environmentally friendly pretreatment step.

3. Operating Principles of the Nexera UC SFE Pretreatment System

The schematic diagrams of the Nexera UC SFE pretreatment system showing material flow and principle of operation are shown in Fig. 4. The extraction process can be roughly divided into four operations:

(1) Extraction vessel delivery and temperature control

An extraction vessel is moved from the rack changer to the SFE unit, and the extraction vessel is subsequently heated to the set temperature (40–80 °C).

(2) Static extraction

Once the extraction vessel has reached the set temperature, the supercritical fluid is introduced into the vessel and static extraction occurs. Parameters such as the extraction vessel temperature, pressure and duration of the extraction, and type and quantity of the modiÿer can be independently controlled during extraction depending on the sample and target constituent to be extracted.

(3) Dynamic extraction

After the static extraction, a dynamic extraction is performed by delivering the fluid through the extraction vessel. This operation allows the extraction of the target material from the extraction vessel and subsequent collection in a trap column located downstream of a back pressure regulator. Downstream of the back pressure regulator is held at close to atmospheric pressure such that carbon dioxide is in a gaseous state while collecting the extraction material in the trap column. ODS and other columns used for HPLC can be used as trap columns. Similar to static extraction, the extraction vessel temperature, pressure and duration of the extraction, and type and quantity of the modiÿer can be adjusted depending on the sample and target constituent to be extracted.

(4) Elution from the trap column and recovery of the extraction material

Once the dynamic extraction has ÿnished, the fluid delivery is stopped and the back pressure regulator is opened, thereby allowing the system pressure to drop to atmospheric pressure. The delivery pump is then switched from the modiÿer to the eluent, which is passed through the trap column to elute the extraction material. The eluate is then recovered into collection tubes using a fraction collector. An organic solvent is used as the eluent to simplify concentration and post-treatment steps of the eluate.

(1) Extraction vessel delivery and temperature control

A speciÿed extraction vessel is transferred to the SFE unit and heated to the set temperature.

(2) Static extraction

When the temperature of the extraction vessel has reached the set temperature, the supercritical uid is introduced and static extraction (i.e., in the absence of uid ow) is allowed.

(3) Dynamic extraction

The extraction is dynamically performed by passing the supercritical fluid through the extraction vessel. The extraction material is taken from the extraction vessel and collected at atmospheric pressure after evaporation of CO_2 in the trap column downstream the back pressure regulator.

(4) Elution from the trap column and recovery of the extraction material

The delivery pump is used to deliver the eluent through the trap column, thereby eluting the extraction material, which is then recovered using a fraction collector.

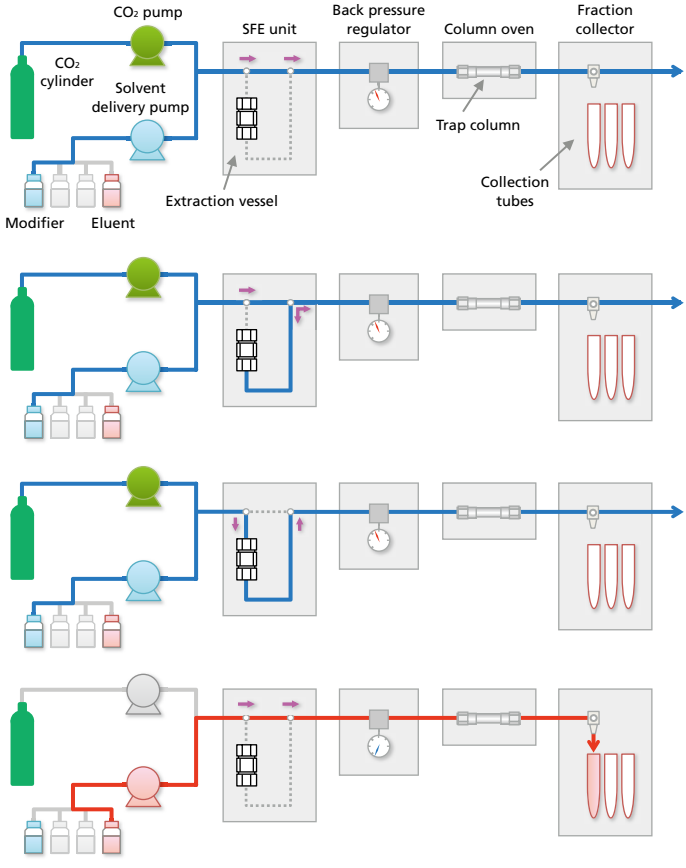


Fig. 4 Material Flow and Principle of Operation

Samples showing high water content complicate the extraction process (i.e., reduce both the extraction efÿciency and the repeatability of the pretreatment) as supercritical carbon dioxide does not mix with water. In these cases, the extraction efÿciency can be increased by mixing the sample with a dehydrating agent before enclosing it into the extraction vessel. Extraction efÿciency can also be low when supercritical carbon dioxide is used for the extraction of highly polar constituents. In this case, the extraction efÿciency can be increased by adding modiÿers such as methanol during the extraction. In the case of samples with constituents showing ionic polar groups, acid (e.g., formic acid, acetic acid), salt (e.g., ammonium formate, ammonium acetate), and bases (e.g., ammonia, diethylamine) can be added during the extraction. Fine pulverization of the sample normally increases extraction efÿciency. For polymer samples, a ÿne freeze-crushing treatment before the extraction often results in increased extraction efÿciencies.

4. Using the Nexera UC SFE Pretreatment System for Extracting Fat-Soluble Vitamins

Vitamin E is a group of fat-soluble compounds widely used as antioxidants and for nutritional support in foods and medicinal products. We present an example of ofšine SFE using the Nexera UC SFE pretreatment system to extract d-*α*-tocopherol, a vitamin E compound, from a nutritional supplement. The sample used was a commercially available soft capsule supplement containing d-*α*-tocopherol. The soft capsule supplement contained a paste, which was mixed with a dehydrating agent before being enclosed into an extraction vessel. The extraction was exclusively performed with supercritical carbon dioxide, and hexane was used as the eluent after trapping. The detailed extraction conditions are shown in Table 1. The extraction liquid recovered by the fraction collector was diluted to 10 mL with hexane in a measuring šask. The sample extraction vessel contained 7.4 mg of d-*α*-tocopherol, and the theoretical concentration of d-*α*-tocopherol in the ÿnal SFE extraction liquid was 0.74 mg/mL.

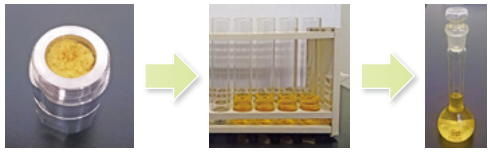


Fig. 5 Sample Before and After the SFE Process

Table 1 SFE Conditions

SFE	
Extraction vessel	: 5 mL
Extraction solvent	: CO_2
Flow rate	: 5 mL/min
Temperature	: 40°C
Back pressure	: 15 MPa
Extraction time	: 15 min (Static extraction → Dynamic extraction)
Trap & Pressure down conditions	
Trap column	: Shim-pack VP-ODS 4.6 mmI.D. × 50 mmL. 5 Žm
Temperature	: 60°C
Pressure down time	: 10 min (15–25 min)
Recovery conditions	
Elution solvent	: Hexane
Flow rate	: 2 mL/min
Fraction time	: 3.5 min (25–28.5 min)

Six extraction vessels were prepared, each containing the same amount of supplement sample. Each extraction vessel was subjected to ofšine SFE after which the recovered liquid was analyzed by SFC at the conditions shown in Table 2. The recovery and repeatability of the process was conÿrmed.

The six chromatograms obtained are shown overlapping each other in Fig. 6.

Table 2 Conditions Used for the Analysis of the Pretreated Samples (SFC)

Column	: Nacalai COSMOSIL Cholester 4.6 mmI.D. × 250 mmL. 3 Žm
Modiÿer	: IPA
Gradient	: 2% (0 min) → 20% (10 min) → 50% (10–12 min)
Flow rate	: 3 mL/min
Temperature	: 40°C
Back pressure	: 15 MPa
Injection volume	: 2 ŽL
Detector	: UV-VIS (@293 nm)

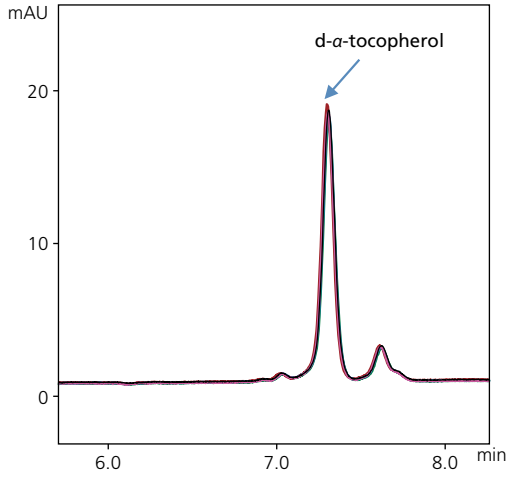


Fig. 6 Results for SFE Extraction Liquid Analyses (The Six Samples Are Shown Overlapping Each Other).

The concentration and recovery of d-*α*-tocopherol in the six SFE extraction liquid chromatograms (Fig. 6) are summarized in Table 3. The extraction pretreatment showed both high repeatability and high recovery, thereby revealing that the Nexera UC SFE pretreatment system can be used for the automated consecutive pretreatment of target constituents in a solid sample with good efÿciency.

Table 3 Repeatability and Recovery of Vitamin E Extraction

No.	Conc. (mg/mL)	Recovery (%)
1	0.776	104.46
2	0.780	105.00
3	0.772	103.92
4	0.790	106.35
5	0.761	102.44
6	0.758	102.04
Average	0.773	
RSD (%)	1.549	

5. Using the Nexera UC SFE Pretreatment System for the Extraction of Residual Pesticides from Agriproducts

Over 800 pesticides are subjected to analysis for their residual presence in food products. Analytical methods that enable rapid and simple testing of a large number of pesticides involving any pretreatment operations required for analysis are needed. Conventional analysis for residual pesticides in food normally involves a solvent extraction method to extract the pesticides, followed by LC/MS or GC/MS analyses. The pretreatment operations employed in these analytical methods are highly labor and time consuming, and they use a large volume of organic solvent. We present an example of utilizing the Nexera UC SFE pretreatment system to extract residual pesticides before analyzing them using a GC/MS/MS system. 1 g of dehydrating agent was added to 1 g of pulverized brown rice*. This mixture was then enclosed into an extraction vessel, and an extraction pretreatment was performed using the conditions shown in Table 4. The extraction liquid recovered by the fraction collector was diluted to 2 mL with an acetone/hexane (1/1, V/V) mixture in a measuring ,ask and then analyzed using GC/MS/MS under the conditions shown in Table 5. The components included in pesticide standard mixture solutions for GC/MS (PL2005 Pesticide GC/MS Mix I to VI and Mix 7, Hayashi Pure Chemical Ind., Ltd.) were analyzed.

* "Miyazaki Hydro-Protect" Patent No. 3645552

Table 4 SFE Conditions

Offline SFE	
Extraction vessel	: 5 mL
Extraction solvent	: CO_2 + Methanol
Flow rate	: 5 mL/min
Temperature	: 40°C
Back pressure	: 15 MPa
Extraction time	: 8 min (Static extraction → Dynamic extraction)
Trap & Pressure down conditions	
Trap column	: Shim-pack VP-ODS 4.6 mmI.D. × 50 mmL. 5 £m
Temperature	: 60°C
Pressure down time	: 4 min (8–12 min)
Recovery conditions	
Elution solvent	: Acetone/Hexane = 1/1 (V/V)
Flow rate	: 2 mL/min
Fraction time	: 2 min (12–14 min)

The pesticide standard solutions were added to an brown rice sample (pesticide concentrations of 100 ng/g). An MRM chromatogram of the extraction liquid obtained from this sample is shown in Fig. 7. The theoretical concentration of each pesticide in the extraction liquid used for GC/MS/MS analysis was 50 ng/mL.

The extraction was performed on six samples to which the abovementioned pesticide standard solutions were added; each pesticide was quantiÿed using a matrix calibration curve created using the SFE extraction liquid obtained from a blank brown rice sample, after which repeatability and recovery were conÿrmed. Good repeatability (relative standard deviation of quantiÿed concentration: <10 %) and good recovery (70 %–120 %) were obtained for the 301 pesticides studied. An excerpt from these results showing the repeatability and recovery for some representative pesticides is shown in Table 6. The Nexera UC SFE pretreatment system can be used for automated consecutive pretreatment of up to 48 samples while consuming low amounts of solvent.

Table 5 Conditions Used for the Analysis of the Pretreated Samples (GC/MS/MS)

Column	: Rxi-5Sil MS 30 m × 0.25 mmI.D., df = 0.25 £m
Column temp.	: 50°C (1 min) → (25°C/min) → 125°C → (10°C/min) → 300°C (15 min)
Carrier gas	: He (Constant linear velocity mode)
Linear velocity	: 47.2 cm/sec
Injection mode	: Splitless (Sampling time 1.00 min)
High press inj.	: 250 kPa (1.5 min)
Injection volume	: 1 £L
Interface temp.	: 250°C
Ion source temp.	: 200°C
MS mode	: MRM
Loop time	: 0.3 sec

Table 6 Repeatability and Recovery of Representative Pesticide Extraction

Compounds	Repeatability (%RSD, n=6)	Recovery (%)
Cyhalofop-butyl	4.2	93
Etofenprox	3.8	90
Iprodione	2.5	93
Malathion	3.2	93
Piperonyl butoxide	3.8	89

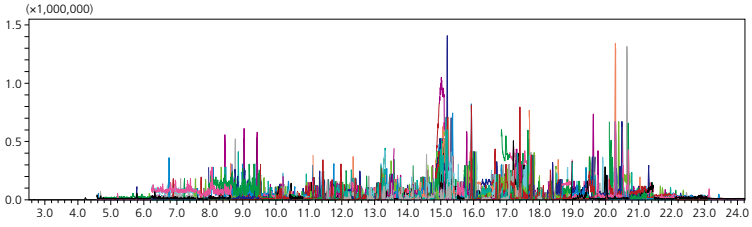


Fig. 7 MRM Chromatogram of the Brown Rice Extraction Liquid

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Solution 4: Impurity Detection and Purity Confirmation

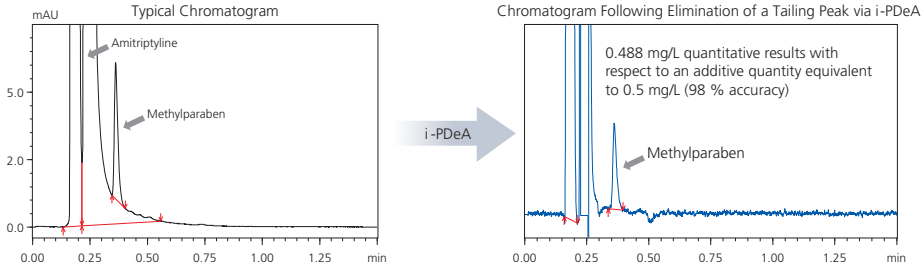
Compositional analysis of drugs is an integral part of drug development and production. The purity of the active ingredient is crucial and varies at different production stages. Other than the active ingredient, impurity analysis is critical to determine the quality of drugs.

If the chromatographic conditions are not sufficiently optimized, or if the column efficiency is reduced, the impurity peaks may be buried by the major component, resulting in the misinterpretation and inaccurate quantification of the target compound.

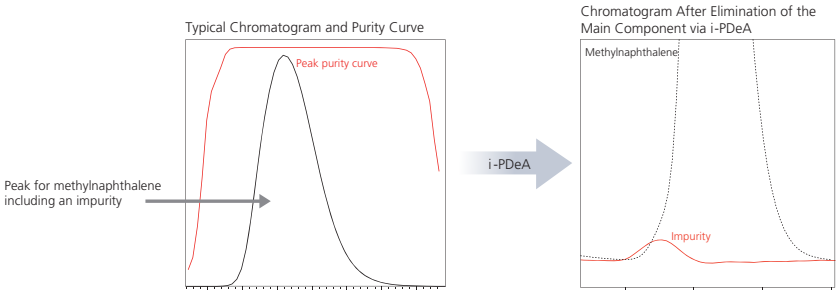
In addition to the commonly used LC/LCMS, Shimadzu also provides advanced algorithms to fully exploit the performance of the hardware to help you identify potentially hidden impurities.

i-PDeA

1) Separation of Eluted Peaks on Tailing Peaks



2) Detection of Unresolved Impurities

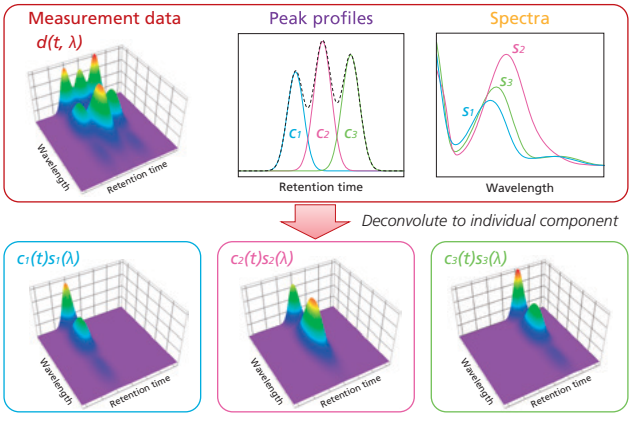


New data processing technique for PDA detector

- The Intelligent Peak Deconvolution Analysis (i-PDeA) function of the Shimadzu PDA Detector can extract unseparated target peaks using spectral differences.
- The iPDeA function can deconvolute co-eluting components to generate individual peak profiles by using the derivative spectrum chromatogram method and the chemometrics multivariate curve resolution alternating least squares (MCR-ALS) technique.

Obtain accurate compositional and purity information

- The i-PDeA functionreduces the need for complete chromatographic separation of impurities. Even without mass spectrometry, it is easy to detect unexpected impurities. Isomers that are difficult to separate can be identified easily.
- The effect of co-eluting components on quantitation can be effectively eliminated. If the peaks fail to achieve baseline separation, the target component can still be quantified separately, greatly improving the accuracy of the quantitative analysis.



Basic Principle of i-PDeA

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Technical Report

Fractional Determination of Co-eluted Compounds Using a New Data Processing Method for Photodiode Array Detector

Principle and Summary of i-PDeA (Intelligent Peak Deconvolution Analysis)

Toshinobu Yanagisawa[1]

Abstract:

The i-PDeA derivative spectrum chromatogram method was developed as a new data processing technique for photodiode array detectors for HPLC. A derivative spectrum is created by performing differential processing on the UV-Vis absorption spectrum at each measurement time. Plotting the derivative spectrum values at the specified wavelength against retention time creates a derivative spectrum chromatogram that is able to separate co-eluted peaks. The high selectivity of the derivative spectrum chromatogram can detect unexpected impurities and quantitate the target component only, without effects from interfering components that elute simultaneously. This paper formulates the theory of the derivative spectrum chromatogram method into mathematical expressions and reports details of verification of the basic performance using standard samples.

Keywords: PDA data processing, peak deconvolution, derivative spectrum chromatogram, Nexera X2, UHPLC

1. Basic Theory of the Derivative Spectrum Chromatogram Method

1-1. Separation of Two Component Co-eluted Peaks

Fig. 1 shows the absorption spectra of two components (target component x and y), and Fig. 2 shows the derivative spectra differentiated along the wavelength axis. In Fig. 2, the derivative is zero for component x in the derivative spectrum at wavelength λ_x and zero for component y at wavelength λ_y.

Denoting the spectrum for target component x as $s_x(\lambda)$ and the peak profile as $p_x(t)$, and similarly the spectrum for target component y as $s_y(\lambda)$ and the peak profile as $p_y(t)$, the 3D chromatogram $S(t,\lambda)$ for the two-component system in which component x and y both elute can be expressed as:

$$S(t,\lambda) = p_x(t)s_x(\lambda) + p_y(t)s_y(\lambda)$$

Partial differentiation at wavelength λ gives the derivative spectrum chromatogram at wavelength λ_d as:

$$\left.\frac{\partial S}{\partial \lambda}\right|_{\lambda=\lambda_d}(t) = p_x(t)s_x'(\lambda_d) + p_y(t)s_y'(\lambda_d)$$

As the derivative spectrum chromatogram at wavelength λ_x where the component x derivative becomes zero is

$$s_x'(\lambda_x) = 0$$

we get,

$$\left.\frac{\partial S}{\partial \lambda}\right|_{\lambda=\lambda_x}(t) = p_y(t)s_y'(\lambda_x) \quad \text{------ (1)}$$

Similarly, as the derivative spectrum chromatogram at wavelength λ_y where the component y derivative becomes zero is

$$s_y'(\lambda_y) = 0$$

we get,

$$\left.\frac{\partial S}{\partial \lambda}\right|_{\lambda=\lambda_y}(t) = p_x(t)s_x'(\lambda_y) \quad \text{------ (2)}$$

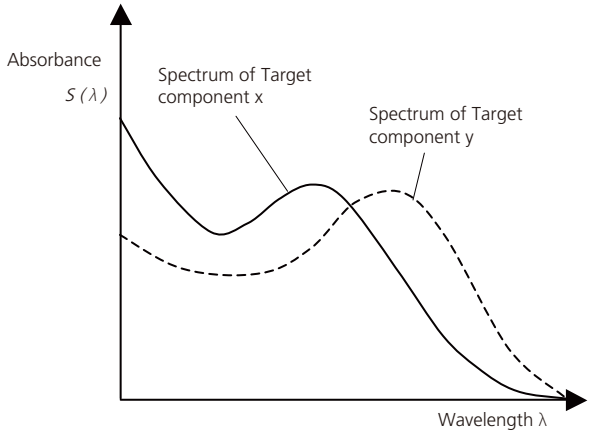


Fig. 1 Spectra of two components

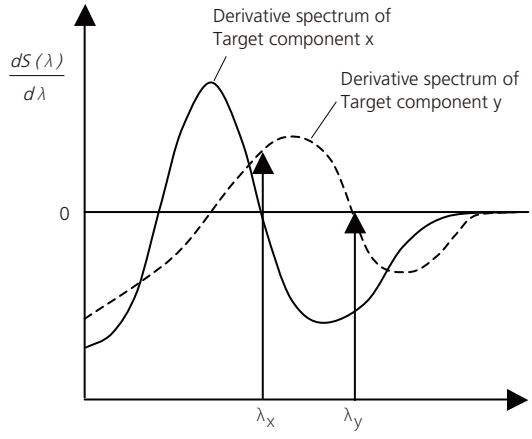


Fig. 2 Derivative spectra of two components

1. Analytical & Measuring Instruments Division

As $s_x'(\lambda_y)$ and $s_y'(\lambda_x)$ are non-zero constants in equations (1) and (2), it can be seen that the derivative spectrum chromatogram at wavelength λ_x shows the elution profile for component y only, and the derivative spectrum chromatogram at wavelength λ_y shows the elution profile for component x only.

That is, the derivative spectrum chromatogram at wavelength λ_x can separate component y only, and the derivative spectrum chromatogram at wavelength λ_y can separate component x only.

1-2. Impurity Detection

i-PDeA can detect whether impurity components exist in addition to the major component. This method can be applied when one major component is mixed with other impurity components. The 3D chromatogram S (t, λ) can be expressed as follows if the major component elution profile is denoted as $p_x(t)$, the impurity component elution profiles as $p_y(t)$, $p_z(t)$…, the major component spectrum as $s_x(\lambda)$, and the impurity spectra as $s_y(\lambda)$, $s_z(\lambda)$…

$$S(t,\lambda) = p_x(t)s_x(\lambda) + p_y(t)s_y(\lambda) + p_z(t)s_z(\lambda)...$$

Then, the derivative spectrum chromatogram at wavelength λ_x at which the major component derivative spectrum chromatogram $s_x'(\lambda)$ value becomes zero is given by

$$\left.\frac{\partial S}{\partial \lambda}\right|_{\lambda=\lambda_x}(t) = p_y(t)s_y'(\lambda_x) + p_z(t)s_z'(\lambda_x) + ...$$

Therefore, the derivative spectrum chromatogram at wavelength λ_x eliminates the major component elution profile and expresses the elution profiles of the impurities besides the major component.

2. Examples of Analysis Using the i-PDeA Functions

2-1. Impurity Detection in Standard Samples

This section demonstrates that an impurity in a methylnaphthalene (MN) standard was detected using the derivative spectrum chromatogram method.

Analytical Conditions

Pump	: Shimadzu LC-30AD×2
Detector	: Shimadzu SPD-M30A
Column oven	: Shimadzu CTO-20AC
Controller	: Shimadzu CBM-20A
Autosampler	: Shimadzu SIL-30AC$_{MP}$
Column	: Shimadzu Shim-pack XR-ODS (30 mmL. × 3.0 mmI.D., 2.2 μm)
Flow rate	: 1 mL/min
Column temp.	: 40 °C
Sampling	: 80 msec
Slit width	: 1 nm
Time constant	: 240 msec
Wavelength range	: 190 nm to 700 nm
Injection volume	: 1 μL

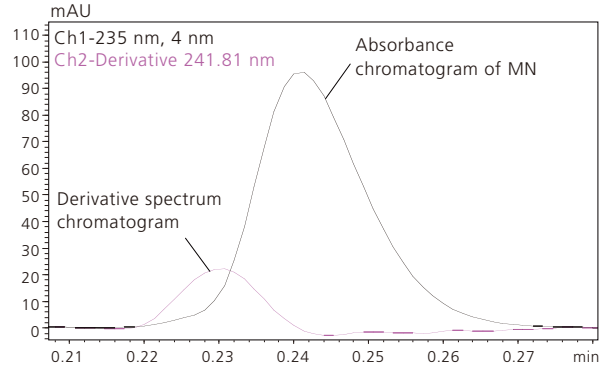
Fig. 3 shows the absorbance chromatogram at 235 nm and derived spectrum chromatogram at 1st derivative zero wavelength under several types of analytical conditions.

In (a), the impurity is detected using i-PDeA, even though MN and its impurity overlap chromatographically.

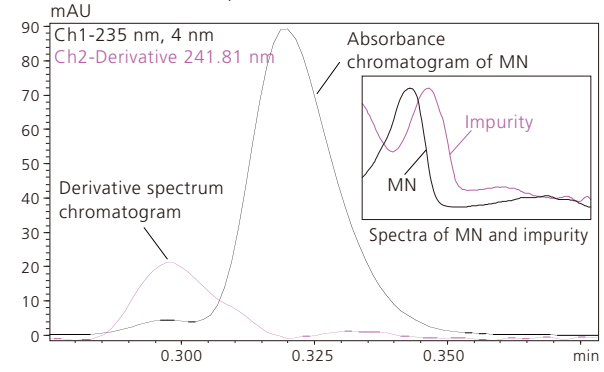
In (b), MN and the impurity begin to separate chromatographically. By comparing the spectra at the apex of each peak, MN and the impurity can be confirmed to be different compounds.

In (c), MN and the impurity are completely separated chromatographically. The derived spectrum chromatogram shows no significant signal at the retention time of the MN peak, which shows the signal of the MN peak does not include a contribution from the impurity.

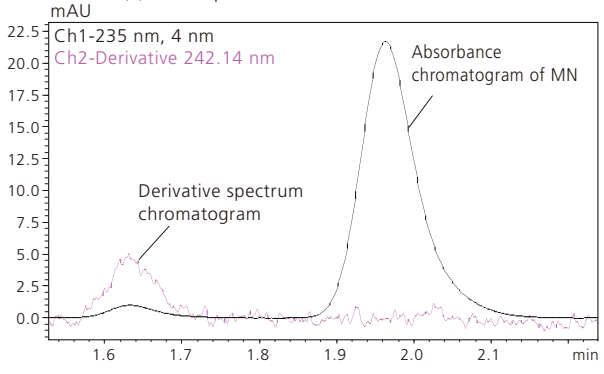
As described in this example, whether impurity peaks are chromatographically resolved or co-eluted with the main component, i-PDeA easily detects their existence.



(a) Mobile phase : Acetnirile95% / Water5%



(b) Mobile phase : Acetonitrile85% / Water15%



(c) Mobile phase : Acetnitrile50% / Water50%

Fig. 3 Absorbance chromatogram and derivative spectrum chromatogram

2-2. Quantitation of a Mixture of Two Components

This section demonstrates that the derivative spectrum chromatogram method can separate and quantitate two chromatographically co-eluted peaks in a data set acquired for a mixture of two components.

Difluorobenzophenone (DFBP) and Valerophenone (VP) standards, in 5 different relative concentrations of 100/1,100/10,100/50, 100/100,100/200, were used to acquire the derivative spectrum chromatograms of DFBP and VP. A calibration curve was created (Table 1) and quantitative analysis of each sample mixture was performed (Tables 2 and 3).

Analytical Conditions

Pump	: Shimadzu LC-30AD×2
Detector	: Shimadzu SPD-M30A
Column oven	: Shimadzu CTO-20AC
Controller	: Shimadzu CBM-20A
Autosampler	: SIL-30AC$_{MP}$
Mobile phase	: Acetonitrile45% / Water55%
Column	: Shimadzu Shim-pack XR-C8 (50 mmL. × 3.0 mmI.D., 2.2 μm)
Flow rate	: 2 mL/min
Column temp.	: 40 °C
Sampling	: 80 msec
Slit width	: 1 nm
Time constant	: 240 msec
Wavelength range	: 190 nm to 700 nm
Injection volume	: 1 μL

Fig. 4 shows the spectrum comparison of DFBP and VP. Fig. 5 shows the absorbance chromatogram of the mixed sample (DFBP/VP= 100/200) at 210 nm and the derived spectrum chromatogram at 255.93 nm (1st derived zero wavelength of DFBP) & 216.93 nm (1st derived zero wavelength of VP).

Fig. 6 shows the absorbance chromatogram of the mixed sample (DFBP/VP=100/1) at 210 nm. Due to the low concentration in the sample, the VP peak is hidden in the DFBP peak. The ellipse in Fig. 6 shows the derivative spectrum chromatograms, which are used by i-PDeA to find and integrate the peak.

The VP calibration curve, created by using the integrated peak area for VP in the derivative spectrum chromatogram, was used to calculate the quantitative amount of VP in each sample. The results are shown in Table 2. In the case of the lowest VP concentration sample (DFBP/VP = 100/1), the concentration was calculated to be 1.023 (2.30% error).

In the same way, the DFBP calibration curve was created and used to calculate the quantitative amount of DFBP in each sample. The results are shown in Table 3. Of note in these results is the reproducibility of peak area for a 1 μL sample injection (<1% RSD) as well as <3% error in the quantitative calculation.

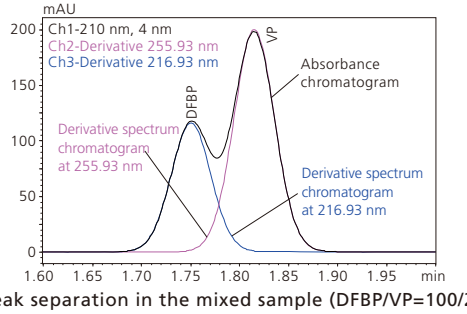


Fig. 5 Peak separation in the mixed sample (DFBP/VP=100/200)

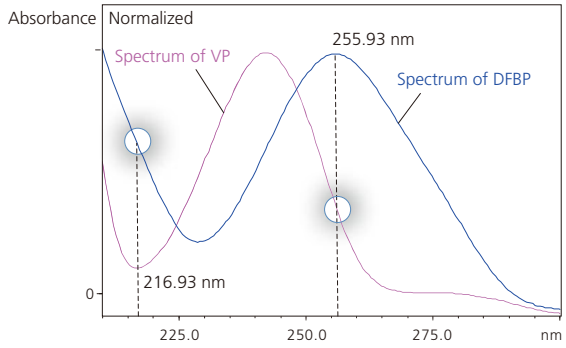


Fig. 4 Spectrum comparison; DFBP and VP

Table 1 Calibration data points created by derivative spectrum chromatogram of VP standard sample (R^2=0.9999309)

Sample relative concentration (VP)	Retention time (min)	Area	Concentration	Error (%)
1	1.804	2,984	0.964	-3.62
10	1.801	30,368	9.876	-1.24
50	1.804	151,922	49.439	-1.12
100	1.802	310,801	101.149	1.15
200	1.802	613,207	199.572	-0.21

Table 2 Quantitation result of VP in DFBP/VP mixed sample

Sample relative concentration (DFBP/VP)	Retention time (min)	Area	Concentration	Error (%)
100/1	1.808	3,167	1.023	2.30
100/10	1.807	30,372	9.878	-1.22
100/50	1.802	153,206	49.856	-0.29
100/100	1.806	309,596	100.757	0.76
100/200	1.815	620,556	201.964	0.98

Table 3 Quantitation result of DFBP in DFBP/VP mixed sample

Sample relative concentration (DFBP/VP)	Retention time (min)	Area	Concentration	Error (%)
100/1	1.746	359,670	102.225	2.23
100/10	1.742	357,969	101.741	1.74
100/50	1.737	357,497	101.607	1.61
100/100	1.742	357,891	101.719	1.72
100/200	1.751	351,528	99.911	-0.09

Area %RSD=0.87 (injection volume:1 μL)

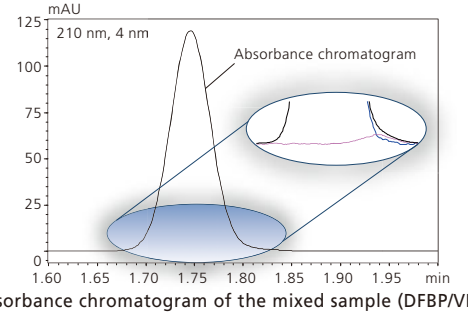
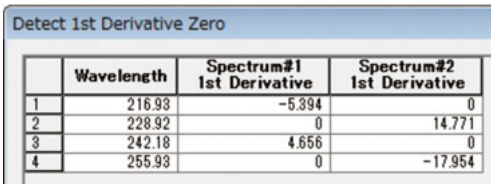


Fig. 6 Absorbance chromatogram of the mixed sample (DFBP/VP=100/1)

3. Summary of i-PDeA Settings

The parameters for the i-PDeA function are set as part of the data processing method of the photodiode array detector. Once the analytical protocol is defined, it can be applied for routine analysis. The following is a brief summary of the i-PDeA parameters.

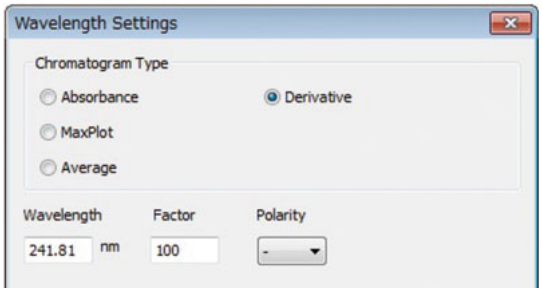
1. The Savitzky-Golay method is used to determine the first derivative spectrum, from which a list of wavelengths where the 1st derivative value is zero is generated. Spline interpolation is applied to calculate the wavelength closest to the 1st derivative zero wavelengths, and the results are displayed in a table.


Detect 1st Derivative Zero

	Wavelength	Spectrum#1 1st Derivative	Spectrum#2 1st Derivative
1	216.93	-5.394	0
2	228.92	0	14.771
3	242.18	4.656	0
4	255.93	0	-17.954

i-PDeA most effectively resolves co-eluted peaks when the peak height in the derivative spectrum chromatogram for one component, taken at a 1st derivative zero wavelength of another component, is sufficiently large. If the shape of the spectra of two components is very similar, i-PDeA cannot be applied.

2. To extract the derivative spectrum chromatogram, plotting the derivative spectrum values at the specified wavelength against retention time, select "Derivative" for Chromatogram Type and using the wavelength obtained by the Detect 1st Derivative Zero function, set the value (with 2 decimal places) of the Wavelength in the Wavelength Settings window of the Multi-Chromatogram table.



The derivative spectrum chromatogram has positive value when the slope of the spectrum is up and negative value when the slope of the spectrum is down. Peak direction in the derivative spectrum chromatogram can be adjusted by setting the polarity, and peak size can be adjusted by setting the factor.

3. Set integration and quantitation parameters for the derivative spectrum chromatogram.

The derivative spectrum chromatogram can be handled the same as other multi-chromatograms for quantitative purposes.

4. Conclusion

The excellent performance and reproducibility of the SPD-M30A photodiode array detector and the Nexera X2 system make this new separation methodology possible. The i-PDeA function helps increase the speed of analysis and enhances laboratory productivity. To summarize the key benefits:

- Co-eluted peaks can be separated mathematically, using derivative spectrum chromatograms
- Poorly resolved peaks are processed and visualized as pure peaks with no contribution from co-eluting components
- Impurity peaks hidden by, or even in, the target peak can be detected
- Fast and accurate quantitative analysis is possible, even without complete chromatographic separation
- Simple post-run analysis procedure

The i-PDeA function provides a new solution, which is useful for identification and quantitation of impurities. The use of this feature is expected to increase laboratory efficiency and produce more reliable analytical data.

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Technical Report

New Data Processing Method for Photodiode Array Detectors

Principle and Overview of Intelligent Peak Deconvolution Analysis (i-PDeA II)

Toshinobu Yanagisawa[1]

Abstract:

An i-PDeA II (Intelligent Peak Deconvolution Analysis II) data analysis technique was developed for extracting target peaks from unseparated peaks by analyzing photodiode array (PDA) detector data using the chemometrics multivariate curve resolution alternating least squares (MCR-ALS) technique. The i-PDeA˜II function can separate peaks for multiple components in absorption spectra and chromatograms by simply specifying the wavelength and time ranges. The i-PDeA˜II function can be used to identify spectra and quantitate peaks after separation of individual components, even for difÿcult-to-separate peaks for which a standard sample cannot be prepared. Furthermore, because i-PDeA˜II separates peaks based only on differences in spectral shape, it can also be used to separate and quantitate peaks for co-eluted isomers. This report explains the principle used by the i-PDeA˜II technique to separate peaks, describes an example of using i-PDeA˜II to analyze a sample with isomers of three components, and evaluates the spectral identiÿcation and quantitation performance.

Keywords: Photodiode array detector, chemometrics, MCR-ALS, and LabSolutions

1. Fundamental Theoretical Basis for Peak Deconvolution Algorithm

1-1. Modeling PDA Detector Data

Given peak profiles and spectra for each component in a three-component mixture, $c_1(t)$, $s_1(\lambda)$, $c_2(t)$, $s_2(\lambda)$, $c_3(t)$, and $s_3(\lambda)$, then measurement data in an ideal system $d(t,\lambda)$ can be described by the following expression.

$$d(t,\lambda) = c_1(t)s_1(\lambda) + c_2(t)s_2(\lambda) + c_3(t)s_3(\lambda)$$

Then spectra $d(t_i,\lambda)$ measured as a function of time t_i can be expressed as follows:

$$d(t_i,\lambda) = c_1(t_i)s_1(\lambda) + c_2(t_i)s_2(\lambda) + c_3(t_i)s_3(\lambda)$$

Assuming spectral components are vectors with discrete values λ_j (where j˜=˜1 to m), then spectra can be described as follows:

$$\boldsymbol{d}_i^T = \alpha_i\boldsymbol{s}_1^T + \beta_i\boldsymbol{s}_2^T + \gamma_i\boldsymbol{s}_3^T = \begin{pmatrix}\alpha_i & \beta_i & \gamma_i\end{pmatrix}\begin{pmatrix}\boldsymbol{s}_1^T\\ \boldsymbol{s}_2^T\\ \boldsymbol{s}_3^T\end{pmatrix}$$

where,

$$\boldsymbol{d}_i^T = \begin{pmatrix}d(t_i,\lambda_1) & \cdots & d(t_i,\lambda_m)\end{pmatrix}$$

$$\alpha_i = c_1(t_i),\ \beta_i = c_2(t_i),\ \gamma_i = c_3(t_i)$$

$$\boldsymbol{s}_1^T = \begin{pmatrix}s_1(\lambda_1) & \cdots & s_1(\lambda_m)\end{pmatrix},\ \boldsymbol{s}_2^T = \begin{pmatrix}s_2(\lambda_1) & \cdots & s_2(\lambda_m)\end{pmatrix},\ \boldsymbol{s}_3^T = \begin{pmatrix}s_3(\lambda_1) & \cdots & s_3(\lambda_m)\end{pmatrix}$$

By summarizing each spectrum measurement at time t_i (where i˜=˜1 to n), measurements can be expressed in matrix form, as follows:

$$\begin{pmatrix}\boldsymbol{d}_1^T\\ \vdots\\ \boldsymbol{d}_n^T\end{pmatrix} = \begin{pmatrix}\alpha_1 & \beta_1 & \gamma_1\\ \vdots & \vdots & \vdots\\ \alpha_n & \beta_n & \gamma_n\end{pmatrix}\begin{pmatrix}\boldsymbol{s}_1^T\\ \boldsymbol{s}_2^T\\ \boldsymbol{s}_3^T\end{pmatrix}$$

or by direct product (outer product), as follows:

$$D = \boldsymbol{c}_1\boldsymbol{s}_1^T + \boldsymbol{c}_2\boldsymbol{s}_2^T + \boldsymbol{c}_3\boldsymbol{s}_3^T \qquad \text{Eq.˜(1)}$$

or alternatively

$$D = CS^T \qquad \text{Eq.˜(2)}$$

where,

$$D = \begin{pmatrix}d(t_1,\lambda_1) & \cdots & d(t_1,\lambda_m)\\ \vdots & \cdots & \vdots\\ d(t_n,\lambda_1) & \cdots & d(t_n,\lambda_m)\end{pmatrix},\ \boldsymbol{c}_1 = \begin{pmatrix}\alpha_1\\ \vdots\\ \alpha_n\end{pmatrix},\ \boldsymbol{c}_2 = \begin{pmatrix}\beta_1\\ \vdots\\ \beta_n\end{pmatrix},\ \boldsymbol{c}_3 = \begin{pmatrix}\gamma_1\\ \vdots\\ \gamma_n\end{pmatrix}$$

$$C = \begin{pmatrix}\alpha_1 & \beta_1 & \gamma_1\\ \vdots & \vdots & \vdots\\ \alpha_n & \beta_n & \gamma_n\end{pmatrix},\quad S^T = \begin{pmatrix}s_1(\lambda_1) & \cdots & s_1(\lambda_m)\\ s_2(\lambda_1) & \cdots & s_2(\lambda_m)\\ s_3(\lambda_1) & \cdots & s_3(\lambda_m)\end{pmatrix}$$

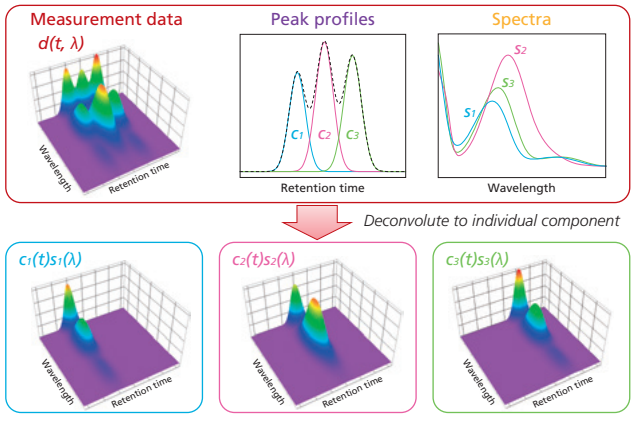


Fig. 1 Measurement Data from Three-Component Mixture Sample

The data can be expressed schematically as follows:

$$\begin{pmatrix}\text{Meaurement spectrum 1}\\ \vdots\\ \text{Meaurement spectrum n}\end{pmatrix} = \begin{pmatrix}\text{Component 1 profile} & \text{Component 2 profile} & \text{Component 3 profile}\end{pmatrix}\begin{pmatrix}\text{Component 1 pure spectrum}\\ \text{Component 2 pure spectrum}\\ \text{Component 3 pure spectrum}\end{pmatrix}$$

Considering measurement error, noise, and unpredictable factors, and given a remainder R, the measurement data can be modeled as follows:[1), 2)]

$$D = CS^T + R$$

This relational expression is valid for any number of components.

1 Analytical & Measuring Instruments Division

1-2. Solutions Using MCR-ALS Technique

The MCR-ALS technique estimates the peak profile or the spectrum with the closest fit to measurement data by repeatedly approximating *C* (peak profiles) or *S* (spectra) in equation (2) using least squares approximation. The following is the typical method for determining solutions by the MCR-ALS technique.[3), 4), 5), 6)]

Step 1 Specify the number of components in measurement data *D*.
Step 2 Calculate initial estimate (for example, by specifying the initial value for *C*).
Step 3 Using the estimate of *C*, calculate the S^T matrix under appropriately chosen constraints.
Step 4 Using the estimate of S^T, calculate the *C* matrix under appropriately chosen constraints.
Step 5 From the product of *C* and S^T found in the above steps of an iterative cycle, calculate an estimate of the original data matrix, *D*.
Step 6 Repeat steps 3, 4, and 5 until convergence is achieved.

Equation (2) generally does not give a unique solution. Therefore, to determine the optimal solution, constraints must be specified based on problem characteristics. Consequently, by specifying appropriate constraints, MCR-ALS can provide valid solutions even without prior information.

1-3. i-PDeA II Peak Separation Algorithm

If equation (1) is expanded for N components, the measurement signal *D* can be expressed by the following equation.

$$D = \boldsymbol{c}_1\boldsymbol{s}_1^T + \boldsymbol{c}_2\boldsymbol{s}_2^T + \cdots + \boldsymbol{c}_N\boldsymbol{s}_N^T$$

This algorithm determines a solution by minimizing the following squared errors, with the chromatogram vector $\boldsymbol{c}_k$ substituted by the chromatogram model function $\boldsymbol{f}_k$.[7)]

$$E = \left|D - \Sigma \boldsymbol{f}_k \boldsymbol{s}_k^T\right|^2 \qquad (k = 1, 2, \ldots, N)$$

In this case, a bidirectional exponentially modified Gaussian (BEMG) function is used as the chromatogram model function. BEMG is the reciprocal of the delay time component of the exponentially modified Gaussian (EMG) function, as defined by the following equations.

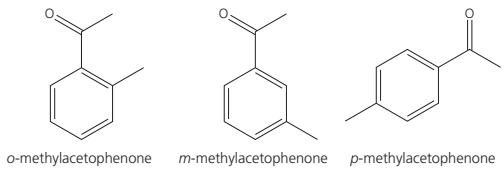
$$bemg(t,a,b) = \int_{-\infty}^{0} e^{ax} \cdot emg(t-x,b)dx$$

$$emg(t,b) = \int_{0}^{\infty} e^{-bx} \cdot exp(-(t-x)^2)dx$$

This algorithm applies the MCR-ALS technique by using an estimated value as the initial value and the BEMG model function as the chromatogram constraint. Since the number of components after separation is unknown, the initial condition starts with a single component and then successively adds components as the presence of unseparated peaks are determined in the residual signal to determine the optimal solution.

2. Example of Using the Algorithm for a Three-Component Mixture Sample

The following describes an example of using the algorithm for a mixture of the positional isomers *o*-methyl acetophenone (*o*-MAP), *m*-methyl acetophenone (*m*-MAP), and *p*-methyl acetophenone (*p*-MAP), shown in Fig. 2.



o-methylacetophenone *m*-methylacetophenone *p*-methylacetophenone

Fig. 2 Structure of Target Substances

400 µg/mL concentration standard samples of the pure isomers were prepared by dissolving pure *o*-MAP, *m*-MAP, and *p*-MAP in 30 vol% methanol-water solution and then data was acquired using the following analytical conditions.

Analytical Conditions	
HPLC System	: Shimadzu LC-2030C 3D
Mobile Phase	: Methanol 30 %/water 70 %
Column Type	: Shimadzu Shim-pack XR-ODS III C18 (3.0 × 50 mm, 2.2 µm)
Mobile Phase Flowrate	: 1.0 mL/min
Oven Temperature	: 40 °C
Sampling	: 240 msec
Slit Width	: 1.2 nm
Time Constant	: 480 msec
Sampling Wavelength Range	: 190 nm to 400 nm
Sample Injection Volume	: 1.5 µL

The methylacetophenone isomers eluted in the order *o*-MAP, *p*-MAP, and *m*-MAP respectively, where the similarity between respective components in spectra obtained from peak tops for each isomer in measurement data was 0.8410 for *o*-MAP/*p*-MAP, 0.9123 for *p*-MAP/*m*-MAP, and 0.9809 for *o*-MAP/*m*-MAP (Fig. 3).

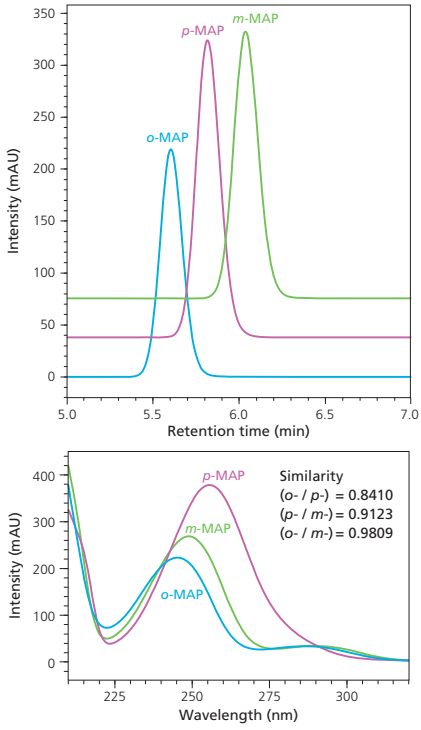


Fig. 3 Measurement Results for Standard Samples of *o*-MAP, *m*-MAP, and *p*-MAP

A mixture solution prepared by mixing *o*-MAP, *m*-MAP, and *p*-MAP standard samples to 400 µg/mL, 400 µg/mL, and 4 µg/mL concentrations, respectively, and then data was acquired using the same analytical conditions. Given the relative concentrations in the order of peak elution (*o*-MAP/*p*-MAP/*m*-MAP =100/1/100), the peak for *p*-MAP (relative concentration of 1) was obscured by the peaks for *o*-MAP and *m*-MAP (relative concentration of 100), which eluted before and after the *p*-MAP peak. Consequently, the presence of *p*-MAP could not be confirmed visually. However, when i-PDeA II was used for measurement data from the time range from 5.0 to 7.0 minutes and wavelength range from 210 to 320 nm, *o*-MAP, *m*-MAP, and *p*-MAP could be separated into independent peaks, as shown in Fig. 4.

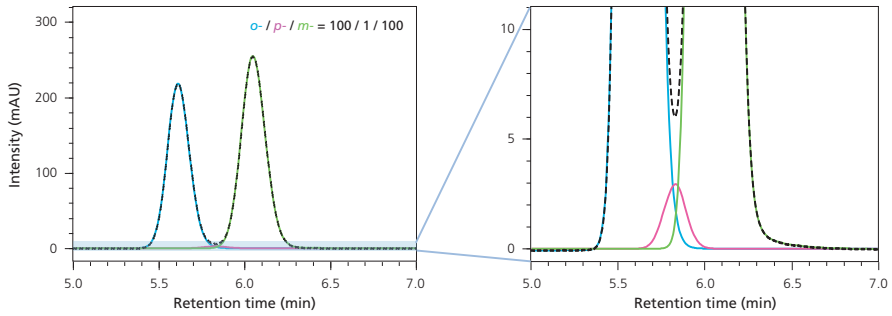


Fig. 4 Separation Results for Mixture Sample of *o*-MAP, *m*-MAP, and *p*-MAP

3. Using i-PDeA II for Spectral Analysis and Quantitative Analysis

3-1. Using i-PDeA II to Measure Purity

When i-PDeA˜II was applied to measurement data to confirm the purity of respective standard samples for *o*-MAP, *m*-MAP, and *p*-MAP, an impurity was detected in the *p*-MAP standard sample (Fig. 5).

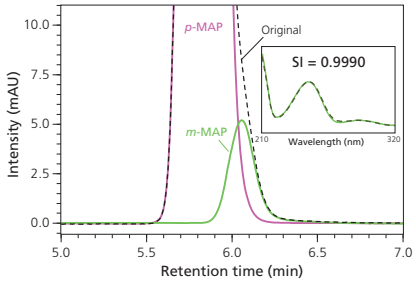


Fig. 5 Impurity Contained in *p*-MAP Standard Sample

Based on the elution time and spectral similarity, the impurity is presumably *m*-MAP.

Therefore, the *p*-MAP standard sample was measured using a Shimadzu Shim-pack XR-Phenyl reversed phase ultra fast analysis column (3.0˜×˜75˜mm, 2.2˜µm packing) to separate the impurity (Fig.˜6).

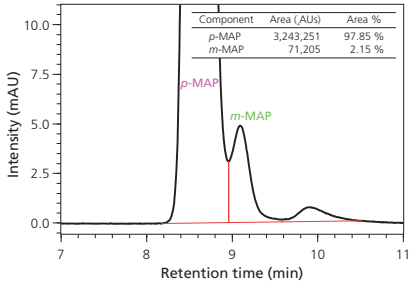


Fig. 6 Measurement of *m*-MAP Content in *p*-MAP Standard Sample

As a result, the *m*-MAP peak was separated and it was confirmed that with the area ratio in results averaged from three measurements the standard sample contained 2.15˜% *m*-MAP.

3-2. Using i-PDeA II for Quantitative Analysis

To evaluate how well the algorithm performs, 400˜µg/mL, 400˜µg/mL, and 4˜µg/mL standard samples of *o*-MAP, *m*-MAP, and *p*-MAP, respectively, were measured individually using the same analytical conditions as used to analyze the three-component mixture sample. Then the area values from measurement results were compared to the area values of separated peaks. In addition to identifying the separated peaks using the spectra in Fig. 3, their similarity was calculated as well.[8)]

A comparison of area values and spectral similarity from averaged results for three analyses is shown in Table˜1. (The true *p*-MAP and *m*-MAP values were calculated by correcting the area value measured from the *p*-MAP standard sample to compensate for the 2.15˜% *m*-MAP content.)

Table 1 Evaluation of i-PDeA II Performance for Quantitative Analysis

Component	Area (µAUs)		Error %	Similarity
	True Value	Mixture Sample (Deconvoluted)		
o-MAP	2,090,806	2,080,405	-0.50 %	1.0000
p-MAP	27,666	26,639	-3.71 %	0.9996
m-MAP	2,658,837	2,656,836	-0.08 %	1.0000

Fig.˜7 shows the normalized *p*-MAP spectrum from the standard sample compared to the spectrum estimated based on the separated peak from the mixture.

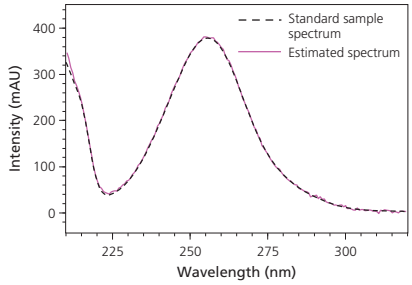


Fig. 7 Shape Comparison of *p*-MAP Spectra

In the case of the three-component mixture sample with relative *o*-MAP, *p*-MAP, and *m*-MAP concentrations of 100, 1, and 100, respectively, there was less than ±1˜% error and over 0.9999 similarity between the area values of separated peaks and the corresponding peaks measured from standard samples with relative *o*-MAP and *m*-MAP concentrations of 100 and less than ±4˜% error and over 0.9996 similarity between the area values of peaks for the relative *p*-MAP concentration of 1.

4. Data Analysis Using LabSolutions

The i-PDeA˜II peak separation algorithm is included in LabSolutions data analysis functionality. Data for separated peaks can be displayed as chromatograms for individual peaks, and also as separated spectra, in the LabSolutions PDA data analysis window (Fig.˜8).

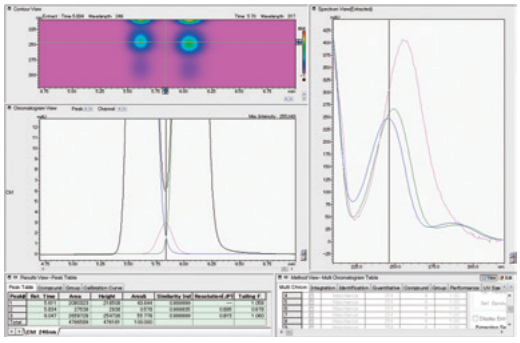


Fig. 8 PDA Data Analysis Window in LabSolutions

The window for i-PDeA˜II settings is shown in Fig.˜9.

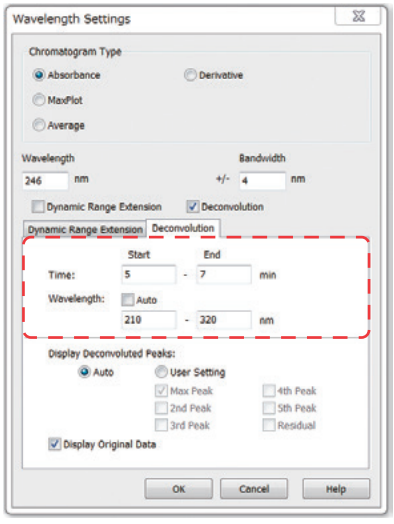


Fig. 9 Window for i-PDeA II Settings

Peaks can be separated using the i-PDeA˜II function by simply specifying the wavelength and time ranges.

By using the data analysis functionality in LabSolutions, the entire process of separating peaks, integrating the areas under separated peaks, and calculating quantitative values can be performed seamlessly without any data conversion and spectra can be identified and libraries searched based on peak-top spectra.

5. Conclusion

A new analytical technique was developed for separating peaks that is based on improved basic photodiode array detector performance, superior HPLC/UHPLC system reproducibility, and incorporation of chemometrics technology, in addition to column technologies even for difficult-to-separate-peaks.

The i-PDeA˜II function can help analyze samples more quickly and improve laboratory productivity. Key points are summarized below.

- An algorithm for separating co-eluted peaks was developed by applying the MCR-ALS technique from chemometrics to photodiode array detector data.
- Fast and accurate quantitative analysis is possible even if components are not fully separated in the column.
- i-PDeA II can even be used to analyze isomers with identical molecular weights.
- Spectral data can be analyzed even after peak separation.

i-PDeA˜II provides a unique solution for peak separation or quantitative analysis of isomer samples that was not possible with previously available techniques. These features can be expected to provide further improvements in analytical efficiency and data reliability.

Acknowledgments

i-PDeA˜II was developed based on results obtained from joint development work with Eisai Co., Ltd. We are especially grateful to Takashi Kato, Kanta Horie, Shuntaro Arase, Hideki Kumobayashi, and the many others involved for their generous cooperation during development. In particular, we are grateful to Naoki Asakawa for providing the development opportunity and generously sharing his extensive knowledge and valuable suggestions during routine discussions.

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Solution 5: Impurity Identification

In recent years, regulations have become increasingly strict with the requirements for impurities in pharmaceuticals. For example, the International Council for Harmonization (ICH) Q3B (R2) (Impurities in New Drug Products) requires identification and characterization of all degradation products, even at trace levels.

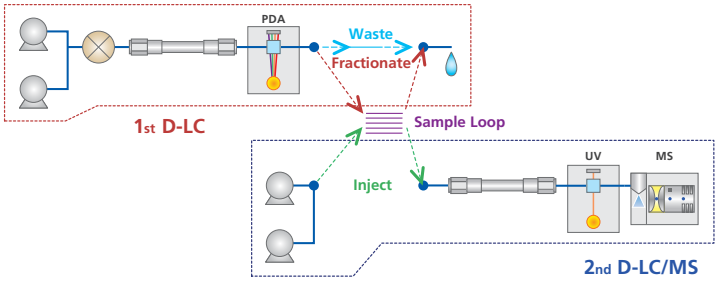
Various mass spectrometry with excellent qualitative capabilities are excellent tools for impurity identification. However, the different operating requirements and compatibility issues of the chromatography and mass spectrometry systems have, to some extent, proved to be challenging and have increased the workload.

Shimadzu offers a sophisticated 2D-LC system and uses it in conjunction with our MS to help you efficiently eliminate the difficulties in impurity identification.

Trap-free 2D LC/MS System

The perfect combination of 2D-LC and MS

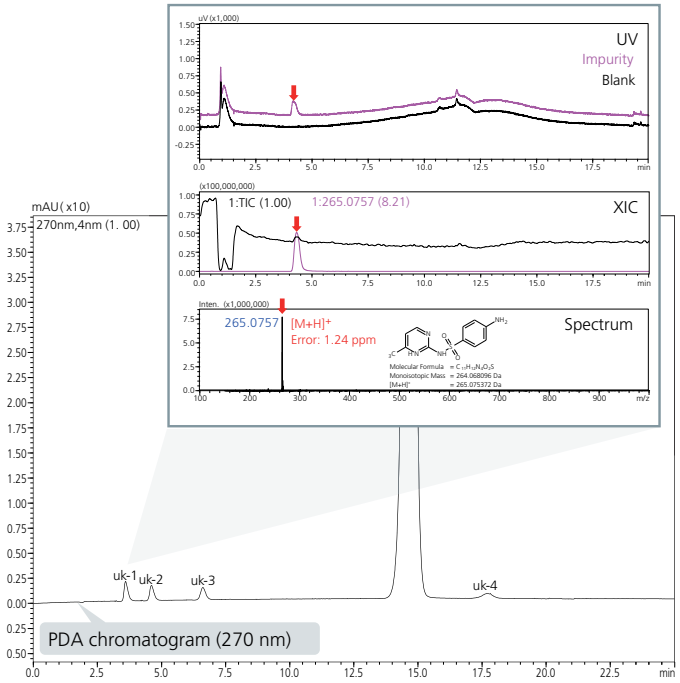
- Using Shimadzu's unique trap-free 2D LC system, combined with dedicated software, the fraction from first dimension LC is automatically subjected to structural elucidation by LC/MS in the second dimension.
- Shimadzu's ultra-fast mass spectrometry LC-MS/MS and high-quality precision multi-stage tandem mass spectrometry LCMS-IT-TOF can be used for structural analysis and confirmation of impurities.



✓ Online switching of mobile phase

From separation to identification in one step

- There is no need to re-develop the method. The initial LC conditions are used to accurately locate the impurity. The fluctuation of impurity peaks retention time caused by the change of buffer system can be avoided.
- Even if a non-volatile buffer is used in the original analytical method, no method changes are required. It can be easily switched to volatile buffer with the use of 2D-LC, and online accurate identification of target impurities can be achieved.



Step 1
Choose impurity peaks to be identified in the principal component UV chromatogram.

Step 2
Using the support tool, specify the impurity retention times, and create a time program and batch schedule.

Step 3
Using the 2DLC-LCMS system, conduct sample analysis and blank analysis.

Step 4
Compare the second-dimension UV chromatogram with the blank analysis data and identify impurity peaks.

Step 5
Confirm the mass spectrum for peaks with the same retention times as both the UV peaks and the MS peaks.

Step 6
Identify the impurities by composition analysis.
(If necessary, acquire MS^n data and conduct structural analysis.)

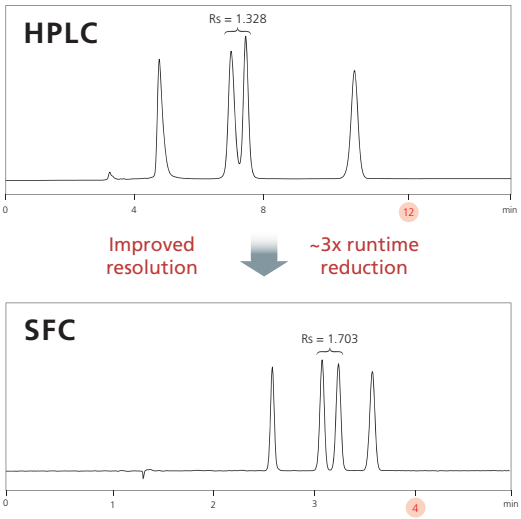
Solution 6: Chiral Separation

Among all the drugs used in the world, chiral drugs account for more than half. These chiral compounds (enantiomers) may exhibit different responses to pharmacological activities and metabolic processes in the human body; some may show therapeutic effects, or be deemed ineffective or in the worst case even harm the body. Accurate analysis of chiral impurities that may occur during drug synthesis is an important part of ensuring drug safety.

When traditional normal phase or reversed phase chromatography is used for chiral separation, difficult column selection, poor separation, long analysis time, and large solvent consumption are common problems.

SFC is widely used for the separation and analysis of chiral compounds. Shimadzu Nexera UC offers a variety of SFC configurations to meet your different analytical testing needs in chiral separations.

Nexera UC SFC System
Chiral Screening System



Comparison of retention time and separation acquired by Conventional LC and SFC
(sample: α-tocopherol, column: Shim-pack UC-X Sil)

✓ Effective GUI for Chiral Screening System

Ideal solution for chiral analysis

- The supercritical fluid with large diffusion coefficient and low viscosity is used as the mobile phase. The modifiers, such as methanol, with different polarities and proportions can meet the separation and detection requirements of chiral compounds with different polarities in a wider range.
- With any combination of columns and modifiers, a large number of analytical methods are automatically generated for screening, perfectly supporting the development of chiral compound methods.

Perfect combination to increase efficiency and reduce cost

- Compared to traditional normal phase and reversed phase chromatography, Nexera UC provides better separation selectivity and faster analysis for difficult-to-separate isomers.
- Nexera UC can help you to minimize organic solvent consumption, and reduce analysis costs while reducing environmental impact.

Nexera UC SFC-UV System

C190-E200

SHIMADZU
Excellence in Science

Technical Report

Supercritical Fluid Chromatography

Hidetoshi Terada[1], Takato Uchikata[1], Takanari Hattori[1], Keiko Matsumoto[1], Yoshiyuki Watabe[1], Tadayuki Yamaguchi[1], Yasuhiro Funada[1]

Abstract:

Advances in column technology have led to a renewed interest in supercritical ˜uid chromatography, which uses a supercritical ˜ uid as its mobile phase. Compared to liquid, supercritical ˜uids have low viscosities and high diffusivities. In this report, starting from the basic principles of supercritical ˜uid chr omatography, we introduce examples of high-speed, high-resolution analysis and chiral separation.

Keywords: supercritical fluid chromatography, SFC

1. Supercritical Fluid

A supercritical fluid is a state of substance wherein the temperature and pressure are both above its critical point (Fig. 1). Supercritical fluids can dissolve substances better than gases and are more diffusive and have lower viscosities than liquids (Table 1). Although various substances have particular critical points, the especially low critical point of carbon dioxide (critical temperature: 31.1°C, critical pressure: 7.38 MPa) makes it easy to handle. As it is non-flammable, inert, low-cost, and non-toxic, it has been widely used in industrial processes, such as for decaffeination of coffee beans and extraction of hops extract and flavor compounds (Fig. 2). Supercritical fluids are also used in analytical fields, including as the main mobile phase in supercritical fluid chromatography (SFC) and the main extracting solvent in supercritical fluid extraction (SFE).

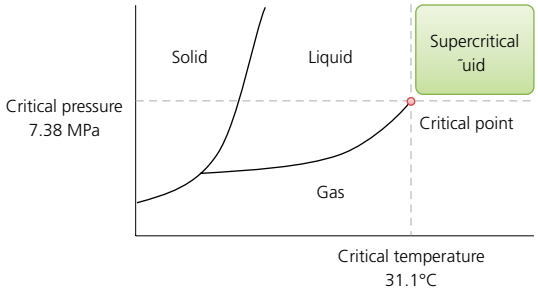


Fig. 1 Phase Diagram for Carbon Dioxide

Table 1 Properties of Supercritical Fluids

	Diffusion coefficient (cm^2/s)	Density (g/cm^3)	Viscosity ($g/cm \cdot s$)
Liquid	10^{-6}	1	10^{-2}
Supercritical fluid	10^{-3}	0.2 to 0.8	10^{-3}
Gas	10^{-1}	10^{-3}	10^{-4}

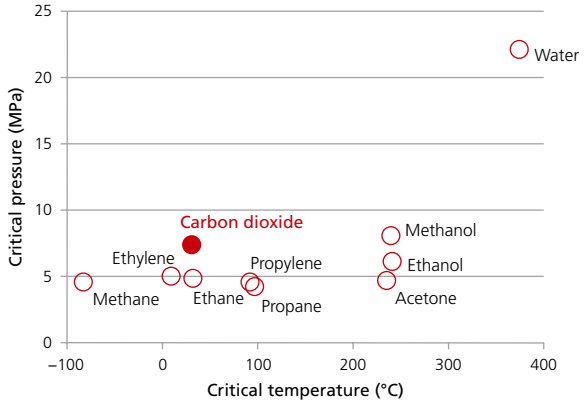


Fig. 2 Critical Points of Various Substances

2. Supercritical Fluid Chromatography

SFC is a separation technique that uses a supercritical ˜uid as its main mobile phase (often supercritical carbon dioxide). Because of the properties of supercritical ˜uids, which include low viscosities and high diffusivities, SFC can be performed at a lower column back pressure than conventional high-performance liquid chromatography (HPLC). Additionally, a high-speed analysis can be performed at high ˜ow rates and a high-resolution analysis can be performed by using a longer column. Also, recent advances in SFC systems and in the packed columns made for SFC allow analyses to be performed with HPLC-like operation.

Although supercritical carbon dioxide has a similar hydrophobicity as hexane, this property alone is often insufÿcient for the elution of target compounds from a column. These target compounds can be eluted by adding an organic solvent, called a modiÿer, to modify the polarity of the mobile phase. Organic solvents that are compatible with carbon dioxide, such as methanol, ethanol, isopropyl alcohol, and acetonitrile, are used as modiÿers. Organic solvents with an added acid (e.g., formic acid or acetic acid), salt (e.g., ammonium formate or ammonium acetate), or base (e.g., diethylamine) are also used as modiÿers for the analysis of highly polar compounds.

1 Analytical & Measuring Instruments Division

3. Nexera UC

The Nexera UC platform can accommodate a wide variety of analyses and pretreatments and includes an (1) SFC system, (2) online SFE-SFC system, and (3) of'ine SFE system. A major difference between a Nexera UC system and a conventional HPLC system is the addition of a back pressure regulator to prevent mobile phase vaporization inside the column and the pump that delivers the carbon dioxide. The Nexera UC platform is based around the Nexera ultra high-performance liquid chromatograph, with each Nexera UC system conÿgured by adding a newly developed carbon dioxide delivery unit (LC-30ADSF), a back pressure regulator unit that allows high-precision pressure control (SFC-30A), and an extraction unit used for SFE (SFE-30A) (Fig. 3). The autosamplers and other units designed for liquid chromatography can be used in the Nexera UC system.

(1) SFC system

SFC systems include an SFC-UV system that uses a UV (or PDA) detector, a UFMS system (SFC-MS) that uses a mass spectrometer (MS) that is suitable for high-speed analyses by SFC, and a chiral screening system that automatically switches between multiple columns and modiÿers to examine the analytical conditions.

(2) Online SFE-SFC system

Online SFE-SFC systems combine SFE and SFC online to automatically perform all steps from target compounds extraction from solid samples to analysis.

(3) SFE pretreatment system

Of'ine SFE pretreatment systems are speciÿcally designed to extract target compounds from solid samples.

The characteristic properties of the supercritical 'uid used in the Nexera UC SFC systems, which include high diffusivity and low viscosity, allow for low column pressures even at high 'ow rates, enabling high-speed analyses while maintaining column efÿciency. Because of these factors, the Nexera UC SFC systems can shorten analysis times to between one third and one ÿfth of the time required for HPLC analysis using the same size column. (Fig. 5).

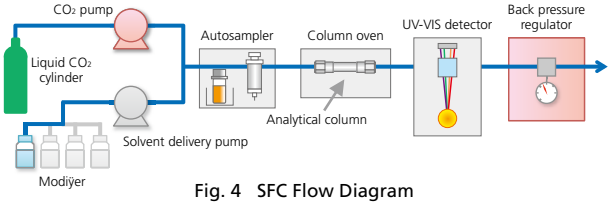
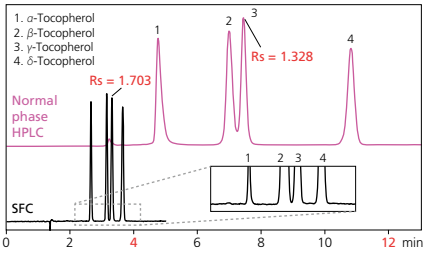


Fig. 4 SFC Flow Diagram



SFC conditions

Column	:	Shim-pack UC-SIL (4.6 mm I.D. × 250 mm L. 5 µm)
Modiÿer	:	MeOH
Modiÿer conc.	:	5 %
Flow rate	:	3.5 mL/min
Temperature	:	40°C
Detection	:	UV 290 nm
Back pressure	:	10 MPa

Normal phase HPLC conditions

Column	:	Shim-pack HRC-SIL (4.6 mm I.D. × 250 mm L. 5 µm)
Mobile phase	:	HEX/IPA 99/1 (V/V)
Flow rate	:	1 mL/min
Temperature	:	40°C
Detection	:	UV 290 nm

Fig. 5 Comparison between HPLC and SFC

SFC-UV System

SFC-MS System

Fig. 3 Nexera UC Systems

By using the same column packing material for separation in the Nexera UC systems as that used in normal phase HPLC analysis (e.g., silica gel), normal phase HPLC analyses can be easily transferred to SFC analyses while improving the resolution and increasing the analysis speed, as shown in Fig. 5. Transferring analyses from normal phase HPLC to SFC can also substantially reduce the volume of organic solvents consumed per analysis, as shown in Fig. 6, which also reduces analysis costs. SFC is an environment- and user-friendly technique as it reduces consumption of toxic organic solvents.

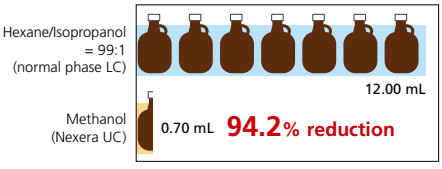
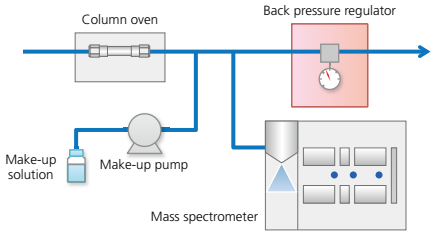


Fig. 6 Comparison between HPLC and SFC of Organic Solvent Consumption

When using a mass spectrometer for SFC, equipment used for LC/MS can be used as is. In SFC, a make-up solution is added after column separation to promote ionization. Conventional SFC systems used pressure regulators that had a large internal volume. This required the £ow path of the column eluate to be split before entering the mass spectrometer to suppress the effect of extra-column dispersion (Fig. 7(a)). The Nexera UC systems use a proprietary low-internal volume design for their back pressure regulator (patent pending). This allows the £ow path to enter the back pressure regulator and mass spectrometer in series, so all the column eluate enters the mass spectrometer (Fig. 7(b)). Increasing the volume of eluate introduced to the mass spectrometer in this way enables higher sensitivity analysis, and precludes the effects of split ratio variation, etc., resulting in highly reproducible SFC/MS analysis (Fig. 8, Table 2).

(a) Conventional SFC/MS flow path



(b) Nexera UC SFC/MS flow path

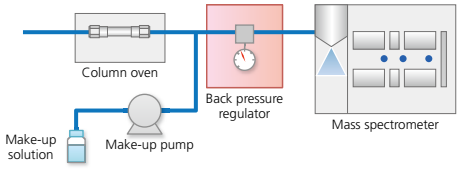
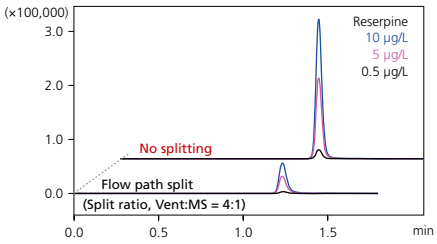


Fig. 7 SFC-MS Flow Path



Column : Shim-pack UC-Diol 4.6 mm I.D. × 150 mm L.
Modifier : MeOH
Modifier conc. : 30 %
Flow rate : 3.0 mL/min
Temperature : 40°C
Back pressure : 10 MPa
Injection volume : 1 µL
Sample : Reserpine (0.5, 5, 10 °g/L)
Detection : TQ mass spectrometer ESI(+) *m/z* 609.3 > 195.0

Fig. 8 Sensitivity With and Without Flow Path Splitting

Table 2 MS Reproducibility With and Without Flow Path Splitting

	Injection volume (µL)	Retention time		Area		Height	
		Ave.	%RSD	Ave.	%RSD	Ave.	%RSD
Flow path split	0.1	0.359	0.64	6,583	18.83	2,361	17.29
	1	0.356	0.25	81,467	4.26	26,656	3.88
	2	0.355	0.32	156,831	2.18	49,721	3.28
No splitting	0.1	0.356	0.09	16,264	6.18	7,673	6.17
	1	0.353	0.05	155,170	2.43	71,971	2.23
	2	0.35	0.07	325,739	1.16	142,350	1.19

Column : Shim-pack UC-Diol 4.6 mm I.D. × 150 mm L. 5 µm
Modifier : MeOH with 0.1 % w/v ammonium formate
Modifier conc. : 30 %
Flow rate : 2.0 mL/min
Temperature : 40°C
Back pressure : 10 MPa
Injection volume : 1 µL
Detection : TQ mass spectrometer ESI(–) *m/z* 351.20 > 271.20 (prostaglandin 100 µg/L)

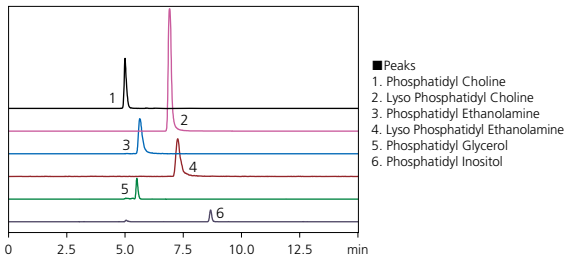
4. Shim-pack UCX Series Columns for SFC

Because of the high diffusivity of the mobile phase used in SFC, the separation behavior substantially changes based on the column stationary phase and modifiers used. The Shim-pack UCX series columns are designed for SFC and encompass eight different stationary phases, as shown in Table 3. This allows the columns to accommodate the separation of a wide variety of compounds.

Table 3 Shim-pack UCX Series Columns

	Functional group	Pore size	Surface area	Carbon content
Shim-pack UC-RP	Octadecyl group + polar functional group	10 nm	—	9%
Shim-pack UC-GIS II	Octadecyl group		450 m^2/g	11%
Shim-pack UC-Diol	Diol group			20%
Shim-pack UC-Sil	—			—
Shim-pack UC-Amide	Carbamoyl group			18%
Shim-pack UC-NH2	Aminopropyl group			8%
Shim-pack UC-Phenyl	Phenethyl group			9.5%
Shim-pack UC-CN	Cyanopropyl group			14%

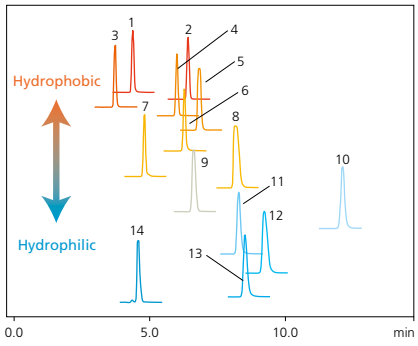
Fig. 9 shows an example analysis of phospholipids using the Shim-pack UCX-Diol column. This column allows separation of phospholipids by class, as with normal phase LC. Phospholipids can also be separated by molecular species using the same modifier conditions paired with a different column, such as the Shim-pack UCX-GIS II, which has an octadecyl group stationary phase. Using different stationary phases but the same mobile phase, SFC can be used to recreate the retention behaviors observed with normal phase and reverse phase HPLC, providing a variety of other separation behaviors. This is of substantial benefit for the analysis of complex samples.



Column : Shim-pack UC-Diol 4.6 mm I.D. × 150 mm L. 5 µm
Modifier : MeOH with 0.1 % w/v ammonium formate
Modifier conc. : 10 % (0 min) → 40 % (10–15 min)
Flow rate : 3.0 mL/min
Temperature : 40°C
Make-up solution : MeOH with 0.1 % w/v ammonium formate
Make-up flow rate : 0.1 mL/min
Back pressure : 10 MPa
Detection : TQ mass spectrometer (ESI)

Fig. 9 Phospholipid Analysis

Fig. 10 shows an example analysis of pesticides of a wide range of polarities—from hydrophobic to hydrophilic—using the Shim-pack UCX-RP column. The Shim-pack UCX-RP column is unique in having a stationary phase that combines octadecyl and polar functional groups. This stationary phase is able to retain a wide range of compounds, including both hydrophobic and hydrophilic compounds. This column allows the simultaneous analysis of pesticides that were previously difficult to analyze without changing the analytical conditions, thereby providing improved analytical efficiency.



No.	Compound	log *P*
1	Carbofuran	7.4
2	Ethofenprox	6.9
3	Fenpropathrin	6.0
4	Pyriproxyfen	5.0
5	Pyraclostrobin	4.0
6	Linuron	3.0
7	Aminocarb	1.9
8	Ethoxysulfuron	1.0
9	Halosulfuron methyl	0.0
10	Bentazone	–0.5
11	Chlorsulfuron	–1.0
12	Rimsulfuron	–1.5
13	Nicosulfuron	–1.8
14	Vamidothion	–4.2

Column : Shim-pack UC-RP 4.6 mm I.D. × 150 mm L. 5 µm
Modifier : MeOH with 0.1 % w/v ammonium formate
Modifier conc. : 0 % (0 min) → 10 % (11 min) → 30 % (14 min) → 40 % (14.01–17 min)
Flow rate : 3.0 mL/min
Temperature : 40°C
Make-up solution : MeOH with 0.1 % w/v ammonium formate
Make-up flow rate : 0.1 mL/min
Back pressure : 15 MPa
Detection : TQ mass spectrometer (ESI)

Fig. 10 Pesticide Analysis

5. Chiral Separation

In the field of pharmaceuticals, research is underway in the area of drug discovery using chiral columns for rapid chiral separation. Finding the appropriate combination of analytical column and mobile phase for a given analyte from the wide variety of chiral columns available requires a substantial amount of time and labor. Therefore, there is a demand to improve the speed of condition scouting for chiral separations.

The speed and labor required for scouting chiral compound separation conditions can be improved by combining Shimadzu's Nexera UC chiral screening system and the wide range of polysaccharide derivative CHIRALPAK and CHIRALCEL series chiral columns (Daicel Corporation).

The Nexera UC chiral screening system includes an SFC system, solvent switching valves, and column switching valves and is able to acquire comprehensive data by automatic and continuous screening of the modifier conditions on a maximum of 12 columns. Its mobile phase blending function can also mix up to four different solvents to user-defined ratios for analysis under a variety of separation conditions, which significantly simplifies the workflow of condition scouting for chiral compounds.

Also, Method Scouting Solution for Nexera UC is software that presents a graphical user interface environment developed to support the process of separation condition scouting for chiral compounds (Fig. 11). This software provides database management for analytical columns, mobile phases, and modifiers, which improves management efficiency and can reduce the number of operating errors that arise with multiple operators. The software provides powerful support for work related to chiral compound analysis, including work such as the calculation of required modifier and sample volumes, column washing, changeover of enclosed liquids at the end of analysis to prevent column degradation, and estimation of analysis completion times.

Here, we present chiral separation screening results for omeprazole obtained from all 36 possible combinations of the 12 chiral columns (Daicel Corporation) and three modifier conditions (Fig. 12, 13).

The Nexera UC chiral screening system utilizes SFC to select the mobile phase and optimize the separation conditions in a short time period, which improves R&D efficiency during the drug discovery stage of pharmaceutical production.

Fig. 11 Configuration Window of Method Scouting Solution Software

Column
(1) CHIRALPAK IA-3/SFC
(2) CHIRALPAK IB-3/SFC
(3) CHIRALPAK IC-3/SFC
(4) CHIRALPAK ID-3/SFC
(5) CHIRALPAK IE-3/SFC
(6) CHIRALPAK IF-3/SFC
(7) CHIRALPAK AD-3/SFC
(8) CHIRALPAK AS-3/SFC
(9) CHIRALPAK AY-3/SFC
(10) CHIRALCEL OD-3/SFC
(11) CHIRALCEL OJ-3/SFC
(12) CHIRALCEL OZ-3/SFC

3.0 mm I.D. × 100 mm L. 3 µm

×

Modifier
(1) MeOH
(2) EtOH
(3) Acetonitrile/EtOH 75/25 (V/V)

Modifier conc	:	20 %
Flow rate	:	3.0 mL/min
Temperature	:	40°C
Back pressure	:	10 MPa
Injection volume	:	1 µL
Detection	:	UV (300 nm)

Fig. 12 Screening Conditions

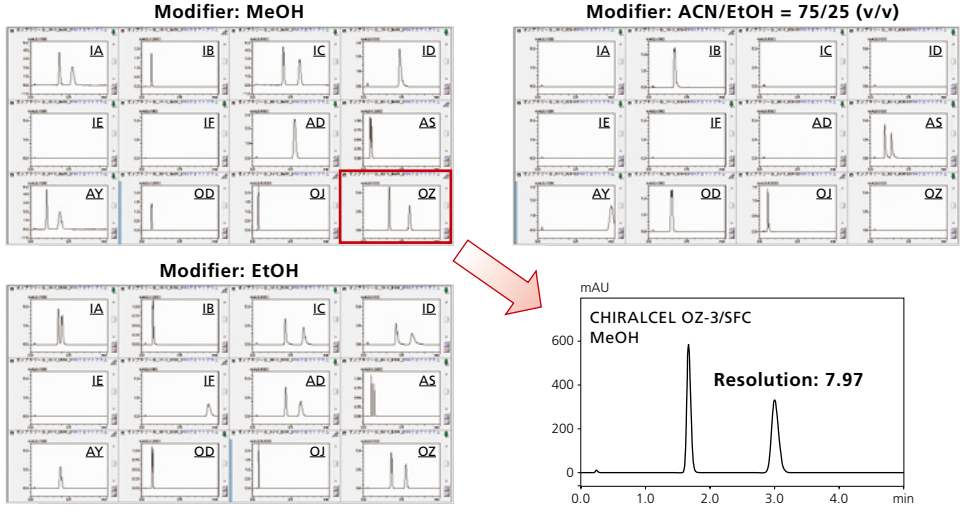


Fig. 13 Screening Results

CHIRALPAK and CHIRALCEL are registered trademarks of Daicel Corporation.

First Edition: September, 2016

Shimadzu Corporation
www.shimadzu.com/an/

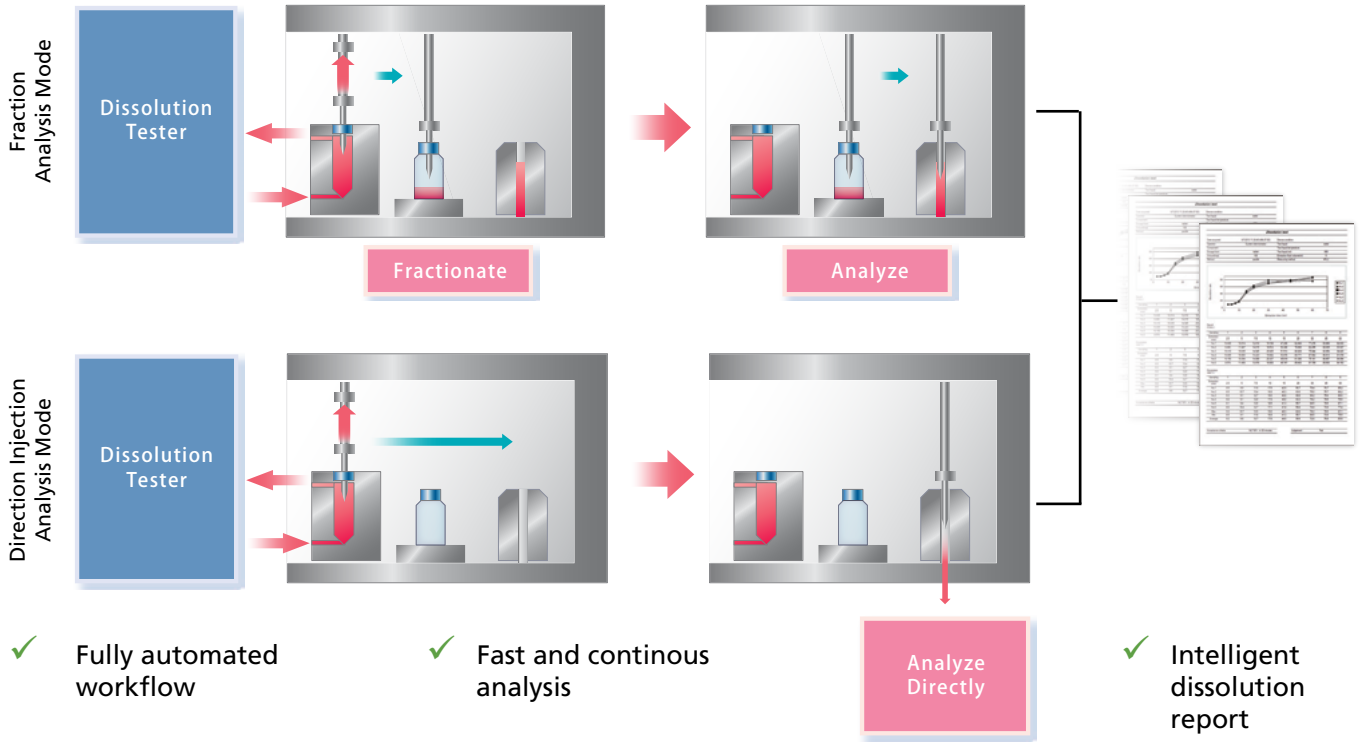
Solution 7: Online UHPLC for Dissolution Testing

Dissolution analysis is widely used in drug development and quality control. The online analysis of dissolution apparatus and UHPLC can greatly simplify the dissolution test operation and improve work efficiency. This combination has been increasingly recognized by chemists in the pharmaceutical industries.

However, the existing online systems in the market still have issues with sample dilution, sample preparation, carryover, etc. Furthermore, the analysis speed of LC cannot match the progress of the dissolution test.

The Nexera FV system, developed exclusively by Shimadzu, really helps you seamlessly connect from dissolution testing to LC analysis.

Nexera FV



✓ Fully automated workflow

✓ Fast and continous analysis

✓ Intelligent dissolution report

New online Dissolution Apparatus - UHPLC System

- The newly designed Nexera FV autosampler is directly connected with the dissolution tester to realize the automation of the whole process from sampling to data acquisition and result report.
- The Nexera FV system, which possesses high speed, stability, powerful automatic dilution and sample preparation function of Nexera X2, is even more powerful and capable in obtaining highly reliable dissolution test results.

Significantly improve the efficiency of dissolution analysis

- Fully automated workflow reduces the amount of manual transfer and dilution of the sample, as well as possible human error.
- The high speed of UHPLC with the online coupling of the Nexera FV allows non-stop and uninterrupted dissolution-LC batch analysis. This online system is proven to be effective for the sustained release preparation.

SHIMADZU
Excellence in Science

Technical Report

Auto Dilution of Standard and Sample Solutions Using the SIL-30AC Autosampler

1. Introduction

When conducting quantitative analysis by HPLC, dilution of standard solutions and sample solutions is typically performed manually. Not only does this operation require some degree of experience and knowledge, the time and effort associated with these tasks are certainly not insignificant. With recent advances in instrumentation technology, there is considerable expectation that labor savings through automation will enable improved throughput. Delivering on this expectation by automating these dilution-related tasks should have a very positive impact on productivity.

The Nexera® SIL-30AC autosampler system incorporates such pretreatment functionality as a standard feature, allowing dilution and reagent addition to be fully automated.

Here, we introduce an example of analysis of caffeine in beverages using the pretreatment feature of the SIL-30AC.

2. Automatic Preparation of Standard and Sample Solutions

Quantitation methods typically use a calibration curve, where multiple standard solutions are prepared at known concentrations, and analysis of these provides an equation that defines the correlation between known analyte concentrations and peak area or peak height. Then, the peak area or peak height obtained in the subsequent analysis of the unknown sample is used to calculate the concentration of the analyte. The concentration range is set to obtain a linear calibration curve, and dilution of the sample solution is sometimes necessary to keep the analyte concentration within the linear range.

The SIL-30AC's pretreatment feature eliminates the manual operations related to preparation of standard solutions (Fig. 1). The standard solutions are automatically prepared at the desired concentration levels, and will be injected automatically. Sample solutions can also be diluted to the appropriate dilution factor and then injected, all automatically.

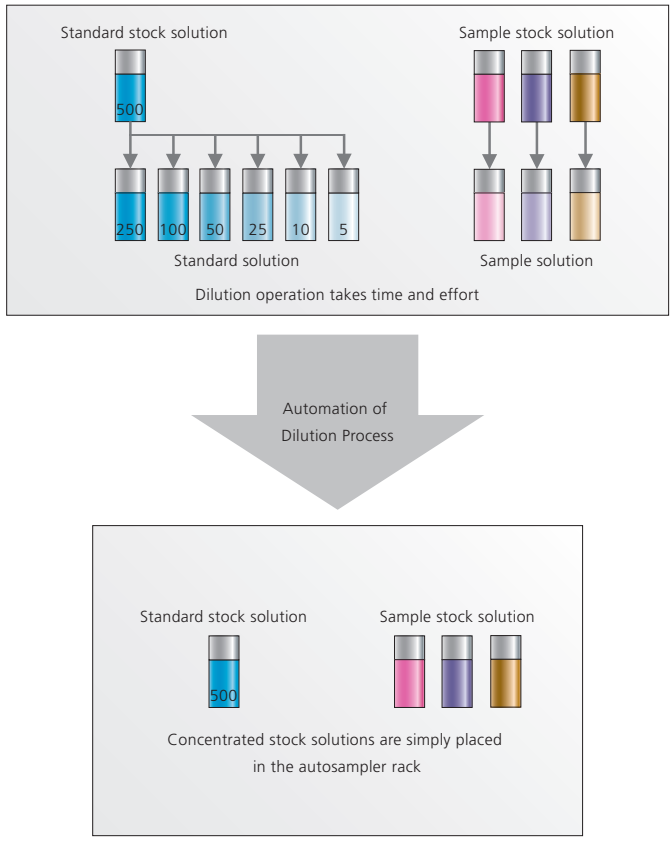


Fig. 1 Advantage of Automated Dilution

3. Templates Facilitating Use of Pretreatment Function

The pretreatment functions of the SIL-30AC are controlled by LabSolutions chromatography software. Common pretreatment functions like dilution and reagent addition are easily accessed through preset templates. Additionally, a custom pretreatment program is available for setting up user-defined commands to accomplish specific operations.

In this example, we present an analysis of caffeine in beverages using the pretreatment template for dilution.

4. Analysis of Standard Solutions

The caffeine standard stock solution will be diluted from 1/2–1/100 of the original concentration using the pretreatment feature of the SIL-30AC, and each dilution level will then be injected onto the column.

4-1. Preparation Items

1) Caffeine standard stock solution
2) Vials for mixing (1.0 mL polypropylene vials)

4-2. Outline of Standard Solution Analysis Operation Procedure

The volume of standard stock solution corresponding to the specific dilution ratio is aspirated from the stock solution vial, then dispensed into an empty vial along with the diluent. Mixing is then conducted in this vial (matching the sample number in the sequence table), and the specified injection volume of mixed solution is aspirated and introduced onto the column (Fig. 2). In this example, one of the SIL-30AC rinse solutions (R0) was defined as the diluent.

These operation steps are shown below.
(1) Aspirate standard stock solution
(2) Dispense standard stock solution with diluent into empty vial
(3) Mixing
(4) Analysis start

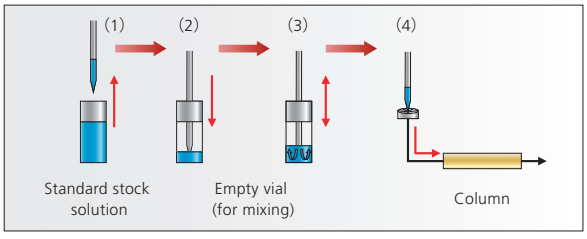


Fig. 2 Standard Solution Injection Operation Procedure

4-3. Entries for Standard Solution Analysis Dilution Template

Fig. 3 shows an example of template entries with placement of the caffeine standard stock solution in rack number 1, vial number 105, and the injection of a 1/20 dilution using rinse solution R0. The final volume following dilution was set to 100 µL.

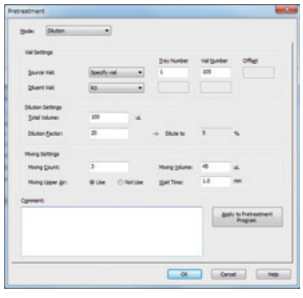


Fig. 3 Template Entry Example for Standard Solution Analysis

The requirements indicated in Fig. 3 are executed via the program below.

n.drain	: Needle is returned to the home position, then moved to the drain position, and then slightly lowered.
disp 600,rs	: Liquid is dispensed at the set volume (600 µL) and speed (rs).
vial.n 1,105	: Needle moves to the specified rack number (1) and vial number (105).
n.strk ns	: Needle is lowered by the set needle stroke (ns) value.
aspir 5.0,ss	: Liquid is aspirated at set volume (post-dilution volume 100 µL / dilution factor 20 =5.0 µL) and speed (ss).
vial.n rn,sn	: Needle moves to specified rack number (rn) and vial number (sn).
n.strk ns	: Needle is lowered by the set needle stroke (ns) value.
disp 100,ss	: Liquid is dispensed at the set volume (100 µL) and speed (ss).
a1=2	: Defines user variable (a1=2).
a2=10	: Defines user variable (a2=10).
mix 3,5,45,a1,a2	: Mixing is conducted the set number of times (3 times), with specified upper air volume (5 µL), mixing volume (45 µL), aspiration speed (a1), and discharge speed (a2).
n.drain	: Needle is returned to the home position, then moved to the drain position, and then slightly lowered.
disp 100,rs	: Liquid is dispensed at the set volume (100 µL) and speed (rs).
d.rinse	: Needle moves to rinse port, and is immersed for the specified immersion time.
inj.p	: Needle moves to injection port, and is lowered to bottom of injection port.
v.inj	: High pressure valve is switched to injection side (flow line in which sample loop connects with solvent delivery pump and column).
wait 1.0	: Operation waits until end of set wait time (1.0 min).
goto f0	: Operation shifts to the set file number (standard injection operation f0).
end	: Pretreatment program ends.

Program variables (ns, rs, etc.) are defined as follows.

ns	: Needle stroke set in the method
rs	: Rinse liquid aspiration speed set in the method
ss	: Sample aspiration speed set in the method
rn	: Specified rack number for batch analysis or single analysis
sn	: Specified vial number for batch analysis or single analysis

4-4. Standard Solution Analysis Example

An example of standard solution analysis is shown below. The settings were performed to dilute a 500 mg/L caffeine aqueous standard stock solution 100-, 50-, 20-, 10-, 5-, and 2-fold in order to obtain standard solutions with final caffeine concentrations of 5 mg/L, 10 mg/L, 25 mg/L, 50 mg/L, 100 mg/L and 250 mg/L, respectively. Pure water was used as the standard stock solution diluent (rinse solution R0). The analytical conditions are shown in Table 1, the analysis results for the various standard solutions in Fig. 4, and the calibration curve obtained from the standard solution analysis results in Fig. 5.

To check the repeatability of the automatic dilution operations, 6 successive analyses were conducted for each automatically diluted standard solution, and the peak area repeatability (%RSD) was calculated. Those results are shown in Table 2.

In addition, to verify the accuracy of dilution(*1) using the SIL-30AC, we compared the peak area values obtained using standard solutions prepared manually by an experienced technician and those prepared using the automatic dilution feature of the SIL-30AC. Those results are shown in Table 3.

*1 Dilution Accuracy (%) = caffeine peak area in standard solution using SIL-30AC ÷ caffeine peak area in standard solution prepared manually × 100

Table 1 Analytical Conditions

Column	: Shim-pack XR-ODS II 75 mmL. x 2.0 mmI.D., 2.2 µm
Mobile phase	: 10 mmol/L (Sodium) phosphate buffer / Methanol = 88 / 12
Flow rate	: 0.6 mL/min
Column temp.	: 40°C
Detection	: 270 nm
Injection volume	: 2 µL

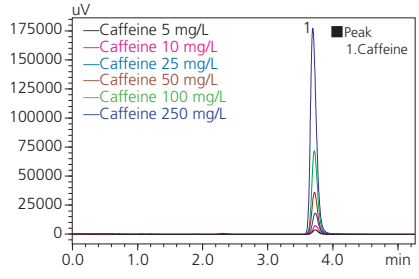


Fig. 4 Overlaid Chromatograms of Standard Solutions (5–250 µg/L)

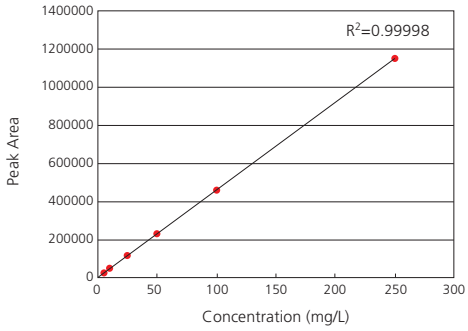


Fig. 5 Caffeine Calibration Curve

Table 2 Repeatability Results of Standard Solution Repeat Analyses (n=6)

Concentration (mg/L)	Area Value %RSD (%)
5	0.425
10	0.505
25	0.167
50	0.297
100	0.068
250	0.145

Table 3 Dilution Accuracy at Each Dilution Ratio

Dilution Ratio (Factor)	Dilution Accuracy (%)
2	100
5	100
10	101
20	101
50	101
100	102

5. Analysis of Sample Solution

Here we describe the procedure for preparing a 1/10 dilution of the sample stock solution using the SIL-30AC pretreatment feature.

5-1. Preparation Items

1) Sample stock solution* (coffee, black tea, green tea)
2) Vials for mixing (1.0 mL polypropylene vials)

* Filtered through membrane filter (0.2 µm pore size)

5-2. Outline of Sample Solution Analysis Operation Procedure

The volume of sample stock solution corresponding to the specific dilution ratio is aspirated from the stock solution vial, and is dispensed into an empty vial along with diluent. Mixing is then performed in the vial, and then the specified injection volume of mixed solution is aspirated and introduced into the column. The operation procedure is the same as that described above for analysis of standard solution.

5-3. Entries for Sample Solution Analysis Dilution Template

Fig. 6 shows an example of template entries assuming auto setting of the sample stock solution vial position, and where the mixing vial position is set to 15 vials offset(*2) from sample stock vial position, and diluent consisting of rinse solution R0 is used to prepare a 1/10 dilution for injection. As in the preparation of the standard solutions, the final volume after dilution was set to 100 µL.

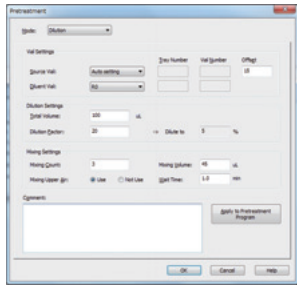


Fig. 6 Template Entry Example for Sample Solution Analysis

The requirements indicated in Fig. 6 are executed via the program below.

```
n.drain          : Needle is returned to the home position, then moved to the drain position,
                   and then slightly lowered.
disp 600,rs      : Liquid is dispensed at the set volume (600 µL) and speed (rs).
a0=sn+15         : Defines user variable (a0=sn+15).
vial.n rn,a0     : Needle moves to specified rack number (rn) and vial number (a0).
n.strk ns        : Needle is lowered by the set needle stroke (ns) value.
aspir 10,ss      : Liquid is aspirated at set volume
                   (post-dilution volume 100 µL / dilution factor 10 =10 µL) and speed (ss).
vial.n rn,sn     : Needle moves to specified rack number (rn) and vial number (sn).
n.strk ns        : Needle is lowered by the set needle stroke (ns) value.
disp 100,ss      : Liquid is dispensed at the set volume (100 µL) and speed (ss).
a1=2             : Defines user variable (a1=2).
a2=10            : Defines user variable (a2=10).
mix 3,5,45,a1,a2 : Mixing is conducted the set number of times (3 times), with specified upper air volume
                   (5 µL), mixing volume (45 µL), aspiration speed (a1), and discharge speed (a2).
n.drain          : Needle is returned to the home position, then moved to the drain position,
                   and then slightly lowered.
disp 100,rs      : Liquid is dispensed at the set volume (100 µL) and speed (rs).
d.rinse          : Needle moves to rinse port, and is immersed for the specified immersion time.
inj.p            : Needle moves to injection port, and is lowered to bottom of injection port.
v.inj            : High pressure valve is switched to injection side (flow line in which sample loop
                   connects with solvent delivery pump and column).
wait 1.0         : Operation waits until end of set wait time (1.0 min).
goto f0          : Operation shifts to the set file number (standard injection operation f0).
end              : Pretreatment program ends.
```

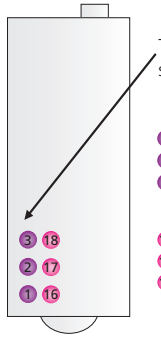
*2 Vial Offset Setting

When the sample stock solution vial position is auto setting, this specifies the distance between the injection vial (empty vial corresponding to sample number SN mixing occurs) and sample stock solution vial as a vial offset number. In the example of Fig. 6 (offset 15), this is defined as follows.

By entering (sample stock solution vial number) = (sample vial number) + 15 offset, it lets the autosampler calculate the position of the stock and sample vials instead of the user manually entering the numbers. This allows the same pretreatment file to be used with different methods and batches with different numbers of samples, and also reduces errors if an incorrect vial number is set.

As shown in Fig. 7 below, if the vials containing sample (for example, No. 1–3) are spaced at the same distance from the empty mixing vials (for example, 16–18), samples can be pretreated and injected sequentially using a single pretreatment program with the variable A0 used to increment vials. This vial position scenario is specified in the program using the following expression:

Arrangement of vials is shown in Fig. 7.



To enter vial numbers for batch analysis or a single analysis, specify the injection vial number (vial numbers 1–3).

1 Empty vial for mixing coffee (vial number 1)
2 Empty vial for mixing black tea (vial number 2)
3 Empty vial for mixing green tea (vial number 3)

16 Coffee stock solution vial (vial number 16)
17 Black tea stock solution vial (vial number 17)
18 Green tea stock solution vial (vial number 18)

Fig. 7 Vial Arrangement Example

5-4. Sample Solution Analysis Examples

Examples of analysis of the sample solutions are shown in Fig. 8–10. All of these were analyzed using a 1/10 dilution of the sample stock solutions prepared using the automatic dilution feature. The analytical conditions were the same as those used for analysis of the standard solutions.

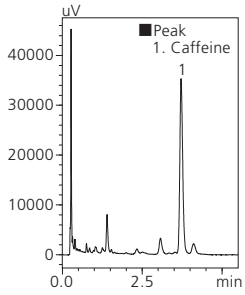


Fig. 8 Chromatogram of Coffee

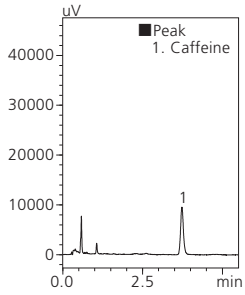


Fig. 9 Chromatogram of Black Tea

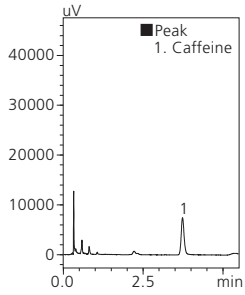


Fig. 10 Chromatogram of Green Tea

6. Overlap Injection Feature

Using the new overlap injection feature of LabSolutions makes it possible to start pretreatment operations for the next analysis while the current analysis is in progress. This permits shortening of the analysis time of the subsequent analysis by one cycle compared to a typical analysis if the analysis is started after the pretreatment (*3). In this analysis, where the pretreatment took 6 minutes and analysis took 7 minutes, utilizing the overlap injection for preparation of the 3 solutions allowed shortening of the analysis time by 31% compared to the standard analysis without overlapping injections (Fig. 11).

*3 Note that the overlap injection cannot be implemented if the autosampler is set for "internal and external needle rinse".

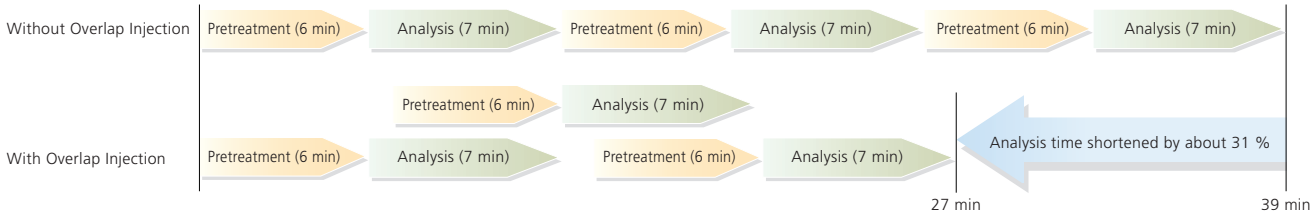


Fig. 11 Shortened Analysis Time Using Overlap Injection

7. Summary

Pretreatment operations were shown to require less time and effort when using the automatic dilution feature of the SIL-30AC. Furthermore, these automated functions allow highly accurate results, free of technician-related errors.

When using the SIL-30AC, automatic dilution and automatic reagent addition can easily be set up by using standard templates. Also, use of pretreatment programs makes it possible to set up very complex operations for a wide range of functions to be executed automatically.

Analyses that are conducted utilizing the autosampler pretreatment features introduced here do not employ a calibrated volumenometer, and are therefore unsuitable if traceability is required.

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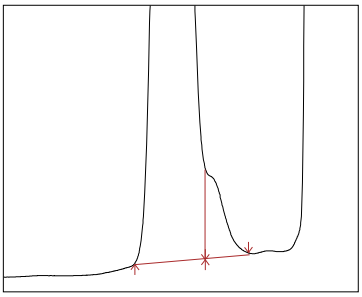
Solution 8: Quantitative Data Processing

As analytical instruments get faster, equipped with automation capabilities and can operate round the clock, data are being generated at a much faster rate. To keep up to speed and improve the laboratory productivity, more efficient, reliable and accurate data processing is required.

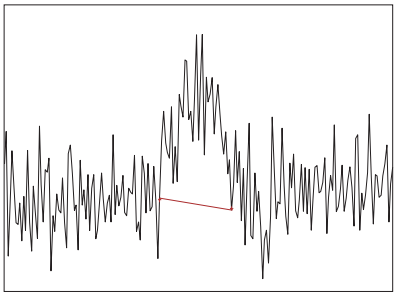
The reliability of chromatographic quantification is greatly influenced by the integration repeatability problems caused by noise, drift and erratic baseline, as well as the efficiency and regulatory compliance issues associated with manual integration. Meanwhile, in drug analysis, the wide disparity and great changes of concentration bring some difficulties to the rapid and accurate quantification of different components.

Shimadzu LabSolutions software provides a variety of automated chromatographic peak integration algorithms and data processing functions, which can easily achieve accurate quantitative analysis of all types of chromatographic data.

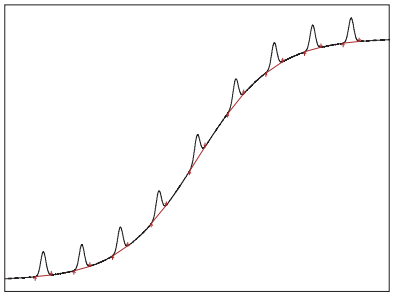
i-PeakFinder



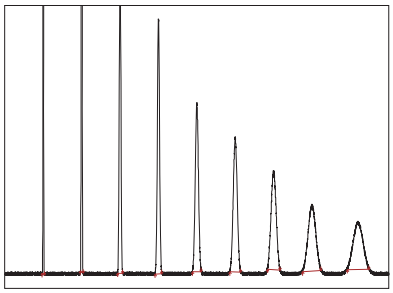
✓ Highly accurate detection of shoulder peaks



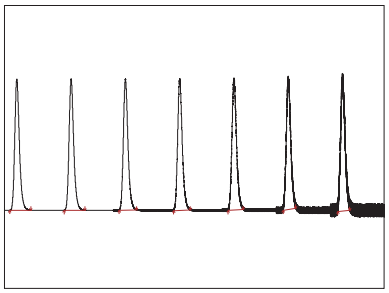
✓ Peak detection at low S/N levels



✓ Automatic determination of baseline undulation and drift



✓ Automatic tracking of peak width variation



✓ Automatic tracking of noise intensity changes

A new automated chromatographic peak integration algorithm

- i-PeakFinder, a new peak integration algorithm for LabSolutions, is a completely automated integration function that can detect peaks with high levels of accuracy without requiring special parameter adjustments.
- This algorithm has adjustable parameters that allow the integration function to be applied to a wide range of complex chromatogram patterns. Furthermore, the adjustable parameters allow to output highly accurate peak integration results from large quantities of data, even for batch analysis.

Easily calculate peak areas in complex baselines

- For hidden shoulder peaks and impurity peaks seriously interfered by main peaks, no complex time program and other parameter settings are needed, and accurate integration results can be obtained quickly.
- Faced with a variety of complex peak shapes, as well as baseline fluctuations and drift, the consistent peak detection sensitivity can be automatically maintained throughout the chromatography, thus obtaining better peak area reproducibility results.

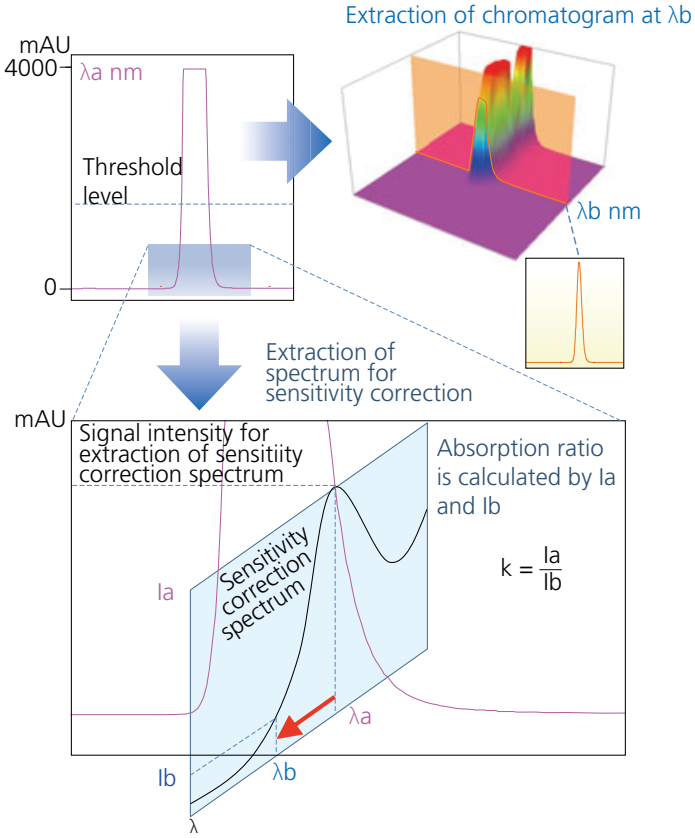
i-DReC

Unique quantitative data processing function

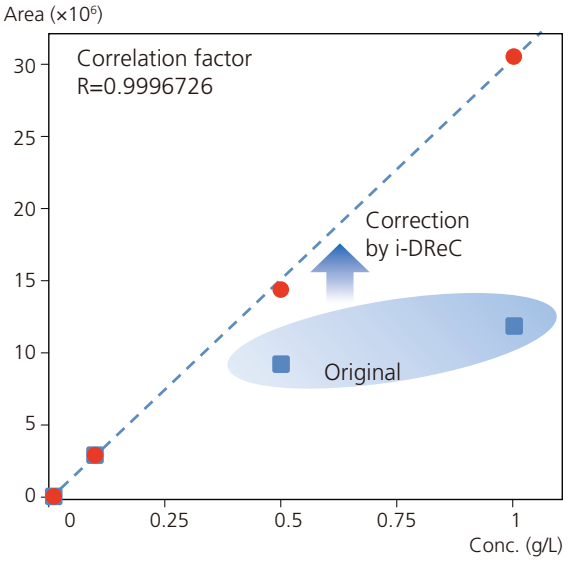
- When the peak intensity of PDA detector data exceeds the user-defined threshold value, i-DReC automatically shifts the chromatographic profile to a wavelength with less UV absorption to prevent signal saturation, so as to avoid wrong quantitative results caused by the chromatographic quantitative peak exceeding the upper limit of the detector.
- The absorption ratio between the original target wavelength and the wavelength used by the i-DReC function is applied as a correction factor to the peak area of the acquired chromatogram, thereby calculating the peak area and height at the original target wavelength.

Accurately quantify high concentration sample

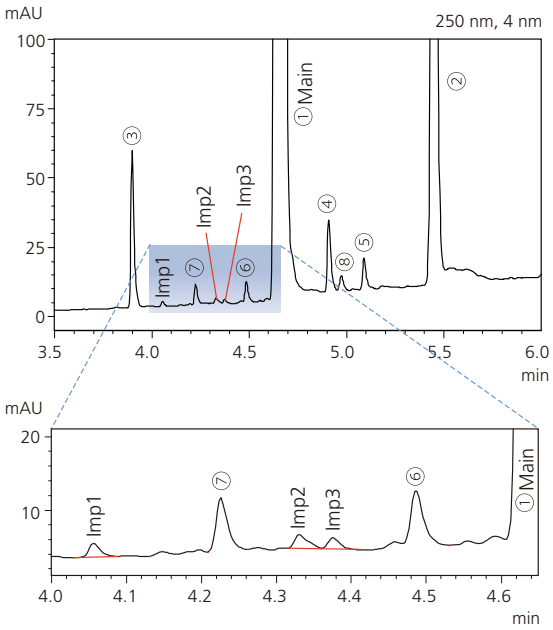
- Faced with high concentration target products in the monitoring of synthetic reaction processes, a broader linear range of standard curves can be established, making it easier to quantify accurately at all concentrations.
- Accurate quantitative results can be obtained with one injection without additional dilution when analyzing the content of active ingredients and impurities in the drug. The efficiency of laboratory analysis can be significantly improved.



Basic principle of i-DReC



Peak area correction and linear dynamic range extension



Peak	Retention Time (min)	Mean Area (μAUsec)	Area %RSD	Area Ratio (%)
1 (Main)	4.634	31,123,746	0.06	--
2	5.448	925,522	0.12	2.974
3	3.900	64,161	0.08	0.206
4	4.910	32,810	0.15	0.105
5	5.091	15,103	0.16	0.049
6	4.487	9,487	0.26	0.030
7	4.226	7,981	0.28	0.026
8	4.975	7,981	0.44	0.026
Imp1	4.056	2,001	0.27	0.006
Imp2	4.331	2,440	0.85	0.008
Imp3	4.376	1,663	0.65	0.005

i-DReC correction provided peak area reproducibility of 0.06% RSD for the saturated Peak1

C191-E044

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Technical Report

A New Peak Integration Algorithm for LabSolutions

Yusuke Osaka[1], Shinji Kanazawa[1], Hiroaki Kozawa[1], Etsuho Kamata[1]

Abstract:

The time required to analyze large quantities of data obtained from the increasingly quick analyses and increasingly prevalent simultaneous multicomponent analyses in recent years has become a challenging issue. In addition, the strengthening of regulations associated with data integrity has given rise to demands for automated and simpliÿed manual methods for integrating the areas of problematic chromatogram peaks. Herein, we introduce a new peak integration algorithm for LabSolutions, i-PeakFinder, which is designed to resolve these issues.

Keywords: Data processing, peak integration, algorithm

1. Introduction

i-PeakFinder, a new peak integration algorithm for LabSolutions, is a completely automated integration function that can detect peaks with high levels of accuracy without requiring special parameter adjustments. In addition, this algorithm has adjustable parameters that allow the integration function to be applied to a wide range of complex chromatogram patterns. Furthermore, the adjustable parameters allow to output highly accurate peak integration results from large quantities of data, even for batch analysis.

2. Features of i-PeakFinder

With Shimadzu's traditional peak integration methods or competitor methods, some complex chromatograms require time programming in addition to the typical parameter adjustments for peak integration. However, i-PeakFinder can perform peak integration of these chromatograms just with simple parameter adjustments. This peak integration function has the following features:

- Highly accurate detection of shoulder peaks
- Simple adjustment of peak-baseline processing
- Improved peak-baseline positioning that results in improved reproducibility
- Performing accurate peak integration even with variabilities caused by baseline drift

Shimadzu also emphasizes on compatibility, so LabSolutions can also be used with the traditional peak integration methods (Chromatopac mode). Switching between the traditional peak integration and i-PeakFinder methods during analysis is easy, allowing the user to select an appropriate peak integration method for the circumstances. This includes selecting a traditional method for compatibility with past data. Fig. 1 shows examples of using the completely automated integration function to analyze typical peaks.

2-1. Highly Accurate Detection of Shoulder Peaks

i-PeakFinder can accurately detect shoulder peaks. In cases wherein manual peak integration is required to distinguish and detect the shoulder and main peaks using traditional peak integration methods, i-PeakFinder can automatically detect shoulder peaks while maintaining consistent peak detection sensitivity throughout the entire chromatogram. In general, it is difýcult to automatically detect considerably small shoulder peaks, as shown in Fig. 2; however, i-PeakFinder can even detect such peaks automatically based on the threshold judgment.

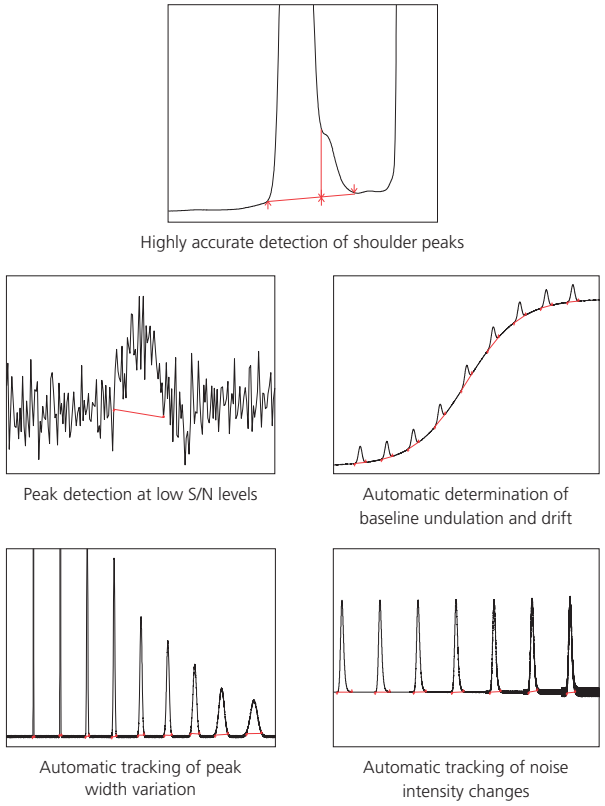


Fig. 1 i-PeakFinder's Completely Automated Integration Function

1 Analytical & Measuring Instruments Division

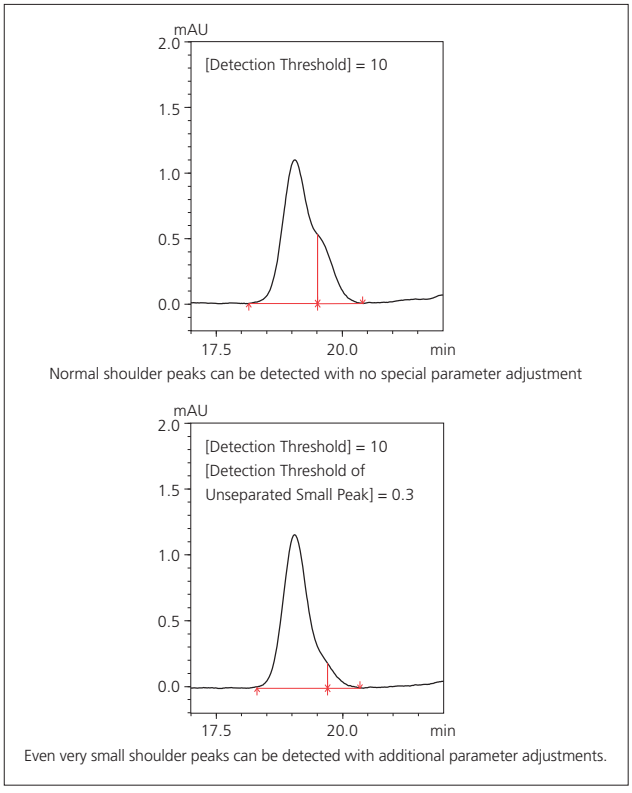


Fig. 2 Examples of Shoulder Peak Detection

2-2. Simple Adjustment of Peak-baseline Processing

Accurate detection of impurity peaks is essential for pharmaceutical quality control and other applications. Impurity peaks are often fused at the base of a major peak, and quantitative results obtained via area normalization can vary depending on the method used for peak-baseline processing. The method used for peak-baseline processing also differs based on the samples and testing objectives. With traditional methods, performing a speciÿc type of peak-baseline processing requires the user to include time programming or perform peak integration manually.

However, i-PeakFinder has adjustable parameters in its basic settings for performing a speciÿc type of peak-baseline processing, allowing the user to easily implement the optimum peak-baseline type in each situation. Fig. 3 shows a list of peak-baseline type in the settings window, Fig. 4 shows the result of peak-baseline type setting of an impurity peak that is fused at the base of a major peak, and Table 1 summarizes the quantitative results obtained via area normalization with different peak-baseline type settings. Peak-baseline processing appropriate for a particular situation can be simply performed by changing a few basic parameters.

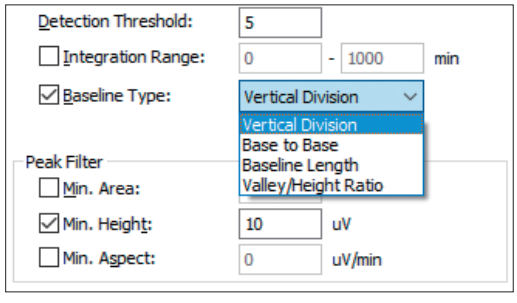


Fig. 3 Peak-baseline Type Settings

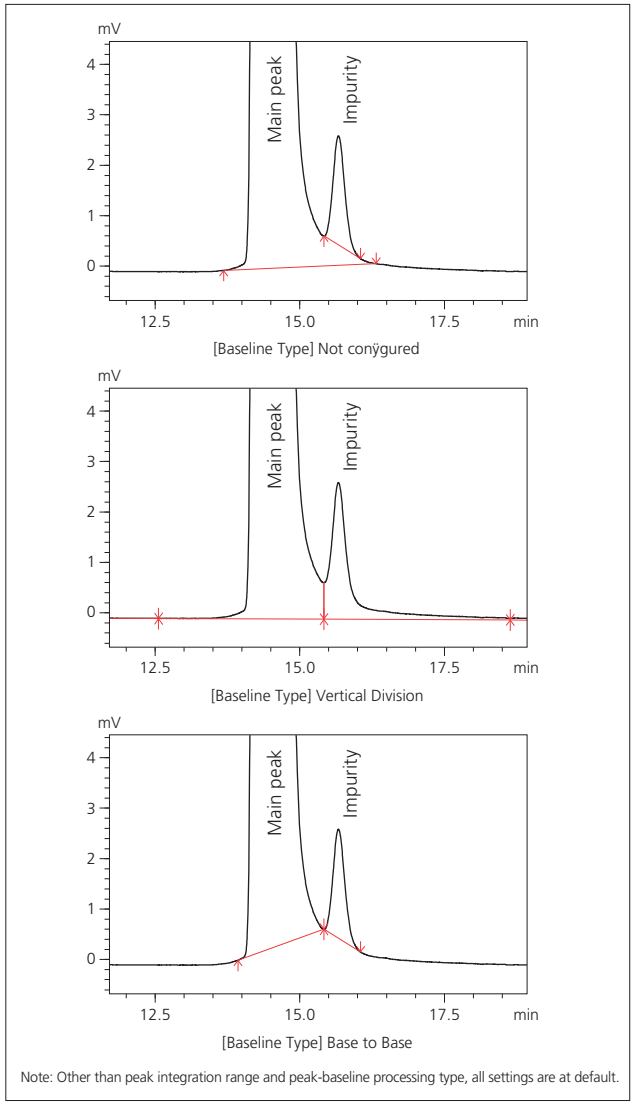


Fig. 4 Peak-baseline Processing Example

Table 1 Quantitative Results Obtained via Area Normalization with Different Methods of Peak-baseline Type

	Not configured	Vertical Division	Base to Base
Main peak	99.681	99.448	99.680
Impurity	0.160	0.338	0.160

2-3. Improved Peak-Baseline Positioning for Enhanced Reproducibility

When a target component is separated with peak tailing or leading in some cases, using a traditional peak integration method would result in variability in the calculated peak areas depending on which point along the trailing and leading edge was deÿned as the peak base. This affected the reproducibility of area results.

With i-PeakFinder, the user can adjust the height at which the start and end of a peak-baseline needs to be deÿned so that peak-baseline processing is consistent even with tailing and leading peaks.

The reproducibility of results obtained using the traditional method and i- PeakFinder is summarized in Table 2 for the chromatogram shown in Fig. 5. In the example below, the peak area results obtained using i-PeakFinder exhibit superior reproducibility compared to the traditional method.

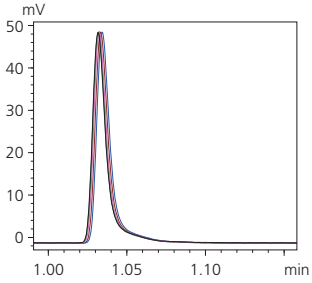


Fig. 5 Chromatogram with Peak Tailing (N=5)

Table 2 Comparison of Area Reproducibility Between the Traditional Method and i-PeakFinder

	i-PeakFinder	Traditional method
%RSD	0.106	0.275
Processing	Peak-baseline Type: Base to Base Peak-Baseline Height* 2	Width: 1 s Slope: 2000 uV/min

* Described in more detail later in the article

3. Setting Parameters

i-PeakFinder not only performs highly accurate peak integration with default settings but also includes more detailed settings for adjusting the peak detection conditions (peak width and peak-baseline start and end heights) and peak-unifying conditions that determine how to combine multiple peaks. The most important of these settings will be explained here.

3-1. Basic Peak Detection Parameters

The basic parameters that allow the user to adjust peak detection conditions are the peak-baseline type mentioned above, peak detection threshold, and peak integration range. Using the detection threshold setting, the peaks below a certain threshold are not detected based on the estimated noise level calculated using a proprietary algorithm. Decreasing the peak detection threshold value allows the detection of smaller peaks. The peak integration range specifies the time range during which peaks will be detected. Fig. 6 shows the change in results produced by changing the peak detection threshold from the default setting of 5 to 2,000. These intuitive controls allow the user to detect or not to detect small peaks with a simple adjustment. Fig. 7 shows an example of adjusting the peak integration range. Without adjusting the peak integration range, all peaks fall within the range and the peak-baseline is affected by negative peaks. Setting the peak integration range to exclude negative peaks allows the user to configure an appropriate baseline.

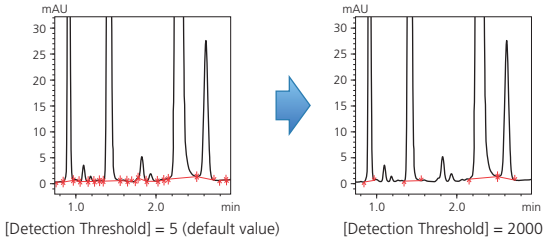


Fig. 6 Peak Detection Threshold

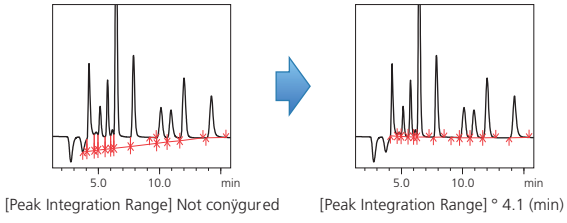


Fig. 7 Peak Integration Range

3-2. Detailed Peak Detection Settings

For complex chromatograms, adjustments of the detection threshold, peak integration range, and peak-baseline type may not be enough to obtain the desired peak integration results. i-PeakFinder is compatible with a wide variety of chromatograms and allows the user to configure more detailed peak detection conditions. Some of these detailed settings are described below:

(1) To Detect Peaks Not Affected by Noise [Minimum Half Width]

Smoothing is sometimes performed on chromatograms obtained via LCMS. If the noise frequency is close to the peak frequency, then it becomes difficult to determine peaks automatically and single peaks may be recognized as multiple peaks. In this situation, configuring the minimum half width setting ignores noise smaller than that value and ensures that peaks with a FWHM value above the set minimum value are detected among the peaks with a wide wave profile due to smoothing. Fig. 8 shows the difference made by increasing the minimum FWHM value. This feature is useful when noise can be observed in a peak.

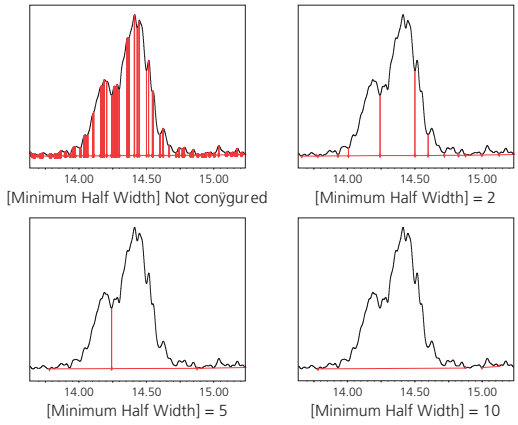


Fig. 8 Example Configuration of Minimum FWHM

(2) To Ensure Peak Area Accuracy and Linearity [Peak Baseline Height]

With tailing peaks and chromatograms with a large amount of baseline noise, the peak start and end points can vary depending on the data, which can reduce area accuracy. By using the peak-baseline height setting, the peak start and end points are recognized as the product of a value entered for peak-baseline height and noise intensity calculated using a proprietary algorithm. Consequently, the larger the peak-baseline height value is, the higher the peak-baseline is positioned.

Using this setting provides good reproducibility in the determination of peak- baseline start and end points. This improves peak area reproducibility, as mentioned earlier in Table 2, and provides linearity across results obtained for a target component at different concentrations. Fig. 9 shows an example configuration of peak-baseline height where the baseline length of the tailing peaks is adjusted by specifying the peak-baseline height.

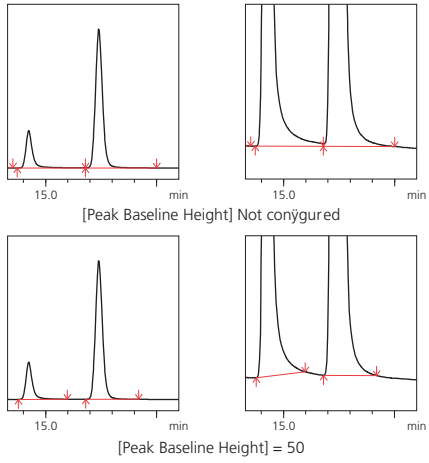
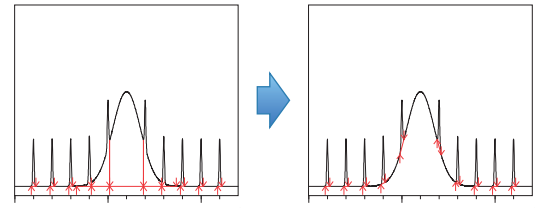


Fig. 9 Example Configuration of Peak-Baseline Height for Tailing Peaks

(3) To Avoid Recognizing Long-Period Undulations as Peaks
[Maximum Half Width]

The maximum half width, which is the opposite of the minimum half width, is a parameter that can be specified to ignore larger peaks as baseline undulations. For example, Fig. 10 shows baseline drift appearing as a large baseline bulge that could be recognized as a peak. This undulation can be removed by specifying a maximum FWHM.

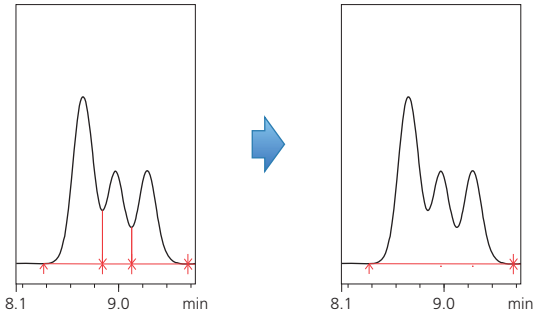


[Maximum Half Width] Not conÿgured [Maximum Half Width] = 20 (sec)

Fig. 10 Example Configuration of Maximum FWHM

(4) To Unify Fused Peaks into a Single Peak
[Unify Peaks by Separation Width]

The minimum half width setting is used to avoid mistakenly recognizing noise as peaks, while unifying peaks by separation factor is used to combine fused peaks into a single peak. Fig. 11 shows three fused peaks. Configuring this setting will unify the two side peaks into the larger peak. Note that this setting is only effective for baseline intervals containing fused peak.

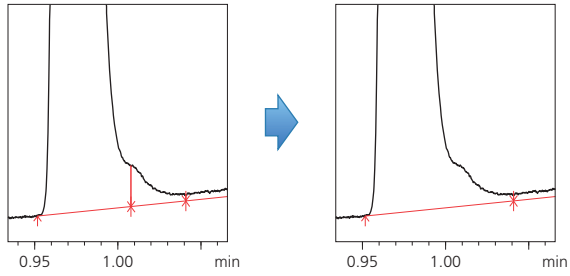


[Unify Peaks by Separation Width] Not conÿgured [Unify Peaks by Separation Width] = 2

Fig. 11 Example of Unifying Unresolved Peaks

(5) To Decide Whether to Recognize Shoulder Peaks
[Unify Peaks by Shoulder Ratio]

When impurities were fused as shoulder peaks at the base of a main component peak, the traditional peak integration method required time programming or manual peak integration to detect the shoulder peak. i-PeakFinder allows easy detection of shoulder peaks and also allows the user to decide whether to recognize (or not) impurity peaks based on a threshold ratio of the main component peak height to the shoulder peak tangential height. Fig. 12 shows an example of shoulder peak detection implemented without time programming or manual peak integration and an example of using the threshold value to unify the shoulder peak with the main component peak. Configuring a threshold value can also be used as a condition for deciding whether to recognize impurity peaks.



[Unify Peaks by Shoulder Ratio] Not conÿgured [Unify Peaks by Shoulder Ratio] = 0.01 (%)

Fig. 12 Example of Setting a Threshold for Recognizing Shoulder Peaks

4. Customizable Default Values

The i-PeakFinder parameters can be configured as the default values of methods. For example, if a user decides to use vertical division as the peak-baseline type, specifying vertical division as the default setting can omit the effort involved in adjusting the parameter for each new analysis. LabSolutions is an integrated workstation capable of controlling a range of different instrument models. However, the HPLC and GC systems occasionally require different default peak integration settings. Accordingly, default settings can be specified for each type of instrument (Fig. 13).

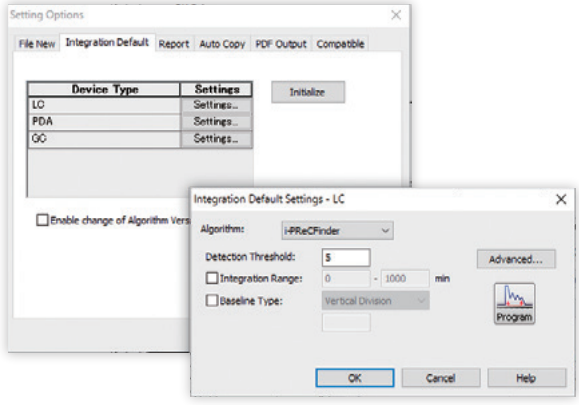


Fig. 13 The Default Settings Window

5. Summary

i-PeakFinder, a new peak integration algorithm developed by Shimadzu, can perform either automated peak integration or peak integration with simple settings adjustments, even for cases in which the traditional peak integration method requires time programming or manual peak integration for individual data sets. i-PeakFinder also allows the user to easily configure the peak-baseline type and perform accurate peak integration of small peaks.

Furthermore, i-PeakFinder can quickly and easily process large quantities of data without errors and improve the efficacy and reliability of analytical operations.

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Technical Report

New Data Processing Method for Photodiode Array Detector

Principle and Summary of i-DReC (Intelligent Dynamic Range Extension Calculator)

Toshinobu Yanagisawa[1]

Abstract:

A new data processing method for a photo diode array (PDA) detector, Intelligent Dynamic Range Extension Calculator (i-DReC) enables the automatic calculation of peak area and height, utilizing spectrum similarity in the high concentration range where UV signal is saturated. When the integrated chromatographic peak area exceeds a user-defined threshold value, i-DReC automatically shifts the chromatographic profile to a wavelength with less UV absorption to prevent signal saturation. The absorption ratio between the original target wavelength and the wavelength used by the i-DReC function is applied as a correction factor to the peak area of the acquired chromatogram, thereby calculating the peak area and height at the original target wavelength. The i-DReC dramatically extends the linear dynamic range of calibration curves, enabling reliable quantitation of high concentration samples without need for sample dilution and reinjection, which would otherwise be required.

Keywords: PDA data processing, dynamic range extension, Nexera X2, UHPLC

1. Basic Principle of i-DReC

High concentration samples can produce saturated UV spectral absorbance, which significantly affects peak area calculation and causes a loss of linearity in the relationship of peak area to concentration. The i-DRec calculates an absorbance ratio between the original target wavelength and another wavelength that provides less absorbance in a spectrum on the down-slope of the chromatographic peak where neither wavelength's absorbance is saturated. The corrected peak area and height are then calculated by multiplying the measured peak area and height by the absorbance ratio.

■Calculation Algorithm

1. i-DRec is automatically applied when the intensity of a target peak exceeds the user-defined threshold value. If the threshold value is not exceeded, i-DReC is not applied.
2. The wavelength used for correction by i-DReC (λb) can be set either manually or automatically. When set manually, λb is a user-defined parameter. When set automatically, λb is determined as follows:
   - A UV spectrum is acquired at the retention time of the target peak.
   - The spectrum is analyzed to determine an appropriate wavelength for which the absorbance is not saturated, which is then set as λb.
3. The chromatogram at λb is extracted from the 3D data and integrated to determine peak area and height.
4. A UV spectrum is extracted from the chromatogram (at the original target wavelength (λa)) at a point on the down-slope of the peak (between the peak apex and peak end) where the absorbance of neither λa nor λb are saturated.
5. An absorption ratio (k) is calculated from the spectrum in (4). The intensity (Ia) of the spectrum at λa is divided by the intensity (Ib) of the spectrum at λb, as follows:

   $k = Ia/Ib$
6. Peak area and height of the measured peak in the chromatogram at λb are corrected by the absorption ratio to determine the effective area and height at λa, as follows:
   Peak area at λa = (peak area at λb) × k
   Peak height at λa = (peak height at λb) × k

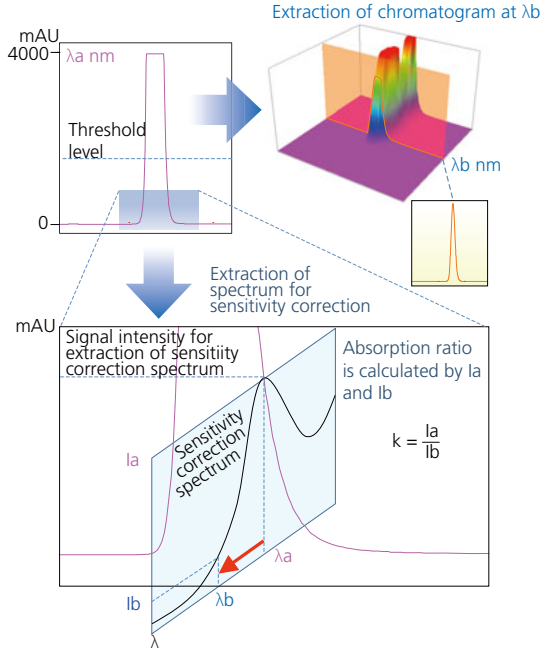


Fig. 1 Basic principle of i-DReC

1. Analytical & Measuring Instruments Division

2. Examples of i-DReC Applications

2-1. Extending the Linear Dynamic Range of Calibration Curves

This section demonstrates the extension of a calibration curve's linearity into a high concentration range, using standard solutions of Rhodamine with concentrations ranging from 0.01 g/L to 10 g/L. The following conditions were used for analysis.

Analytical Conditions

Pump	: Shimadzu LC-30AD×2
Detector	: Shimadzu SPD-M30A
Column oven	: Shimadzu CTO-20AC
Controller	: Shimadzu CBM-20Alite
Autosampler	: Shimadzu SIL-30AC
Mobile phase	: Ammonium formate buffer 45% / ACN 55%
Column	: Shimadzu Shim-pack VP-ODS (4.6 mmL. × 150 mmI.D., 5.0 µm)
Flow rate	: 1 mL/min
Column temp.	: 40 °C
Sampling	: 80 msec
Slit width	: 1 nm
Time constant	: 80 msec
Wavelength range	: 190 nm-700 nm
Cell light path	: 10 mm
Injection volume	: 2 µL

Fig. 2 shows the UV absorbance spectrum of Rhodamine, A Calibration curve was created based on peak area in the extracted chromatogram at 554 nm, the wavelength of maximum absorbance, and is shown in Fig. 3a. At 1 g/L or greater concentration, the calibration curve exhibits the loss of linear relationship between peak area and concentration.

Fig. 3b shows the same calibration curve with i-DReC applied to extend the linearity into the high concentration range. In this example, 347 nm was selected manually as the wavelength for correction, and the spectrum used for sensitivity correction was extracted at an intensity of 700 mAU. The original peak area and the corrected peak area calculated by i-DReC is shown in Table 1. After correction by i-DReC, the calibration curve based on the corrected peak areas exhibited excellent linearity with an unweighted correlation factor of 0.9999078 and 0.9995750 weighted by 1/(concentration)^2 over the concentration range of 0.01 g/L to 10 g/L.

Fig. 3C shows the error in concentration values obtained by inverse estimation using the i-DReC corrected calibration curve with weighting of 1/(concentration)^2. Even though the i-DReC corrected calibration curve extended the linear range of the original calibration curve by an order of magnitude, over the full range of concentration, the error in calculated concentration value was within 5%.

Table 1 Calibration points of Rhodamine samples

#	Conc.(g/L)	Peak area avarage (uAUsec) (n=2)	
		Original	i-DReC
1	0.01	267,847	267,847
2	0.02	544,266	544,266
3	0.08	2,089,341	2,089,341
4	0.1	2,622,781	2,622,781
5	0.2	5,255,999	5,255,999
6	0.5	12,072,748	**12,282,271**
7	0.8	18,539,104	**19,887,814**
8	1	21,823,608	**24,644,792**
9	2	33,708,885	**49,250,552**
10	5	53,883,445	**126,813,723**
11	8	65,182,276	**198,990,013**
12	10	71,500,307	**245,336,353**

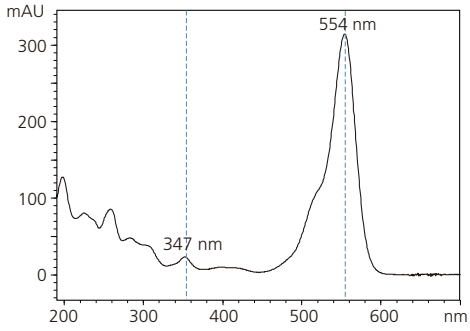
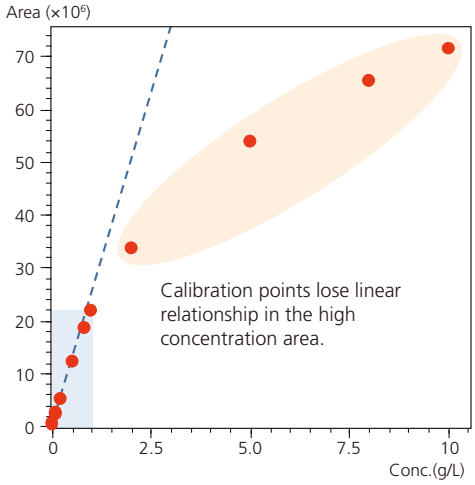
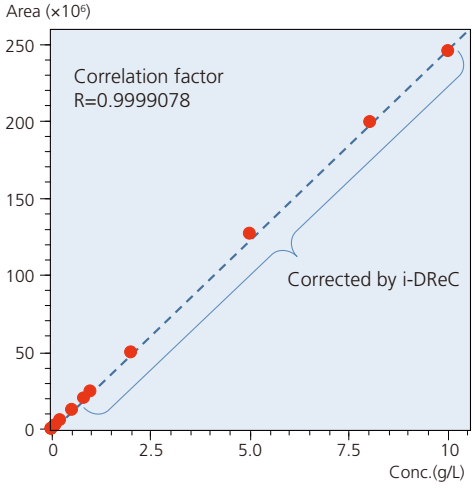


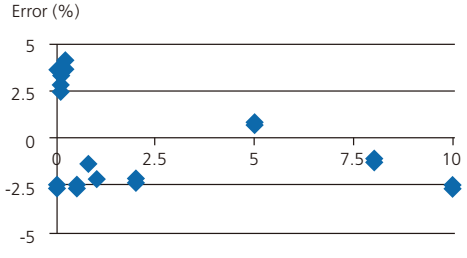
Fig. 2 Spectrum of Rhodamine



(a) Calibration curve at 554 nm



(b) Linearity range extended by i-DReC



(c) Error of corrected calibraition points

Fig. 3 Calibration curve of Rhodamine

2-2. Simultaneous Quantitation of Main Component and Minor Impurities

This section demonstrates the use of i-DReC to simultaneously quantify a relatively high concentration major component in a pharmaceutical sample and the relatively low concentration impurities. Samples in which the concentration of the main component ranged from 0.01 g/L to 1 g/L where analyzed using the SPD-M30A photodiode array detector equipped with the high sensitivity cell. Fig. 4 shows the calibration curve for the main component based on peak area in the extracted chromatogram at 250 nm.

Analytical Conditions

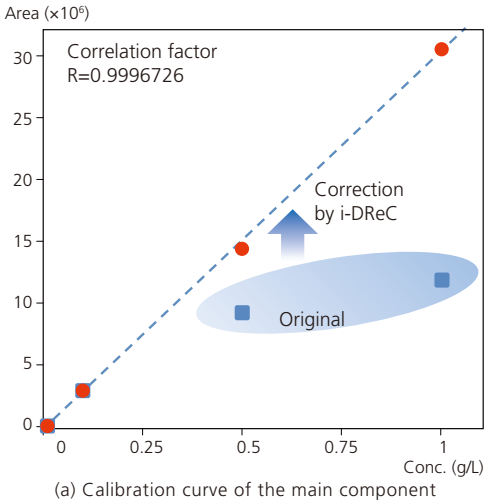
Pump	: Shimadzu LC-30AD×2
Detection	: Shimadzu SPD-M30A
Column oven	: Shimadzu CTO-20AC
Controller	: Shimadzu CBM-20Alite
Autosampler	: Shimadzu SIL-30AC
Column	: Shimadzu Shim-pack XR-ODS (150 mmL. × 3.0 mmI.D., 2.2 µm)
Mobile phase A	: 5% MeCN + 0.05% TFA
Mobile phase B	: 95% MeCN + 0.05% TFA
Time program	: 2% (0–1.2 min) →2–98% (1.2–8.9 min) → 98% (8.9–10.8 min) → 98–2% (10.8–11.1 min) →STOP (14 min)
Flow rate	: 1 mL/min
Column temp.	: 40 °C
Sampling	: 160 msec
Slit width	: 8 nm
Time constant	: 160 msec
Wavelength range	: 190 nm-700 nm
Cell light path	: 85 mm
Injection volume	: 1 µL

As shown in Fig. 4a, calibration points for concentrations above 0.5 g/L deviate from linear relationship. In this example, 280 nm was selected manually as the wavelength for correction, and the spectrum used for sensitivity correction was extracted at an intensity of 200 mAU. The absorption ratio was calculated and used to correct the peak areas and extend the linear dynamic range of the calibration curve, resulting in a correlation factor R of 0.9996726 weighted by 1/(concentration)^2 over the concentration range of 0.01 g/L to 1 g/L.

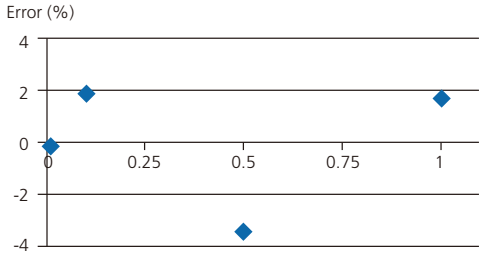
Fig. 4b shows the error in concentration values obtained by inverse estimation using the i-DReC corrected calibration curve with weighting of 1/(concentration)^2 is within 4%. The reproducibility of the peak area (n=6) of the main component and impurities, as well as the peak area ratio between the main component and impurities, is shown in Table 2. An example chromatogram for the sample is shown in Fig. 5. In this example, i-DReC was only applied to correct the peak area of the main component, which provided a saturated signal at 250 nm.

Table 2 Peak area reproducibility of the pharmaceutical sample

Peak	Retention Time(min)	Mean Area (µAUsec)	Area %RSD	Area Ratio(%)
①Main	4.634	31,123,746	0.06	--
②	5.448	925,522	0.12	2.974
③	3.900	64,161	0.08	0.206
④	4.910	32,810	0.15	0.105
⑤	5.091	15,103	0.16	0.049
⑥	4.487	9,487	0.26	0.030
⑦	4.226	7,981	0.28	0.026
⑧	4.975	7,981	0.44	0.026
Imp1	4.056	2,001	0.27	0.006
Imp2	4.331	2,440	0.85	0.008
Imp3	4.376	1,663	0.65	0.005



(a) Calibration curve of the main component



(b) Error of corrected calibration points

Fig. 4 Linearity evaluation

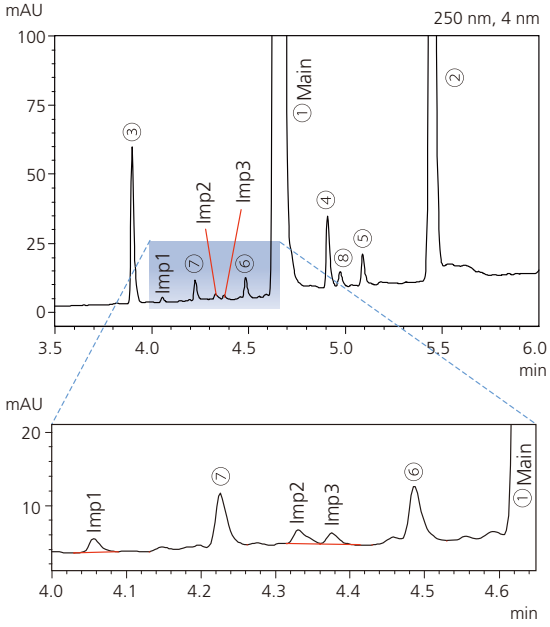


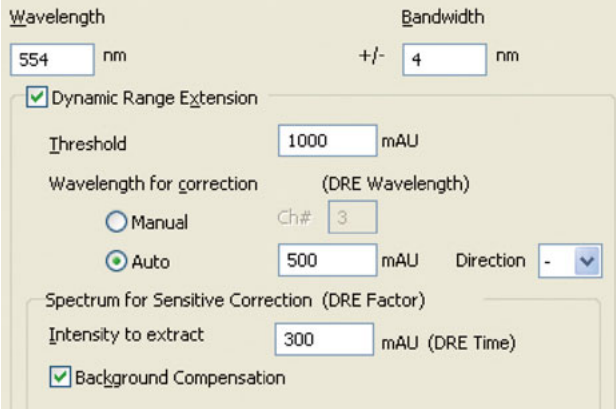
Fig. 5 Chromatogram of the pharmaceutical sample

As shown in Table 2, i-DReC correction provided peak area reproducibility of 0.06% RSD for peak 1, the main component. Peak area reproducibility for impurity peak 3 (Imp3), whose peak area was 0.005% of the main component, was less than 1%.

3. Summary of i-DReC Settings

i-DReC parameters are set as part of the data processing parameters for the photodiode array detector and can be applied in data processing methods for routine analysis without requiring post-run operations. The following is a summary of parameters and their descriptions.

1. i-DReC parameters are set in the multi chromatogram table.



Parameter	Description
Dynamic range extension	Select whether i-DReC is applied or not.
Threshold	When intensity at peak top is over the threshold, i-DReC is applied to the peak for correction.
Wavelength for correction (manual/auto)	Select whether wavelength for correction will be set manually or automatically.
Ch#	When "Manual" is selected, set the channel number of the chromatogram that will be used.
Intensity for correction wavelength	When "Auto" is selected, set the target intensity for determining an appropriate correction wavelength.
Direction (+/-)	When "Auto" is selected, set the direction to search for an appropriate correction wavelength. (+ = longer wavelength, – = shorter wavelength)
Intensity to extract	Set intensity for extraction of sensitivity correction spectrum.
Background compensation	Select whether background compensation is used or not for sensitivity correction spectrum.

The concentration range over which i-DReC effectively extends the linear dynamic range is dependent upon the shape of the spectrum of target peaks. The lower the slope of the spectrum around the correction wavelength, the more reliable the correction.

2. A mark indicating whether i-DReC has been applied, the wavelength used for correction, the retention time of the spectrum used for sensitivity correction, and the calculated sensitivity correction factor can be shown in the peak table and compound table.

Item	Description
Mark	C = peaks to which i-DReC was applied E1-E4 = error in i-DReC calculation
DRE wavelength	For peaks to which i-DReC was applied, this is the wavelength used for correction.
DRE factor	For peaks to which i-DReC was applied, this is the absorption ratio used for sensitivity correction.
DRE time	For peaks to which i-DReC was applied, this is the retention time of the spectrum used for sensitivity correction.

i-DReC requires that spectrum similarity is maintained across the peak. When peak separation is insufficient, i-DReC may not be able to be applied.

3. The peak area and height corrected by i-DReC can be used in normal quantitation processes. The simple implementation allows the seamless use of i-DReC for routine analysis.

4. Conclusion

i-DReC's ability to calculate corrected peak area and height for high concentration samples is made possible by the improved performance of the SPD-M30A photodiode array detector and the excellent reproducibility of the Nexera X2 system. The key features of i-DReC can be summarized as follows.

- Extension of the linear dynamic range using spectral similarity.
- Simultaneous quantitation of both low and high concentration compounds in a single injection.
- Requires the use of only one PDA detector.
- Standard samples are not necessary for correction.
- Simple method settings allow the use of i-DReC in routine anaysis.

i-DReC can be applied to samples containing a wide range of compound concentrations. The use of i-DReC improves the efficiency of sample pretreatment processes and laboratory productivity.

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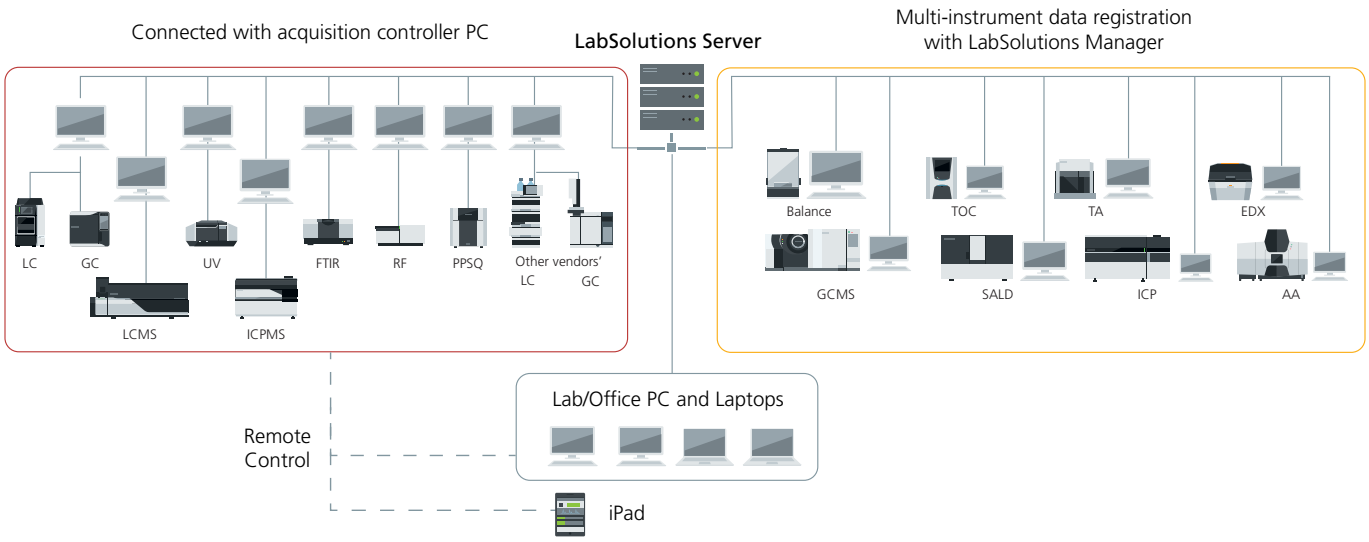
Data Integrity

In recent years, as the FDA has become increasingly strict with compliance requirements, there are more and more pharmaceutical companies that have received FDA warning letters. In response to increasingly diverse GMP/GLP security control management requirements, as well as CSV, FDA 21 CFR Part 11 and other regulations and guidelines, the need for laboratory equipment and analytical data management services has increased significantly. More accurate and efficient implementation of multi-channel analysis operations, as well as the proper management of equipment and data, is the current trend of the pharmaceutical industry.

In addition to a large number of chromatographic instruments, analytical laboratories use a wide variety of non-chromatographic instruments. The secure management and integration of these instruments and their data poses a challenge for laboratory managers.

Shimadzu's complete data management system provides secured data, and a fully-integrated network and laboratory. With Shimadzu's system, pharmaceutical industries are well-equipped for this data-and technology-driven environment.

LabSolutions CS

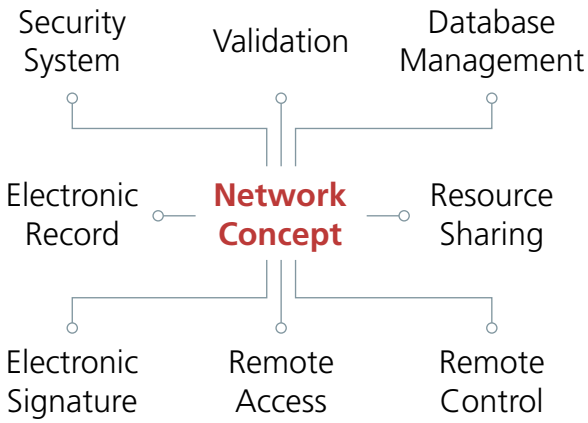


Shimadzu's unified data management platform

- LabSolutions CS allows seamless integration of labs and offices with control commands, instrument monitors, and remote control via other client PCs on the network.
- In addition to chromatographs, spectrometers and mass spectrometers, instruments from other analytical manufacturers can also be controlled and operated by LabSolutions CS, and all data can be managed in the integrated database.

Ideal for high efficiency and low risk

- With a unified platform established, you can accomplish instrument control, account setting, data management, report generation, all functions and operations more efficiently.
- Powerful database management functions and security strategies minimize the risk of data loss and malicious tampering, and strictly follow data integrity management requirements.



C191-E041

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Technical Report

Data Integrity Compliance Using the LabSolutions Report Set

Mamoru Kikumoto[1]

Abstract:

A recent topic related to analytical data is the lack of data integrity due to data modification and replacement. Whether caused intentionally or accidentally, such problems are often the result of incorrect operating procedures. Accordingly, the question of how to ensure data integrity has become a pressing issue for analysis laboratories.
In addition to the sophisticated security functions provided in the previous version, LabSolutions DB/CS version 6.50 includes a new Report Set function that enables the visibility of software operations. Therefore, not only can it help ensure the reliability of the analysis data required by analysis laboratories but, it can also cut decrease the amount of time needed to check analysis results to a half of or a third of that previously required.

Keywords: data integrity, Report Set

1. Data Modification

A recent topic related to analytical data is the lack of data integrity due to data modification and replacement. Whether caused intentionally or accidentally, such problems are often the result of incorrect operating procedures. Accordingly, the question of how to ensure data integrity has become a pressing issue for analysis laboratories.

2. Actual Pharmaceutical Company Case

The FDA (the U.S. Food and Drug Administration) currently issues a large number of warning letters and Form FDA 483s related to data integrity. These notifications have reportedly been triggered by a case of fraud committed by a generic drug manufacturer based in the state of New Jersey in the United States. An FDA audit of an actual pharmaceutical company in 2005 revealed inconsistencies between paper-based and electronic data at the analysis laboratory and revealed that non-conforming test results were never investigated. Consequently, the company halted shipments, recalled all products, and stopped manufacturing. Furthermore, they withdrew seven generic drug applications. Then, after filing bankruptcy in October 2005, they were purchased later that year by a different pharmaceutical company. The chairman and CEO resigned and four responsible persons were accused of criminal activity.[1), 2), 3)]

3. Form FDA 483

The FDA issued Form FDA 483 to Able Laboratories and posted it on the FDA website.[1)]

In that form, the following was included as "OBSERVATION 1."

OBSERVATION 1

"... The Quality Unit failed to: review electronic data as part of batch release, review computer audit trails in the Waters Empower Data Acquisition System and provide adequate training to analytical chemists. ..."

This indicates that the chromatography data system is being called into question.

The form also included the following.

- OOS results were substituted with passing results by Analysts and Supervisors.
- Changed chromatogram headers by cutting and pasting, so during review all sample injections would appear to be in sequence. ..."

This indicates that:

- Non-conforming test results were replaced with passing results and
- Chromatogram headers were modified by cutting and pasting.

4. FDA Response

According to the GMP News[5)] report from the ECA[4)], the FDA responded to the above case as follows.

Triggered by the cases of fraud at Able Laboratories in 2005, the requirements for audits of data integrity during FDA Pre-Approval inspections have been set in the Compliance Programme Guide (CPG) 7346.832. Moreover, FDA's inspectors have been explicitly trained on computer systems and the data they contain.

1 Analytical & Measuring Instruments Division

Table 1 Categories and Remedies for Issues Raised by the FDA

Category	Description	Examples of Issues	Remedies
1	Problems with inadequate recognition	• Paper-based test results did not contain all analytical data.	• Regulatory requirements should be interpreted correctly.
2	Functional deficiencies, inadequate settings, and usage issues	• There were no audit trail functions. • Login IDs and passwords were being shared. • Data deletion was not restricted using user rights.	• Systems should be updated to enable compliance with regulations. • System settings should be specified appropriately.
3	Testing process reliability issues	• Tests were repeated until acceptable results were obtained. • Out-of-specification (OOS) data was neither investigated nor reported.	• Operations should be checked for any improper actions.

5. Categories and Remedies for Issues Raised by the FDA

There are multiple data integrity issues that have been raised by the FDA, however, they can generally be categorized as indicated in Table 1. Category 1 includes issues that result from a fundamental lack of recognition, which requires properly interpreting regulatory requirements. Category 2 includes issues related to functional deficiencies, setting inadequacies, or usage factors. These issues require updating systems to be able to comply with regulations or specifying proper settings. Category 3 includes issues related to the reliability of the testing processes. These issues require verification to confirm that no invalid operations are being performed. Because FDA investigations are currently focused on data integrity, importance has shifted toward providing evidence that no improper operations were performed with respect to analytical results. This approach of the investigators based on suspicion, which is a major departure from the approach used in previous investigations.[6)]

6. Relationship Between Inputs and Outputs

Using HPLC analysis as an example, consider what is lacking in current practices, in terms of data integrity. Based on the prerequisites indicated in Fig. 1, it appears that current practices are acceptable as long as security settings, such as login IDs, audit trail settings, and user rights for deleting data, are configured in compliance with established regulations. Normally, only the printed chromatograms are checked, the instrument conditions (instrument parameters) used for the analysis, the data analysis conditions (data processing parameters), the batch analysis conditions, or other factors. However, reliability can be ensured only by checking all these factors in addition to the chromatogram report.[7)]

It is easier to understand this as a relationship between the input and output processes. Fig. 2 shows that, even if stronger security measures are implemented for outputs (analysis data), they are meaningless without proper inputs (such as acquisition and data analysis conditions). While computers can apply security measures, they cannot judge the malicious intent of humans.

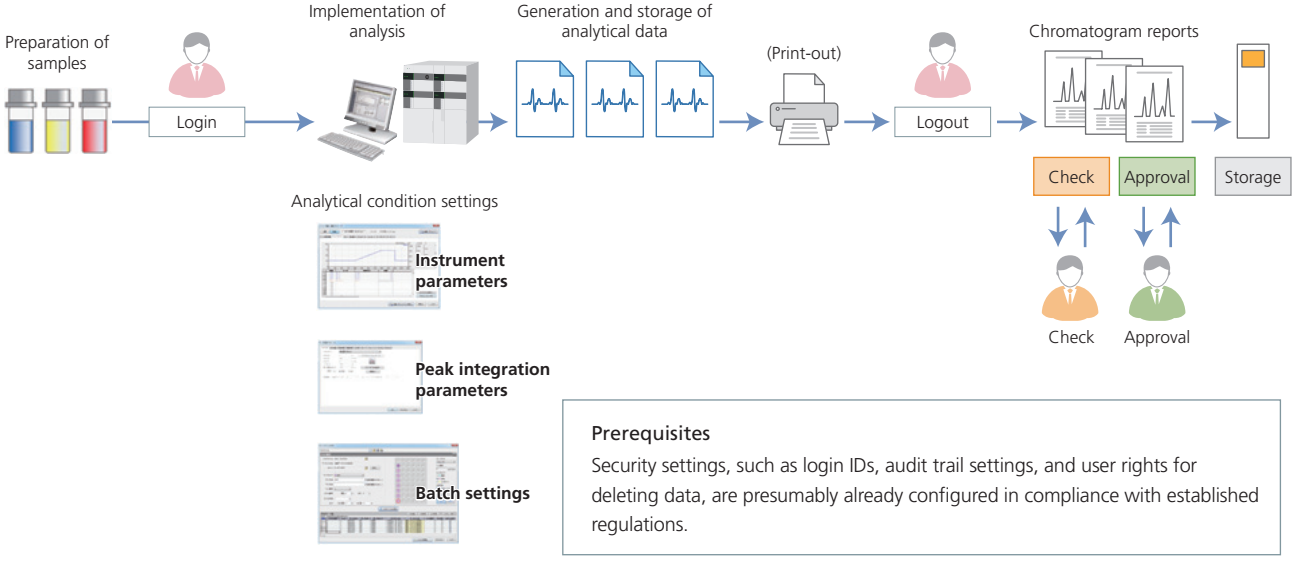


Fig. 1 HPLC Analysis Process Flowchart

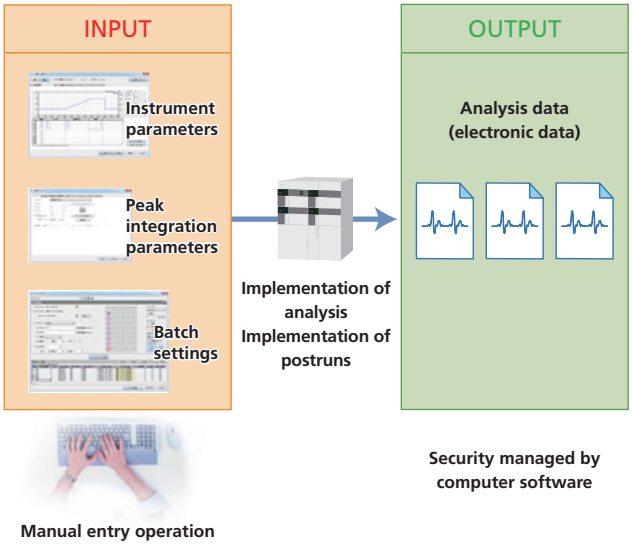


Fig. 2 Relationship Between Inputs and Outputs for HPLC Analysis

Therefore, even if security is strengthened in the computer, it is difficult to prevent improper operations for processes that require human intervention (such as specifying analytical conditions or analyzing analysis data).

7. Achieving Visibility for Computer Operations

Consequently, what techniques should be used to provide evidence that no improper operations were performed with regards to analysis results?

This can be accomplished by ensuring that computer operations are easily visible.

The visibility of computer operations refers to retaining the results of operations that require human intervention, such as setting analytical conditions or analyzing data, and presenting them in a form that is easily visible. Visibility makes it possible to provide evidence that no improper operations were performed, such as modifying or replacing data.

8. LabSolutions DB/CS Report Set

Fig. 3 shows such computer operations presented in a visible form using the Report Set function in LabSolutions DB/CS version 6.50. The Report Set function converts a set of reports, such as batch analysis reports, operation log reports, and chromatogram reports, to PDF format and then digitally consolidates the reports into a single PDF file. This report set, which includes the results of operations involving human intervention, provides visibility of the software operations, and makes it easy to provide evidence that no improper operations were performed, such as modifying or replacing data.

The following are three key features of the report sets.

《Feature 1》

Visibility of the Series of Analysis Operations Reduces the Work Involved in Checking Results and Ensures Reliability

The newly included Report Set function digitally converts batch analysis reports, operation log reports, chromatogram reports, and other reports into a single PDF file. In this case, batch analysis reports do not refer to the batch analysis schedule but rather to an analysis ledger that summarizes (lists) the actual series of analyses and the corresponding postrun analyses performed. The operation log report consists of an analysis (and postrun analysis) computer operation log that records all analysis operations (and a postrun analysis) performed between the start and finish of the analysis processes.

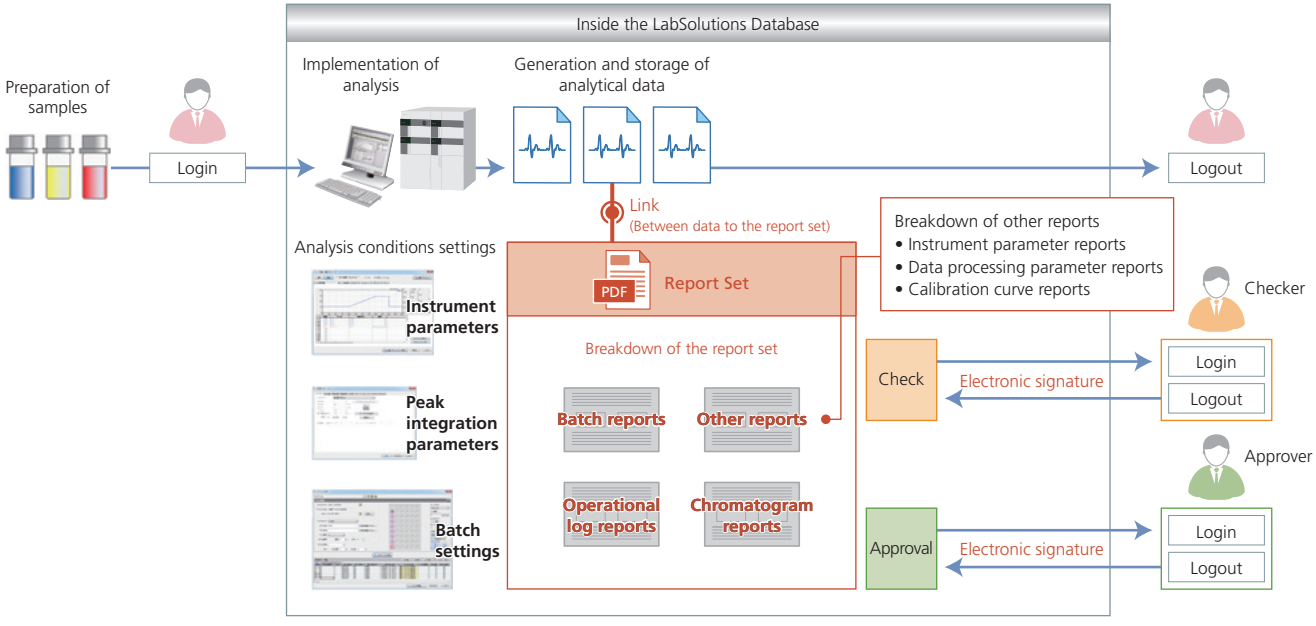
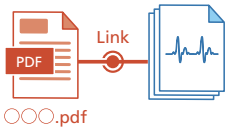


Fig. 3 Data Integrity Compliance Using LabSolutions DB/CS Report Set

Report sets consolidate all the necessary information in a single PDF file, so that the entire series of operations involved in the analysis (and post-run analysis) are easily visible. With the same feel as an electronic book, you can check the details while turning the pages. As a result, it is not necessary to switch between a number of windows or tabs to check operations and settings as in the past. In this way, the Report Set function reduces the work involved in checking results and ensures reliability.

《Feature 2》

The Series of Analysis Results Is Automatically Protected Against Modification



Once a digital link is created between the series of analysis results (electronic data) and the report set for which visibility is being provided, editing is automatically disabled (locked). This will help prevent any data modification, such as replacing or deleting the analysis results.

The digital link created between the data not only ensures a unique relationship between the report set the analysis results (electronic data), but it also enables analysis results (electronic data) to be searched and checked quickly.

《Feature 3》

Enhanced Productivity Thanks to Digitization of the Confirmation Process for the Analysis Results Report

The Report Confirmation function can be used to retain evidence that the content of the chromatogram report included in the PDF file was reviewed. This evidence can be left anywhere in the chromatogram report in the same way as with printouts. A confirmation assistant function is included to ensure content reliability by emitting an error to provide notification of unchecked items.

Problem 1 Associated with Printouts

The significant amount of time needed for analysis report printing, summarization, checking, and storage tasks can interfere with daily operations.

Problem 2 Associated with Printouts

The increasing number of binders required to store printouts can cause storage space problems.

Problem 3 Associated with Printouts

Analysis results might be replaced or discarded.

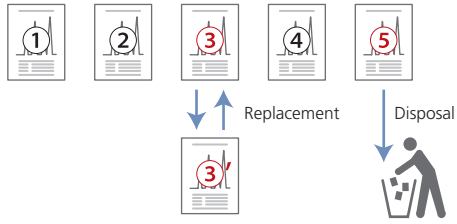


Fig. 4 Problems Associated with Printouts

Electronic signatures can be used for report set review and approval processes, with the original source electronic data (analysis results data) also being reviewed and approved at the same time. Using electronic signatures means electronically signed reports do not necessarily need to be printed out and signed by hand. Consequently, migrating to a paperless work flow can solve the problems associated with printouts (see Fig. 4) and reduce the time required to check various results by one half to a third of that previously required.

The Report Set function is included in LabSolutions, which increases CSV efficiency because a separate validation process is not necessary.

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Technical Report

Data Integrity Compliance: An Innovative Solution for Molecular Spectroscopy

Mamoru Kikumoto[1]

Abstract:

A recent topic related to analytical data is the lack of data integrity due to data modification and replacement. Regulatory authorities for analytical instruments are not only interested in chromatography systems, such as liquid chromatographs (LC) and gas chromatographs (GC), but are also turning their interest to spectroscopy systems, such as UV and IR systems. Consequently, many analytical laboratories are urgently considering how to ensure data integrity for spectroscopy systems. This report describes an innovative solution for ensuring data integrity for such spectroscopy systems.

Keywords: Data integrity, spectroscopy system, and orphan data

1. References to Spectroscopy Systems by Regulatory Authorities

Compliance with data integrity requirements is already a pressing issue for companies that require GxP compliance. In addition to chromatography systems, such as LC and GC systems, regulatory authorities have now turned their attention to spectroscopy systems, such as UV and IR spectrophotometers. (See below.)

• MHRA (Medicines and Healthcare products Regulatory Agency) [1)]

It is common for companies to overlook systems of apparent lower complexity. However, with these systems, it may be possible to manipulate data or repeat testing to achieve a desired outcome with limited opportunity for detection (e.g. stand-alone systems with a user-configurable output such as ECG machines, FTIR, UV spectrophotometers).

• FDA (U.S. Food and Drug Administration) [2)]

For example, a spectral file created by FT-IR (Fourier transform infrared spectroscopy) can be reprocessed, but a static record or printout is fixed. This does not satisfy CGMP requirements to retain original records or true copies (§ 211.180(d)).

• WHO (World Health Organization) [3)]

Original dynamic electronic spectral files created by FT-IR, UV/Vis, and chromatography instruments can be reprocessed, but a pdf or printout is fixed or static and the ability to expand baselines, view the full spectrum, reprocess and interact dynamically with the data set would be lost in the PDF or printout.

• PIC/S [4)]

QC supervisors and managers should not be assigned as the system administrators for electronic systems in their laboratories (e.g., HPLC, GC, UV-Vis).

• PMDA (Pharmaceuticals and Medical Devices Agency) [5)]

Ensuring appropriate data integrity:

The following practices never occur in workplaces, do they?
PDF files of past IR test results are modified and printed.

⇒ Recycling test results

The above shows that regulatory authorities are not only interested in chromatography systems, such as LC and GC systems, but are also turning their attention to spectroscopy systems, such as UV and IR systems.

1 Analytical & Measuring Instruments Division

Table 1 Typical Data Integrity Compliance

		Chromatography (Data Acquisition and Management)	Spectroscopy *1	
			(Data Management)	(Data Acquisition)
Data	Acquisition	Yes	—	Yes
	Management	Yes	Yes *2	—
Audit Trail (Metadata)		Yes	—	Yes *2
Users		Yes	Yes *3	Yes *3
Security		Yes	Yes	Yes
Time (Time Stamp)		Yes		
Remarks		• Requires two servers, one for chromatography and one for spectroscopy. • Requires three types of software: (1) For chromatography data acquisition and management (2) For spectroscopy data management (3) For spectroscopy data acquisition • Not compatible with electronic data		

*1: This table is based on a case of using a data management system to manage spectroscopy data that differs from the brand of the spectroscopy system.
*2: In this example, spectroscopy data and audit trail data cannot be reviewed and managed in a unified manner.
*3: In this example, users that acquire spectroscopy data cannot be managed in the same manner as users that manage spectroscopy data.

2. FDA Warning Letters

The FDA website includes several examples of warning letters for spectroscopy systems that show how warning letters are now being issued specifically for UV and IR systems. (See below.)

• Warning Letter—Example (1) [6]

In response to this letter, provide details of your retrospective review of the HPLC and other laboratory data, such as Fourier transform infrared spectroscopy, gas chromatography, UV spectrophotometry, and (b)(4) analyzer data.

• Warning Letter—Example (2) [7]

You lacked controls to prevent the unauthorized manipulation of your laboratory's electronic raw data. Specifically, your infrared (IR) spectrometer did not have access controls to prevent deletion or alteration of raw data.

3. Data Integrity Compliance for Spectroscopy Systems

So, what sort of compliance is required for ensuring the integrity of data from spectroscopy systems?

In terms of the form of the data, spectroscopy data are considered dynamic data, as indicated in the FDA and WHO excerpts on the previous page, just like it is for chromatography data. Therefore, presumably a key point for compliance will be ensuring equivalence with chromatography systems.

4. Obstacles for Ensuring Data Integrity Compliance for Spectroscopy Systems: Audit Trail and User Management

As illustrated in Fig. 1, compliance is based on Good Manufacturing Practice (GMP), which is premised on the validation of systems and analytical methods. Furthermore, data, audit trail (metadata), and user operations must be correctly time-stamped in a secure environment. If a laboratory has both chromatography and spectroscopy systems, the elements shown in Fig. 1 apply to both chromatography and spectroscopy systems, such that compliance with data integrity requirements results in the complicated operations indicated in Table 1.

Typical systems retain audit trail data (metadata) within the spectroscopy data acquisition system as indicated in Table 1, but cannot manage spectroscopy and audit trail data in a unified manner. (That means the data cannot be managed in a linked state.) The same applies to the user management. Typical systems cannot manage data management users and data acquisition users in a unified manner.

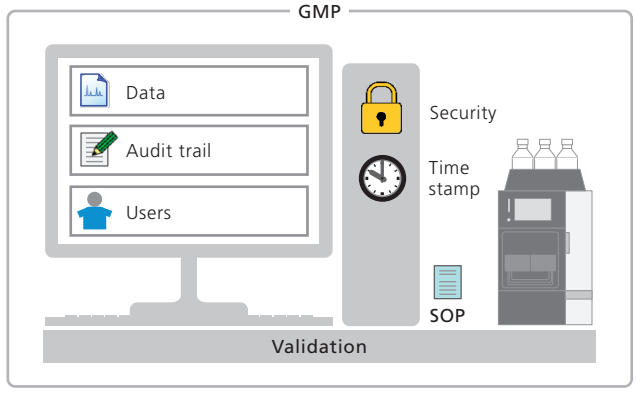

Fig. 1 Illustration of Data Integrity Compliance

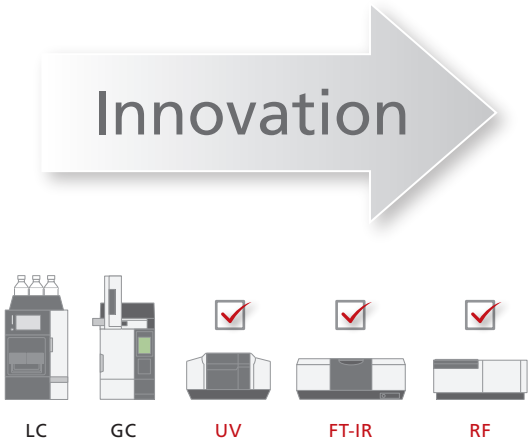

Table 2 Innovative Data Integrity Compliance

		Chromatography and Spectroscopy (Data Acquisition and Management)
Data	Acquisition	Yes
	Management	Yes
Audit Trail (Metadata)		Yes *4
Users		Yes
Security		Yes
Time (Time Stamp)		Yes
Remarks		• One server • One software program *5 • Compatible with electronic data

*4: Spectroscopy data and audit trail data can be reviewed and managed in a unified manner.
*5: In this case, "one software program" means all the elements indicated in Fig. 1 can be managed in a unified manner.

Due to the current attention on data integrity, focus has shifted toward providing evidence to reviewers that no improper operations were performed with respect to analytical results. However, this approach represents a policy of punishing any practice that appears suspicious, which is a major departure from the approach used in previous investigations.[8)] This approach applies to both chromatography and spectroscopy systems, which means the conventional approach cannot be used to ensure appropriate compliance.

5. Innovative Data Integrity Compliance

Therefore, considering that data integrity compliance for spectroscopy systems could present a major obstacle for operating analytical laboratories in a regulated environment, there is a need for an innovative approach to ensuring data integrity that solves such problems. That is exactly what LabSolutions achieves.

LabSolutions takes full advantage of Shimadzu's unique position as a developer and manufacturer of a wide variety of analytical instruments. Therefore, Shimadzu is able to offer a unique solution for data integrity compliance that is not limited to chromatography systems, but can also comprehensively include UV and IR systems and other spectroscopy systems in the LabSolutions family. (See Table 2.)

In other words, Shimadzu successfully integrated operations for ensuring data integrity by deploying the LabSolutions Report Set function [9)], which received excellent reviews for ensuring compliance with data integrity requirements for chromatography systems, for use in ensuring data integrity compliance for spectroscopy systems. A key feature of the report set is that it includes all the information necessary for validating data integrity, as shown in Fig. 2, step (3) Complete a set of reports.

6. Procedure for Using LabSolutions to Create a Report Set for Spectroscopy Systems

The procedure for creating a report set for spectroscopy systems corresponds to the procedure for chromatography systems, so the report set can be created using the same simple operations for both types of systems. As shown in Fig. 2, the procedure steps are (1) select the desired files, (2) right-click on the files and click [Create Report Set] on the right-click menu, and (3) complete the report set. Completed report sets are automatically stored in a database to prevent replacing, altering, destroying, or otherwise tampering with the data.

When a report set is created, it generates an electronic link between the electronic data and the report set, which also automatically disables (locks) editing the electronic data. That means editing or otherwise tampering with electronic data can be prevented after reports are created.

In this case, electronic signatures can be used not only for reviewing and approving report sets, but also for reviewing and approving the electronic data (analytical results) on which report sets are based. Using electronic signatures also eliminates the need to print out and sign reports manually and enables a paperless operation, which eliminates the need for redundant management of both electronic and paper records. That also solves the problems associated with paper records, such as replacement, alteration, or disposal of records.

Additionally, as shown in Fig. 2, data lines are color-coded when electronically approved, so they can be easily differentiated from remaining data that has not been reviewed or approved. That ensures orphan data can be identified easily, just as it can be for chromatography systems.

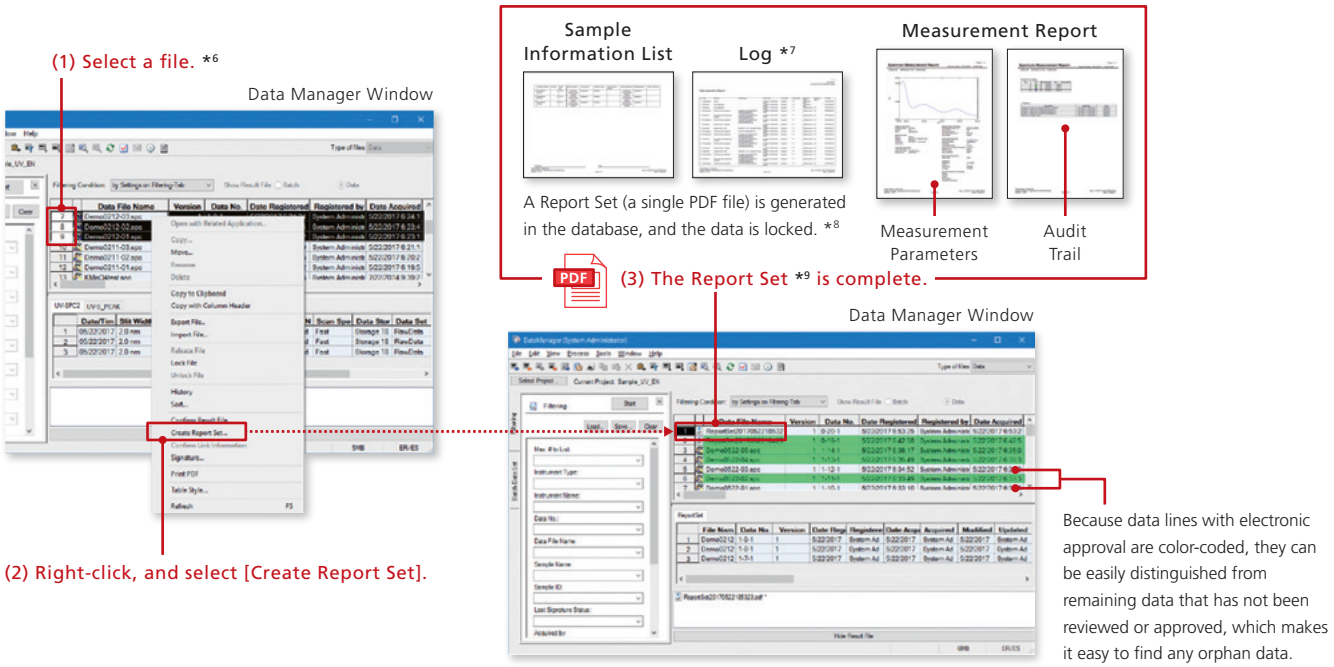

Fig. 2 Procedure for Using LabSolutions to Create a Report Set for Spectroscopy Systems *6

*6: If one UV photometric data file is selected for creating a report set, then the created report set will include files related to the selected file.
If there are multiple related files for UV, FTIR, or RF confirmation testing, then the related files are selected manually.
*7: The log file includes information recorded during measurements (a log record of operations performed between logging in for measurements and logging out). If postrun analysis is performed, a postrun analysis log is recorded (a log record of operations performed between logging in for postrun analysis and logging out) separately from the measurement log.
*8: Locked files can only be unlocked by people with the right to do so.
*9: For UV, FTIR, and RF report sets, support for functions (1) to (3) will be available in the future.
(1) Assigning ID codes to measurement methods (2) Avoiding superimposed printing of measurement methods for multiple sets of data
(3) Measuring sequences. However, this is already supported for UV quantitative testing (photometric). See *6.

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Technical Report

Improving Efficiency in the Preparation of Test Reports for Chemistry, Manufacturing, and Control (CMC) Using Multi Data Report

Satoru Watanabe[1] and Tadayuki Yamaguchi[1]

Abstract:

In order to demonstrate the quality of pharmaceutical products, pharmaceutical manufacturers must perform process studies into the active pharmaceutical ingredients (API) and studies into the pharmaceutical preparation process, and quality assessment for both of these as part of their chemistry, manufacturing, and control (CMC) activities. HPLC is used widely in such investigations for reaction tracing and impurity identiÿcation in API process studies, uniformity testing, and dissolution testing in pharmaceutical preparation development studies, and for analytical method validation in assay development for quality assessment studies.
The results of these studies must be tabulated according to the objectives and summarized in reports. It is often the case that these results are copied into Excel or similar software before a report is created. This article describes three examples of how the LabSolutions Multi Data Report feature is used in pharmaceutical development studies.
(1) API process studies: Outputting scouting results for chiral compounds
(2) Pharmaceutical preparation development studies: Outputting trend plots for dissolution testing
(3) Quality assessment studies: Outputting results from analytical method validation

Keywords: LabSolutions DB/CS, Multi Data Report, analytical method validation, method scouting, dissolution testing

1. Introduction

LabSolutions offers a Multi Data Report function that can combine multiple types of analytical data and create an Excel-like report. Using this function provides substantial efficiency improvements during the preparation of test reports for CMC.

Although Excel has long been used to create these reports, this method requires the manual copying of analytical data, which is both labor-intensive and can introduce errors. Excel document change control is also often left to the individual operator, which introduces the risk of multiple templates existing simultaneously, and the possibility of tampering with formulas or results.

The Multi Data Report function uses report templates that allow formulas similar to Excel to be included, so report formats previously used in Excel can be used in the Multi Data Report function. Report templates are managed securely in the LabSolutions database, and the change history for report templates can be saved as an audit trail.

When reports are created using the Multi Data Report function, they are populated with analytical results in a seamless process that saves on labor and prevents the introduction of transcription errors when Excel is used, thus enabling substantial improvements in data reliability and work efficiency to be achieved.

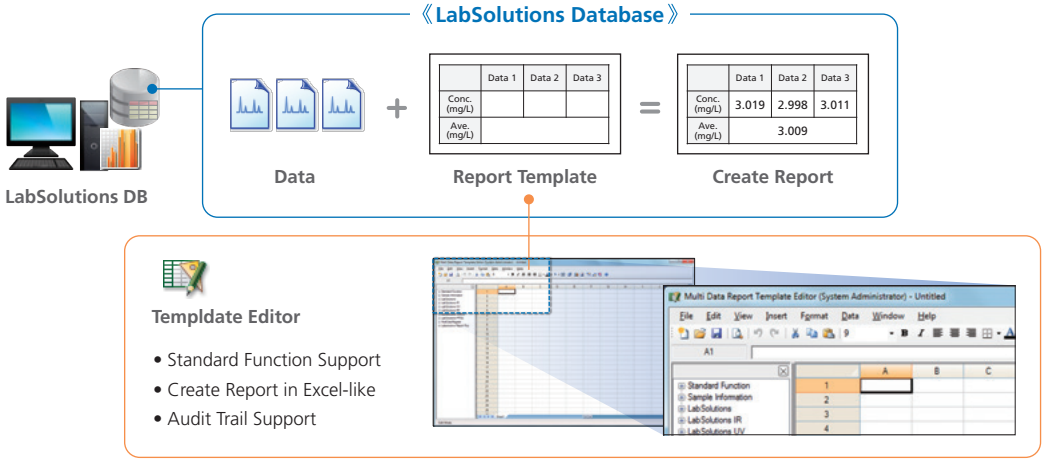


Fig. 1 Outline of the Multi Data Report Function

1 Analytical & Measuring Instruments Division

2. Usage-Case Examples

2-1. API Process Studies: Outputting Scouting Results for Chiral Compounds

In the API research area, chiral columns are being studied for quick and efficient resolution of optical isomers. Finding the appropriate column and mobile phase conditions for a given analysis from the wide variety of chiral columns available is a time-consuming and labor-intensive process, so there is a demand for more efficient means of developing separation conditions for chiral compounds.

Shimadzu offers a "Method Scouting System," which by combining solvent switching valves and column switching valves, is capable of automatically and continuously acquiring comprehensive data from up to 192 column and mobile phase combinations. However, determining optimum resolution conditions from the large volumes of data obtained during method scouting is time-consuming work that has its own set of issues, such as different operators generating different results from the same dataset.

The Multi Data Report function described in this article facilitates quantitative analysis of the large volumes of data acquired during method scouting.

Fig. 2 shows data obtained from methylclothiazide screening and Fig. 3 compares different resolution conditions. Data are displayed as graphs ranked in the order of degree of resolution, so the user can quickly determine the most appropriate column and resolution conditions for a given chiral compound.

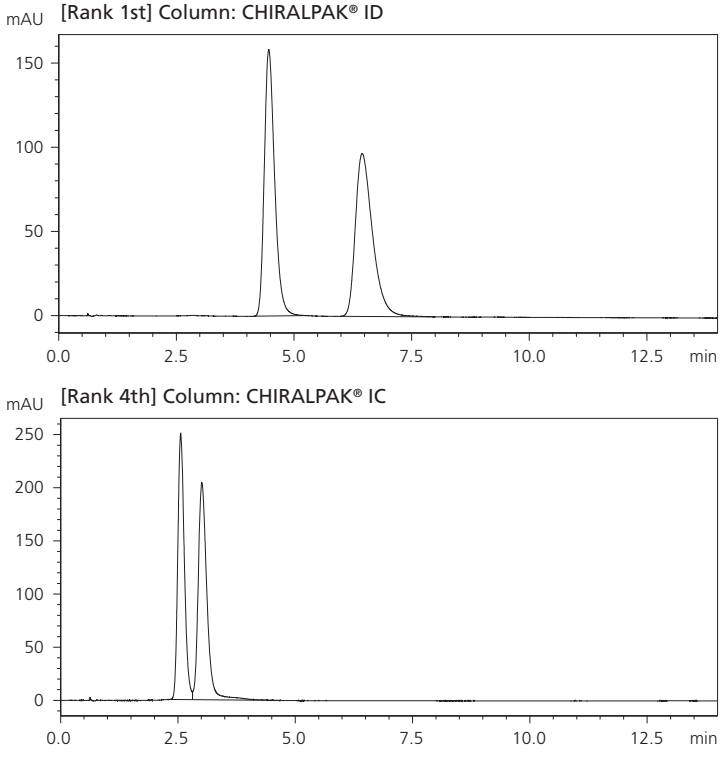
In addition to the degree of resolution, other parameters obtained during analysis such as the symmetry factor, number of peaks detected, and number of theoretical plates can be used freely to create evaluations according to the application.

This method removes operator influence from column scouting, and through the use of qualitative data also improves the reliability of scouting results.

Scouting Report Summary

Rank	Data File	Evaluation Value	Peak Count	Separated Peak Count	Resolution	Resolution Factor	Tailing Factor1	Tailing Factor2	k'1	k'2	Area%1	Area%2
1	Methylclothiazide_ID_n-Hex_EtOH_3_analysis_B20%_14min_035.lcd	7.569	2	2	3.785	1.523	1.310	1.463	5.665	8.626	49.777	50.223
2	Methylclothiazide_IF_MC_EtOH_6_analysis_B2%_4min_078.lcd	6.173	2	2	3.086	1.858	1.127	1.094	1.390	2.583	52.748	47.252
3	Methylclothiazide_IB_MC_EtOH_6_analysis_B2%_4min_070.lcd	4.912	2	2	2.456	2.248	0.715	1.094	0.443	0.995	45.633	54.367
4	Methylclothiazide_IC_n-Hex_EtOH_3_analysis_B20%_14min_033.lcd	3.155	2	2	1.577	1.238	1.264	1.300	2.821	3.493	47.960	52.040
5	Methylclothiazide_IF_n-Hex_EtOH_4_analysis_B100%_18min_052.lcd	3.030	2	1	1.515	2.759	1.465	-	0.102	0.282	48.153	51.847
6	Methylclothiazide_IF_MTBE_EtOH_8_analysis_B2%_4min_104.lcd	2.602	2	0	1.301	1.327	-	-	1.361	1.806	48.306	51.694
7	Methylclothiazide_IF_n-Hex_IPA_2_analysis_B40%_14min_026.lcd	2.433	2	1	1.217	1.807	1.854	-	1.436	2.595	48.504	51.496
8	Methylclothiazide_IA_n-Hex_EtOH_3_analysis_B20%_14min_029.lcd	2.326	2	0	1.163	1.156	-	-	4.943	5.714	48.006	51.994
9	Methylclothiazide_IC_n-Hex_IPA_2_analysis_B40%_14min_020.lcd	2.295	2	0	1.147	1.296	-	-	1.962	2.543	48.356	51.644
10	Methylclothiazide_IA_MTBE_EtOH_8_analysis_B2%_4min_094.lcd	2.202	2	0	1.101	1.209	-	-	2.128	2.573	46.618	53.382

Fig. 2 Screening Results Summary



Analytical conditions

- Mobile phase : Hexane/Ethanol=8/2 (v/v)
- Flowrate : 3 mL/min
- Analysis time : 14 min
- Column temperature : 40 °C
- Injection volume : 10 µL

Fig. 3 Comparison of Resolution Conditions

2-2. Pharmaceutical Preparation Development Studies: Outputting Trend Plots for Dissolution Testing

Dissolution testing is widely used in the field of pharmaceutical development for development and quality control activities, and also in the field of generic drugs for bioequivalence testing. With an increasing number of working hours being accounted for by the growing numbers of test samples, there is demand for a means of reducing the time spent in determining results from dissolution testing.

In pharmaceutical preparation development, the dissolution of pharmaceutical preparations is checked by creating a report in the form of a time-series plot of dissolution rate at short sampling intervals. Since dissolution rates must be calculated using formulas in the Pharmacopoeia, reports are commonly created using Excel and so validation and the control of templates used in this work often presents problems.

The Multi Data Report function described in this article can be used alongside the dedicated dissolution testing software "DT Solution," which reduces the work involved in creating complex reports and in file management.

"DT Solution" offers the ability to not only create an analysis sequence starting with the System Suitability Test (SST), but it also makes it possible to include information needed to calculate the dissolution rate, such as sample interval and measured component weight, within the data. The Multi Data Report function then uses this information to calculate the dissolution rate.

Fig. 5 is a report with a plot showing the trend in dissolution rate. This method offers the ability to automate operations, from analysis to report. In addition, Multi Data Reports also make it possible to automatically calculate the dissolution rate from the measured weight and display this information graphically as a trend plot.

Report templates can be stored securely in the LabSolutions database, and report template change histories can be saved as an audit trail, freeing the operator from administrative tasks.

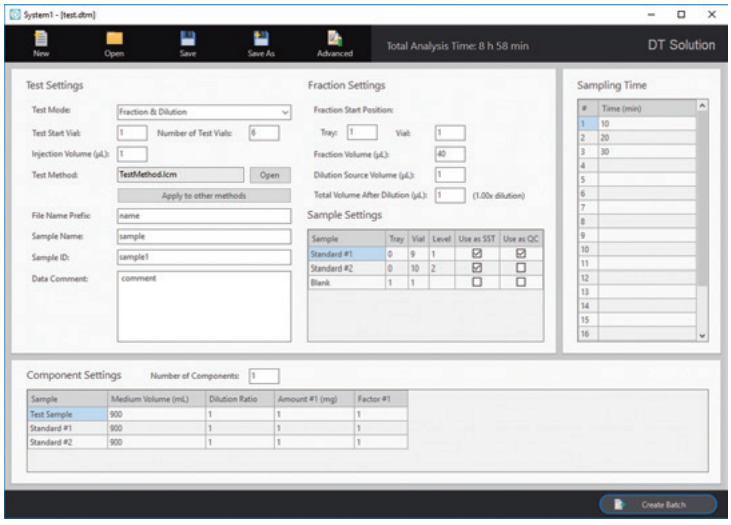


Fig. 4 DT Solution

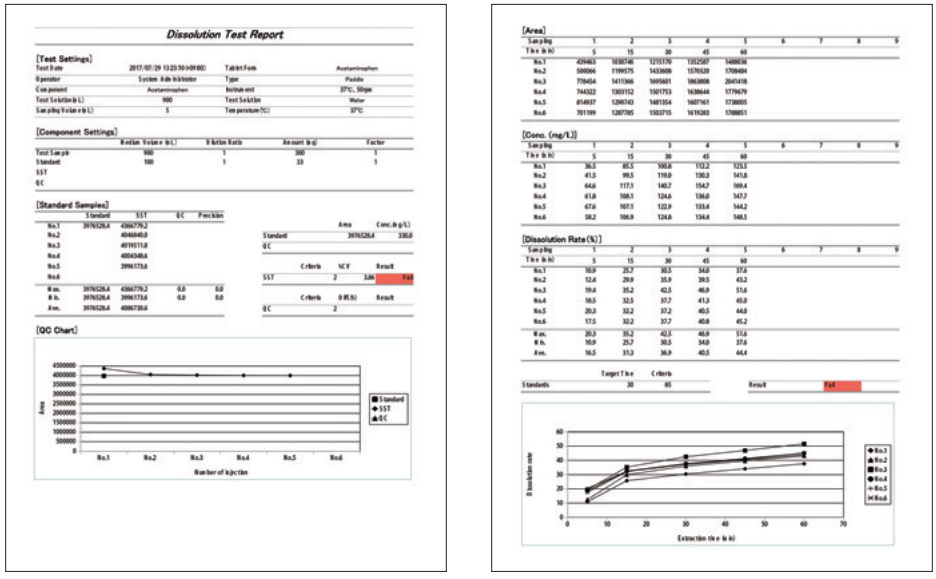


Fig. 5 Trend Graph Report

2-3. Quality Assessment Studies: Outputting Results from Analytical Method Validation

Analytical method validation is an important task specified in ICH guidelines that is used to demonstrate the validity of an assay method. Analytical method validation involves verifying the accuracy, precision, specificity, detection limits, quantifications limits, linearity, and range of an analytical method. As with dissolution testing, Excel is often used during analytical method validation since results must be calculated with formulas. Component area data and concentration data obtained from chromatograms must be transcribed by hand into Excel — a time-consuming task that comes with the risk of error.

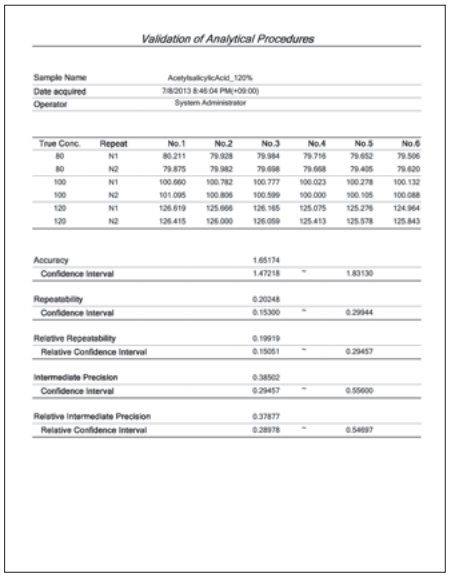
This article describes an example in which the Multi Data Report function is used to accurately incorporate all analytical method validation parameters in full, offering a huge improvement in validation work efficiency.

Fig. 6 shows a report on accuracy and precision. Accuracy represents deviation of mean (measured) concentration from the theoretical value, and precision represents relative standard deviation from (measured) concentration. Precision is expressed in terms of intra-assay precision (repeatability) and within-laboratory reproducibility (intermediate precision).

Fig. 7 shows a report on detection limits. A detection limit is calculated based on standard deviation (σ), which is the error distribution of measured values, and the slope (S) of the standard curve for concentration near the limit of detection.

$$LOD = 3.3\sigma/S$$

There are two methods of calculating standard deviation (σ). The standard deviation can be calculated as the residual error of a regression curve, or as the standard deviation of measured values at concentration zero as estimated from a regression curve. This article shows results calculated using both methods.



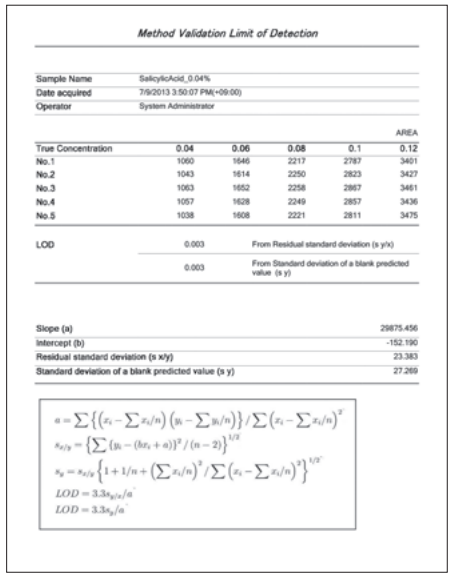
Validation of Analytical Procedures

Sample Name	AcetylsalicylicAcid_120%
Date acquired	7/8/2013 8:46:04 PM(+09:00)
Operator	System Administrator

True Conc.	Repeat	No.1	No.2	No.3	No.4	No.5	No.6
80	N1	80.211	79.928	79.984	79.716	79.652	79.506
80	N2	79.875	79.982	79.698	79.668	79.405	79.620
100	N1	100.660	100.782	100.777	100.023	100.278	100.132
100	N2	101.095	100.806	100.599	100.000	100.105	100.088
120	N1	126.619	125.666	126.165	125.075	125.276	124.964
120	N2	126.415	126.000	126.059	125.413	125.578	125.843

Accuracy		1.65174	
Confidence Interval		1.47218	~ 1.83130
Repeatability		0.20248	
Confidence Interval		0.15300	~ 0.29944
Relative Repeatability		0.19919	
Relative Confidence Interval		0.15051	~ 0.29457
Intermediate Precision		0.38502	
Confidence Interval		0.29457	~ 0.55600
Relative Intermediate Precision		0.37877	
Relative Confidence Interval		0.28978	~ 0.54697

Fig. 6 Accuracy and Precision Report



Method Validation Limit of Detection

Sample Name	SalicylicAcid_0.04%
Date acquired	7/9/2013 3:50:07 PM(+09:00)
Operator	System Administrator

					AREA
True Concentration	0.04	0.06	0.08	0.1	0.12
No.1	1060	1646	2217	2787	3401
No.2	1043	1614	2250	2823	3427
No.3	1063	1652	2258	2867	3461
No.4	1057	1628	2249	2857	3436
No.5	1038	1608	2221	2811	3475

LOD	0.003	From Residual standard deviation (s y/x)
	0.003	From Standard deviation of a blank predicted value (s y)

Slope (a)	29875.456
Intercept (b)	-152.190
Residual standard deviation (s x/y)	23.383
Standard deviation of a blank predicted value (s y)	27.269

$$a = \sum\left\{\left(x_i - \sum x_i/n\right)\left(y_i - \sum y_i/n\right)\right\} / \sum\left(x_i - \sum x_i/n\right)^2$$
$$s_{x/y} = \left\{\sum\left(y_i - (bx_i + a)\right)^2 / (n-2)\right\}^{1/2}$$
$$s_y = s_{x/y}\left\{1 + 1/n + \left(\sum x_i/n\right)^2 / \sum\left(x_i - \sum x_i/n\right)^2\right\}^{1/2}$$
$$LOD = 3.3s_{y/x}/a$$
$$LOD = 3.3s_y/a$$

Fig. 7 Detection Limit Report

3. Conclusion

This article has described how the Multi Data Report function can be used to achieve substantial efficiency improvements during the creation of test reports for CMC by outputting trend plots for chiral compound scouting and dissolution testing, and by saving time during analytical method validation.

In addition, the use of the Multi Data Report function introduced in this article, combined with the LabSolutions database, encourages the adoption of paperless procedures and the computerization of documentation control of reports during pharmaceutical development. It also allows quality test data to be used and managed over the long term while helping pharmaceutical lifecycle management (the strategy of maximizing total sales of pharmaceutical products by considering the pharmaceutical product lifecycle).

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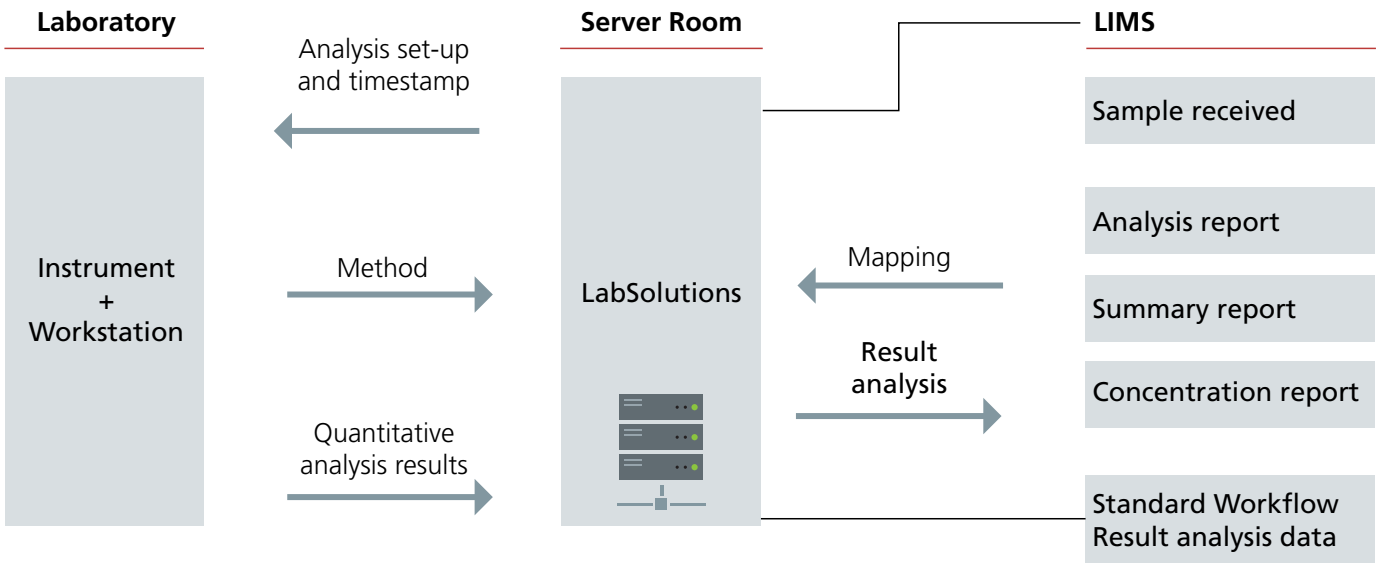
Workflow Management with LIMS

Laboratory Information Management System (LIMS) is an indispensable informatics product for large laboratories. LIMS not only ensures data compliance but also achieves automated laboratory management. This creates a more convenient and efficient laboratory environment for pharmaceutical industries.

As different kinds of analytical instruments and corresponding operating software are used in pharmaceutical laboratories, it is a challenge to build a well compatible LIMS in these laboratories.

Based on the LabSolutions CS, Shimadzu can build a broad laboratory automation network system twith an unified communication interface compatible with various LIMS manufacturers.

LabSolutions LIMS Interface



Integration of Shimadzu CDS and LIMS

- Through collaboration with LIMS vendors, Shimadzu CDS can be integrated to allow data acquisition and report generation to happen in alignment with laboratory task list imported from the LIMS.
- The integrated network system can therefore support automated data management and operation for various analytical devices as required by GMP/GLP.

Simpler laboratory management solution

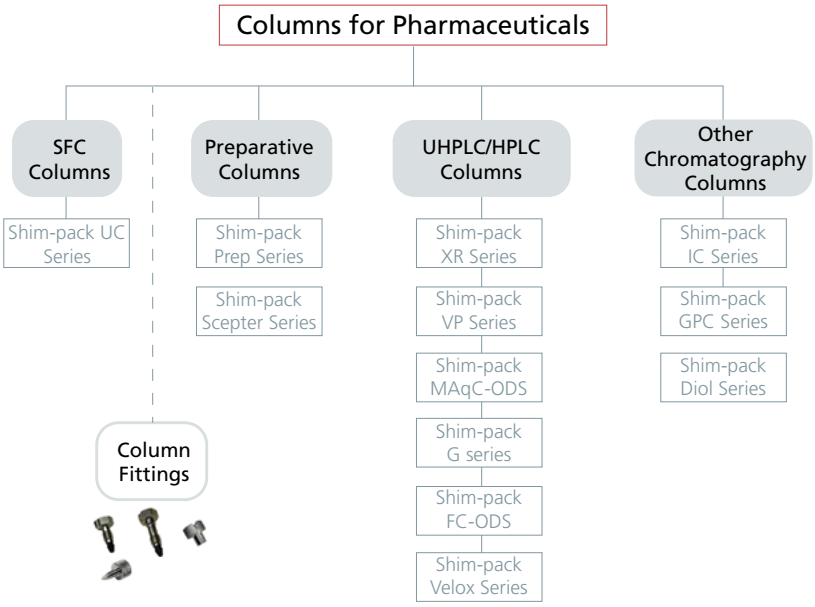
- It enables the automatic management of laboratory equipment, personnel, sample, quality and other all-round automation management. It improves the efficiency of laboratory operation by timely notification of task progress.
- The LabSolutions CS fully covers chromatographic and non-chromatographic instruments, making the connection between CDS and LIMS more convenient, and the data statistics of various instruments more simple and intuitive.

Consumables

Shimadzu is committed to providing the most complete solutions for users in the pharmaceutical industry. Columns and related consumables matched with Shimadzu's full line of LC/SFC products, and a rich and reliable solution to ensure you get the best analytical performance and reproducibility.

Columns

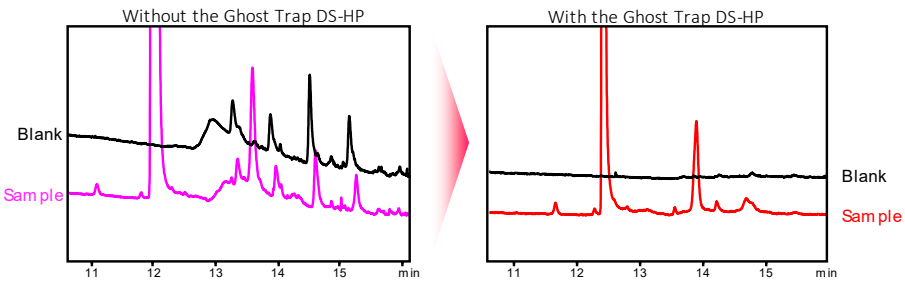
- ✓ Comprehensive product range
- ✓ Wide range of stationary phases
- ✓ High resolution and sensitivity
- ✓ Strict uniformity and high stability
- ✓ High durability and stable reproducibility



Mobile Phase Cleaner for UHPLC/HPLC

- The Ghost Trap DS/DS-HP works for both water solutions and organic solvents.
- The Ghost Trap DS/DS-HP eliminates impurities / contaminants from the mobile phase and system, including wetted parts as it is positioned between the gradient mixer and autosampler.

Ghost Trap DS/DS-HP



LabTotal Vial

- It reduces the adsorption of basic compounds on the surface of the glass vial, provides good consistent and recovery rate of compounds.

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First Edition: December, 2018

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Nexera series

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