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Application News

Shimadzu High performance liquid chromatograph The LC-2050 series

Organic Impurity Analysis of Levofloxacin Drug Material Following USP Monograph

Peiling Hou and Jie Xing

User Benefits

- Following USP monograph 41, impurity analysis of levofloxacin drug material was performed on the i-series LC-2050. System suitability test meets the requirements.
- Six organic impurities of trace levels were found with 4 being identified via matching the RRTs to the listed impurities in the monograph.

■ Introduction

Levofloxacin (Figure 1) is a synthetic broad spectrum antibacterial agent which is active against Gram-positive and Gram-negative bacteria. Levofloxacin is currently used in adults for the treatment of respiratory tract infections, urinary tract infections, chronic bacterial prostatitis and skin and soft tissue infections, etc.

Monitoring impurities in drug active pharmaceutical ingredient (API) and products is crucial for drug development and throughout the manufacturing process. In this application note, Shimadzu i-Series LC-2050 was used for system suitability test and organic impurities analysis of levofloxacin API following the USP monograph 41[1]. The separation was performed on a Shim-pack GIST C18 column (L1). This method with high sensitivity and selectivity can eventually be transferred and validated for QA/QC in pharmaceutical laboratories.

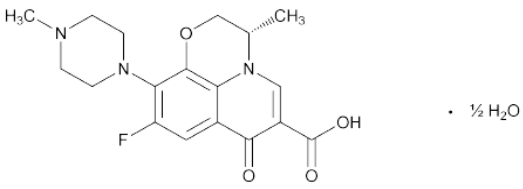

Figure 1. Chemical structure of levofloxacin

■ Experimental

Chemicals and preparation of standards

Ammonium acetate, cupric sulfate pentahydrate and L-isoleucine were obtained from Sigma Aldrich with ≥ 98 % purity. Levofloxacin standard used in this study was provided by a collaboration laboratory. The levofloxacin standard solution of 1.0 mg/mL was prepared using the mobile phase as diluent. It worked as system suitability test solution. Levofloxacin standard solution of 0.3 µg/mL was obtained by serial dilution from the stock, which is used for sensitivity test.

Analytical conditions

Shimadzu i-Series LC-2050 system was employed for analysis. The details of analytical conditions are listed in Table 1.

Table 1. Analytical conditions of levofloxacin

Column	Shim-pack GIST C18 (250 mm x 4.6 mm I.D. 5µm) packing L1
Mobile Phase	A: 8.5 g/L ammonium acetate, 1.25 g/L cupric sulfate (pentahydrate) and 1.3 g/L L-isoleucine in ultrapure water
	B: Methanol
Elution mode	Isocratic, A:B = 7:3 (Vol/Vol)
UV wavelength	360 nm
Flow Rate	0.8 mL/min
Oven Temp.	45 °C
Injection vol.	25 µL

■ Results and discussion

System suitability test

The USP method for levofloxacin was set up on the i-series LC-2050 system. Figure 2 shows the chromatogram of levofloxacin standard of 0.3 µg/mL for the system sensitivity test. The total run time is 25 minutes and levofloxacin elutes at 14.7 minutes.

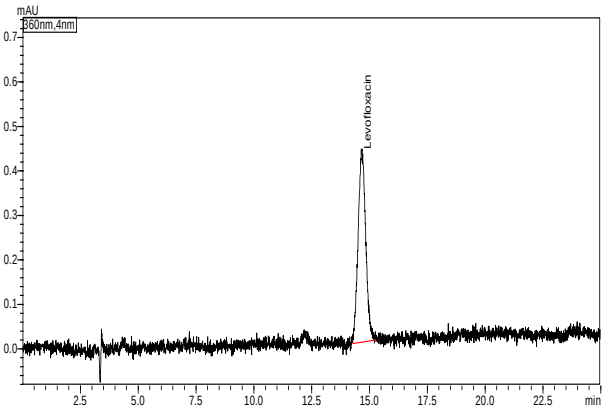

Figure 2. HPLC chromatogram of levofloxacin standard at 0.3 µg/mL

The S/N ratio of 14.4 obtained meets the criteria of NLT 10 (Table 2).

Repeatability test was conducted with the system suitability solution of 1 mg/mL. The %RSD (n = 6) of peak area and retention time of levofloxacin are shown in Table 2, which meet the system suitability requirement of %RSD NMT 1.0 % following USP requirement. The result for tailing factor was within the criteria as listed in Table 2.

Table 2. System suitability test parameters of levofloxacin

Test factors	Solutions (µg/mL)	Acceptance Criteria	Values (n=6)	Results
Area %RSD	1000	NMT 1.0%	0.07%	Pass
R.T. % RSD	1000	NMT 1.0%	0.12%	Pass
Signal-to-noise ratio	0.3	NLT 10	14.4	Pass
Tailing factor	1000	0.5-1.5	0.61	Pass

Identification of organic impurities in levofloxacin

The 1 mg/mL of levofloxacin solution was used for impurity profiling analysis. The HPLC chromatogram is shown in Figure 3.

Trace level impurity peaks of levofloxacin from same solution could be seen on the zoomed chromatogram as shown in Figure 3 (bottom). Six impurities were detected as listed in Table 3. Four of the impurities were identified by matching to the relative retention time (RRT) listed in the Table 1 of USP monograph 41. Two unknown impurities with RRT of 0.55 and 0.67 were found. The total impurity was 0.29%.

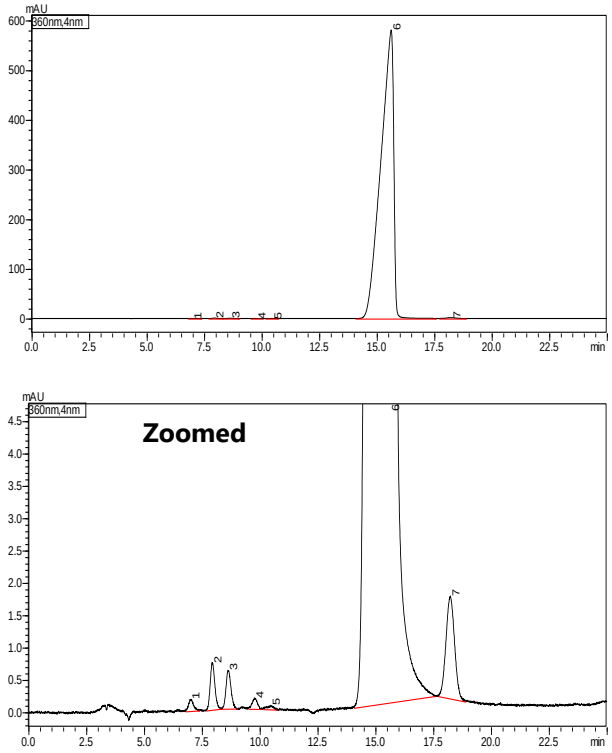

Figure 3. Chromatogram of levofloxacin of 1 mg/mL (top). The zoomed chromatogram (bottom) shows the smaller impurity peaks.

Table 3. Impurities identified in levofloxacin solution of 1.0 mg/mL

Peak#	RT (min)	Area (%)	RRT	Name
1	6.987	0.011	0.447	N-Desmethyl levofloxacin
2	7.941	0.045	0.508	Diamine derivative
3	8.624	0.039	0.552	unknown impurity
4	9.771	0.011	0.625	Levofloxacin N-oxide
5	10.464	0.005	0.670	unknown impurity
6	15.627	99.708	1.000	Levofloxacin
7	18.235	0.181	1.167	D-isomer
Total Impurities		0.292		

■ Conclusion

The USP method for impurity analysis of levofloxacin was tested on the i-series LC-2050 system with Shim-Pack GIST C18 column. The results of system suitability test are sufficient as per the requirements in the USP 41 monograph. The impurities were identified by matching to the relative retention time listed in Table 1 of USP monograph 41. Four out of five impurities listed in USP monograph were identified in the levofloxacin standard solution at 1 mg/mL.

■ Reference

1. USP (USP 41, May 1, 2018) official monograph / Levofloxacin 2395

■ Acknowledgment

We thank *Lin Chiat Teng* from National University of Singapore for her assistance on this work during her internship program at Shimadzu (Asia Pacific) Pte Ltd.

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Application News

High Performance Liquid Chromatograph LC-2050C

Analysis of Eight Carbonyl Compounds in E-Cigarette Liquid using High-Performance Liquid Chromatography

Zhiru Li
Analysis Center, Shimadzu (China) Co., Ltd.

User Benefits

- Enabled simultaneous analysis of eight carbonyl compounds in E-Cigarette Liquid.
- This method can be applied for monitoring the risks of e-cigarettes.

■ Introduction

An e-cigarette is an electronic product that simulates a cigarette. It has the same look, smoke, taste, and feel as a cigarette. It uses a rechargeable lithium polymer battery to power an atomizer that heats e-liquid in the chamber/tank to turn nicotine and other substances into a vapor for the user to inhale. Over the last few years, e-cigarettes have become a new option for many smokers as a substitute for cigarettes, and they are highly sought after as a "healthy" smoking cessation tool. However, e-cigarettes release carbonyl compounds such as formaldehyde, acetaldehyde, and other harmful substances during the heating process, and they can also change the composition of certain chemicals, resulting in new potential hazards. The E-Liquid Safety and Technical Specifications (Draft for Comment) are organizational standards of the China Electronics Chamber of Commerce. Appendix C (E-liquids: Testing for carbonyl compounds) of those specifications stipulates that limit value of formaldehyde, acetone, acrolein, propionaldehyde, crotonaldehyde, 2-butanone, and butyraldehyde in e-cigarette liquid must be ≤20 mg/kg while acetaldehyde must be ≤180 mg/kg.

In this experiment, a method was developed based on Appendix C (E-liquids: Testing for carbonyl compounds) to analyze eight carbonyl compounds in e-cigarette liquids by using high-performance liquid chromatography.

■ Experiment

System

The Sample was analyzed Using LC-2050C 3D, shimadzu high performance liquid chromatograph, and chromatography workstation LabSolutions Ver. 5.98.

Analytical Conditions

Analytical Conditions are shown in Tabel 1.

Table 1 Analytical Conditions

Column	Shim-pack™ GIST C18-HP (150 mm × 4.6 mm I.D., 5 µm)*1
Mobile Phase A	Water/acetonitrile/tetrahydrofuran/isopropyl alcohol = 63:27:9:1
Mobile Phase B	Water/acetonitrile/tetrahydrofuran/isopropyl alcohol = 40:58:1:1
Mobile Phase C	Acetonitrile
Flow rate	1.25 mL/min
Column Temp.	50 °C
Wavelength	UV at 365 nm
Elution mode	Gradient elution, time program is shown in Table 2.

*1 P/N: 227-30041-05

Table 2 Gradient Time Program

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)	Mobile Phase C (%)
0.00	95	0	5
1.00	95	0	5
16.00	70	30	0
19.00	40	60	0
27.00	40	60	0
33.00	0	100	0
35.00	0	0	100
37.00	0	0	100
37.01	95	0	5
41.00	95	0	5

Sample Preparation

- **Mix standard reference solution:** Based on the Draft for Comment, properly transfer DNPH(2,4-Dinitrophenylhydrazine)-derivatized standard solutions of the eight carbonyl compounds to prepare standard stock solutions at a concentration of 10 mg/L. Properly transfer each standard stock solution to prepare a series of standard working solutions with a concentration of 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, and 5.0 mg/L. Prepare at the time of use.
- **The derivatization reagent:** Weigh 4.5 g of DNPH dissolved in about 200 mL of acetonitrile, add 20 mL of 10 % phosphoric acid in aqueous solution, transfer to a 500-mL volumetric flask, add about 250 mL of water, and adjust to volume with acetonitrile. The prepared solution should be stored in a brown reagent bottle away from sunlight, and it can be stored for up to six months.
- **sample solutions:** Accurately weigh 0.1 g of e-cigarette liquid in a brown 1-mL volumetric flask to 0.1 mg, add about 0.8 mL of derivatization reagent, shake well, leave to stand at room temperature for 20 minutes, add 50 µL of pyridine, dilute with acetonitrile, and adjust the volume to reach the mark. Filter through a PTFE membrane and place in a chromatography vial for analysis.

Fig. 1 Appearance of LC-2050C 3D

■ Results and discussion

Chromatogram:

The standard working mixtures (2.0 mg/L) were tested in accordance with the conditions described in Table 2. The chromatogram is shown in Fig. 2. Characteristic peaks of DNPH derivatives of the eight carbonyl compounds were confirmed. Peak resolution of DNPH derivatives of acetone and acrolein was 1.636, which met the requirements.

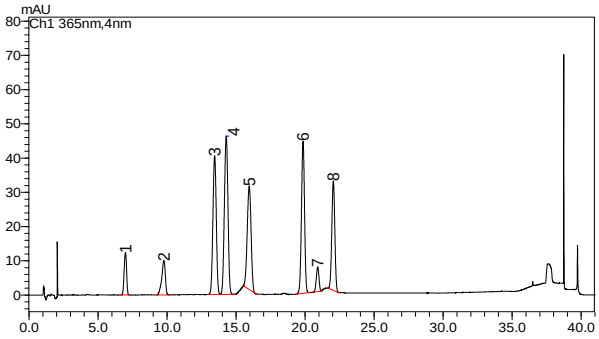

Fig. 2 Chromatogram for Mix standard reference solution (2.0 mg/L)
1. Formaldehyde, 2. Acetaldehyde, 3. Acetone, 4. Acrolein, 5. Propionaldehyde, 6. Crotonaldehyde, 7. 2-butanone, 8. Butyraldehyde

Linearity:

Calibration curves for eight carbonyl compounds were calculated from analysis data of standard working solutions. A good linear correlations were acquired in the concentration range of 0.05 ~ 5.0 mg/L. These results are shown in Fig. 3. Correlation coefficients were all greater than 0.999. Specific results are shown in Table 3.

Table 3 Calibration Curve Parameters

No.	Compound	Linear Equation	Correlation coefficient	Accuracy (%)
1	Formaldehyde	Y = 76218.3X +162.744	0.9997	85.6-116.4
2	Acetaldehyde	Y = 87274.6X -949.451	0.9996	92.8-117.4
3	Acetone	Y = 333463X -2749.83	0.9999	93.5-110.9
4	Acrolein	Y =406256X -2084.23	0.9998	92.1-113.7
5	Propionaldehyde	Y = 338068X -5562.75	0.9998	92.9-112.3
6	Crotonaldehyde	Y = 327883X -3356.25	0.9999	92.2-110.8
7	2-butanone	Y = 39911.6X -547.297	0.9997	86.3-109.8
8	Butyraldehyde	Y = 206013X -1614.52	0.9998	93.2-106.8

Sensitivity:

Sensitivity was calculated at 0.05 mg/L, the lowest concentration of the standard working solutions (the method's detection limit: 0.5 mg/kg). Limit of detection (LOD) and limit of quantitation (LOQ) were calculated using the LabSolutions software and results are shown in Table 4.

Table 4 Sensitivity with Respect to the Eight Carbonyl Compounds

No.	Compound	LOD (mg/kg)	LOQ (mg/kg)
1	Formaldehyde	0.023	0.077
2	Acetaldehyde	0.036	0.120
3	Acetone	0.009	0.030
4	Acrolein	0.008	0.027
5	Propionaldehyde	0.013	0.045
6	Crotonaldehyde	0.010	0.033
7	2-butanone	0.063	0.210
8	Butyraldehyde	0.013	0.044

Recovery:

A standard stock solution was precisely added to a sample of e-cigarette liquid (spike: 20.0 mg/kg), and the sample was prepared like the sample solutions prepared. Recovery results are shown in Table 5. Results revealed that recovery rates of the eight carbonyl compounds were 87.4 to 99.9 %, which met the requirements.

Table 5 Recovery Results (n = 4)

No.	Compound	Volume Added (mg/kg)	Recovery Rate %
1	Formaldehyde		99.9
2	Acetaldehyde		98.4
3	Acetone		97.3
4	Acrolein	20.0	97.2
5	Propionaldehyde		96.0
6	Crotonaldehyde		96.3
7	2-butanone		89.7
8	Butyraldehyde		87.4

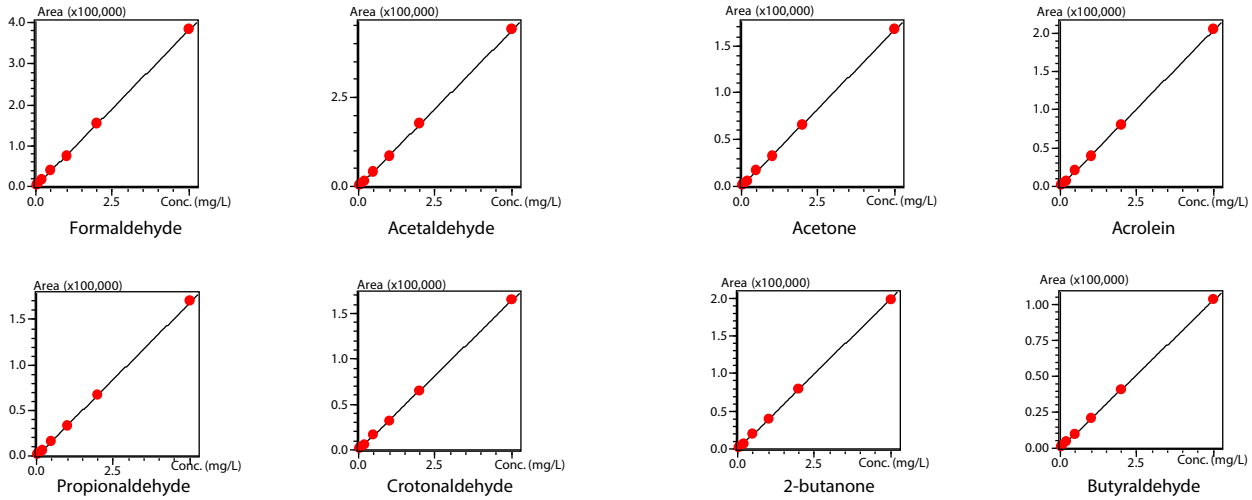

Fig. 3 Calibration Curves for Eight Carbonyl Compounds

Precision:

Standard working solutions (0.1 mg/L, 0.5 mg/L, and 2.0 mg/L) were injected six times consecutively in order to examine instrument precision. Results are shown in Table 6. The retention time RSD% for the eight carbonyl compounds was 0.03 to 0.22 % and the peak area RSD% was 0.10 to 4.29 %. Instrument precision was good.

Testing of e-cigarette liquids:

Six samples of e-cigarette liquids prepared were analyzed in accordance with analytical conditions described in Table 2. The chromatogram for the sample solutions is shown in Fig. 4 and test results are shown in Table 7.

The E-Liquid Safety and Technical Specifications (Draft for Comment) are organizational standards of the China Electronics Chamber of Commerce. Pursuant to Appendix C (E-liquids: Testing for carbonyl compounds) of those specifications, a method is required to meet a detection limit of 0.5 mg/kg for each carbonyl compound. The regulated values for formaldehyde, acetone, acrolein, propionaldehyde, crotonaldehyde, 2-butanone, and butyraldehyde are ≤20 mg/kg while its regulated one for acetaldehyde is ≤180 mg/kg.

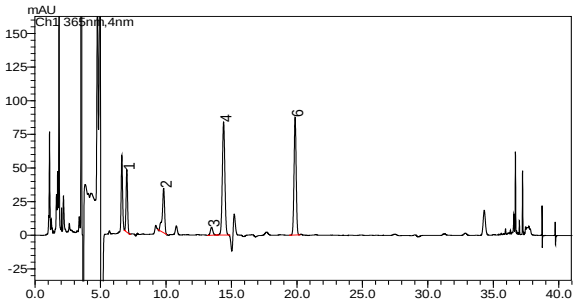

Fig. 4 Chromatogram for E-Cigarette Liquid Sample Solutions (grapefruit, mango, and coconut milk)
1. Formaldehyde, 2. Acetaldehyde, 3. Acetone, 4. Acrolein, 6. Crotonaldehyde

■ Conclusion

In this experiment, a method of using high-performance liquid chromatography to analyze eight carbonyl compounds in e-cigarette liquids was devised based on Appendix C (E-liquids: Testing for carbonyl compounds) of the E-Liquid Safety and Technical Specifications (Draft for Comment). The excellent linearity, repeatability and recovery were confirmed. Additionally, this method was highly sensitive and accurate, so it can provide the tobacco industry with a reference with which to monitor the risks of e-cigarettes.

Table 6 Results for Precision (n = 6)

No.	Compound	0.1 mg/L		0.5 mg/L		2.0 mg/L	
		Retention Time RSD (%)	Peak Area RSD (%)	Retention Time RSD (%)	Peak Area RSD (%)	Retention Time RSD (%)	Peak Area RSD (%)
1	Formaldehyde	0.18	2.52	0.07	1.28	0.13	0.34
2	Acetaldehyde	0.19	3.36	0.09	0.86	0.08	0.27
3	Acetone	0.22	1.04	0.04	0.42	0.10	0.21
4	Acrolein	0.21	2.59	0.05	0.63	0.10	0.18
5	Propionaldehyde	0.21	4.29	0.05	1.31	0.09	0.19
6	Crotonaldehyde	0.10	0.48	0.04	0.35	0.05	0.10
7	2-butanone	0.10	4.10	0.03	0.53	0.04	0.51
8	Butyraldehyde	0.07	0.88	0.03	0.19	0.05	0.17

Table 7 Sample Analysis Results (mg/kg)

No.	Compound	Grapefruit, Mango, and Coconut Milk	Kiwi and Apple	Series B Strawberry Marshmallow	Mango	Icy Banana	Icy Lychee Berry	Limits
1	Formaldehyde	47.6	44.3	13.6	47.6	12.5	40.1	≤20
2	Acetaldehyde	44.6	83.7	22.2	6.0	31.7	33.2	≤180
3	Acetone	2.3	17.2	1.3	16.5	0.8	6.0	≤20
4	Acrolein	26.9	2.4	96.0	3.1	78.3	1.5	≤20
5	Propionaldehyde	N.D.	10.6	1.2	291.1	N.D.	N.D.	≤20
6	Crotonaldehyde	29.0	8.5	14.1	N.D.	5.5	6.6	≤20
7	2-butanone	N.D.	N.D.	N.D.	N.D.	0.9	1.5	≤20
8	Butyraldehyde	N.D.	7.5	N.D.	N.D.	N.D.	0.5	≤20

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Application News

High Performance Liquid Chromatograph LC-2050C 3D

Optimization of Ion Analytical Conditions in Pharmaceuticals Using LabSolutions MD

Hiromasa Iboshi

User Benefits

- Using software, each parameter can be varied comprehensively and easily, enabling efficient analysis method development.
- Resolution and analysis parameter relationships can be visually assessed and the valid parameter areas can be confirmed.

■ Introduction

The physicochemical and pharmacokinetic properties of active pharmaceutical ingredients change depending on the counterion. In the drug development stage, various counterions are tested and selected as appropriate salts. Residual inorganic impurities such as catalysts and ions used the synthesis stage can affect product safety, solubility, and stability, so it is very important to analyze ions as impurities.

In this article, an analysis example using ion exclusion chromatography is described. Formic acid, acetic acid, fumaric acid, and maleic acid, which are organic acids frequently used for drug counterions, were analyzed in the example. Response was visualized by drawing design spaces while comprehensively changing each parameter, and analytical conditions were optimized by using LabSolutions™ MD and LC-2050C 3D.

■ Analytical Conditions

In ion exclusion chromatography, retention strength mainly depends on column temperature and acid concentration. In addition, depending on the analytical conditions, there are components that greatly change the retention time, so it is necessary to consider analytical conditions. In this article, we examined the analytical conditions that can achieve good separation of the four components of formic acid, acetic acid, fumaric acid, and maleic acid by using LabSolutions MD. Table 1 shows the analytical conditions used for the separation study of each component.

The resolution of 4 organic acids was comprehensively examined by changing the column temperature and acid concentrations in the mobile phase that affect separation. Acid concentration was changed from 1 to 5 mmol/L in 1 mmol/L increments, and column temperature from 30 °C to 50 °C in 5 °C increments.

Table1 Analytical Conditions

Mobile Phase A:	Water
Mobile Phase B:	10 mmol/L perchloric acid
Column:	Shim-pack™ Fast-OA (100 mm × 7.8 mm I.D., 5 µm)*1 × 2
	Shim-pack Fast-OA (G) (10 mm × 4.0 mm I.D., 5 µm)*2
B Conc.:	10, 20, 30, 40, 50 % (5 patterns)
Column Temp.:	30, 35, 40, 45, 50 °C (5 patterns)
Flowrate:	0.8 mL/min
Vial:	SHIMADZU LabTotal™ for LC 1.5 mL, Glass*3
Injection Vol.:	10 µL
Detection:	PDA at 210 nm

*1 P/N: S228-59942-41
*2 P/N: S228-59942-42
*3 P/N: 227-34001-01

■ Peak Tracking

LabSolutions MD has a function to identify peaks using multiple parameters. In this article, each peak was identified and peak tracking was performed by combining the two parameters of height % and peak elution number for each component (Fig. 1).

It was found that the retention time of fumaric acid changed significantly compared with other peaks. In this case, each peak could be identified automatically by filtering by peak number and peak height % (maleic acid), or by peak height % only (other components). It was also possible to automatically identify each peak for fumaric acid, for which the peak number changed.

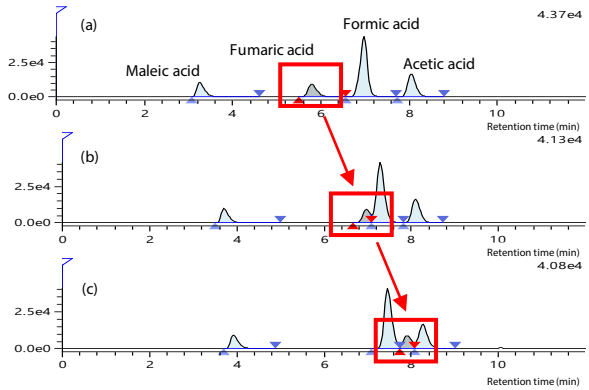

Fig. 1 Chromatogram for Each Analytical Condition

a. Column Temp. : 50 °C, B Conc. : 10 %
b. Column Temp. : 50 °C, B Conc. : 30 %
c. Column Temp. : 35 °C, B Conc. : 40 %

■ Visualizing Separation by Design Space

LabSolutions MD can visually evaluate the relationship between analytical conditions and separation by drawing design spaces. Based on the identified retention time, a design space was produced that shows the minimum separation of each peak in the height direction, with mobile phase B concentration in the vertical axis and the column oven temperature on the horizontal axis (Fig. 2). The warm areas indicate a high response and minimum resolution, allowing the effective analytical conditions to be visually determined.

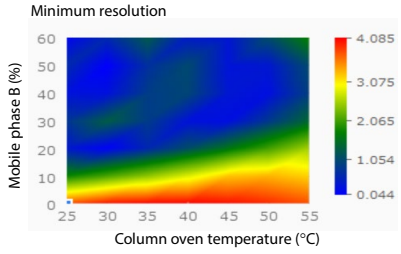

Fig. 2 Design Space for Parameters and Responses

LabSolutions MD also can describe design spaces that focus on specific compounds. Fig. 3 shows the design space when the lower limit of resolution for formic acid and acetic acid is set to 1.5. For formic acid, the region with a resolution of 1.5 or higher is confirmed in the upper left, but with acetic acid, it can be seen that the resolution from other components is not good in the corresponding region. In this way, it is also possible to evaluate the resolution and analytical conditions for each component. In addition, by overwriting the resolution with 2D contour lines, it is possible to evaluate the effective area from multiple perspectives. This time, it was possible to visually confirm the relationship between the effective region showing a resolution of 1.5 or more and the relationship for each component (Fig. 4).

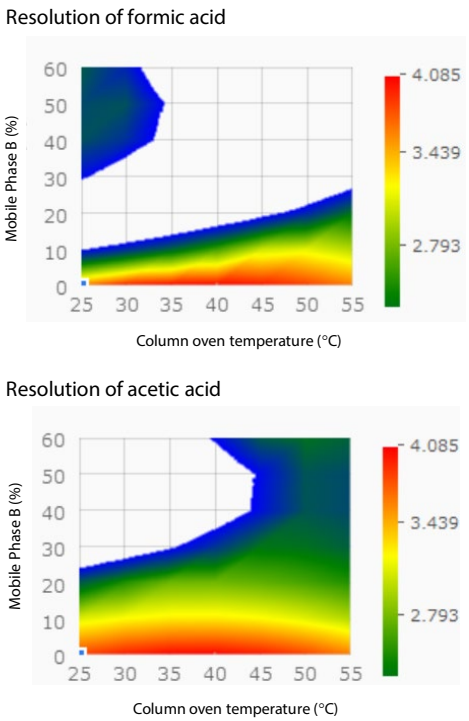

Fig. 3 Design Space for the Resolution of Each Component

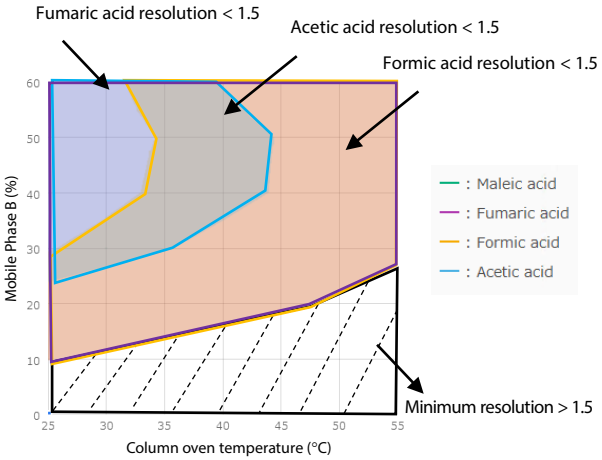

Fig. 4 Overlay 2D Contour Lines

■ Proposal of Optimal Analytical Conditions

LabSolutions MD has a function to search for optimal conditions based on model analysis results. By using this function, it is possible to propose analytical conditions with good separation and high robustness in the entire variation region of various analysis parameters. This time, a search was performed for the optimal point for the minimum resolution, and the corresponding parameters were confirmed (Fig. 5). The predicted and measured chromatograms for the presented analysis parameters are shown in Fig. 6. It was confirmed that there were no large discrepancies in the separation and retention time of each component.

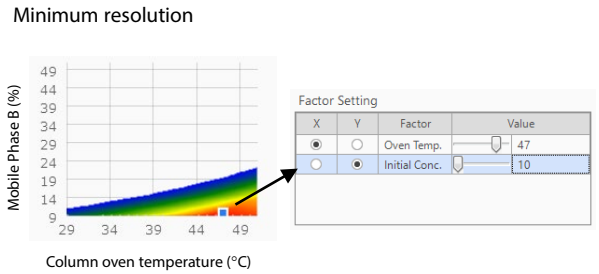

Fig. 5 Optimal Analysis Parameters Proposed

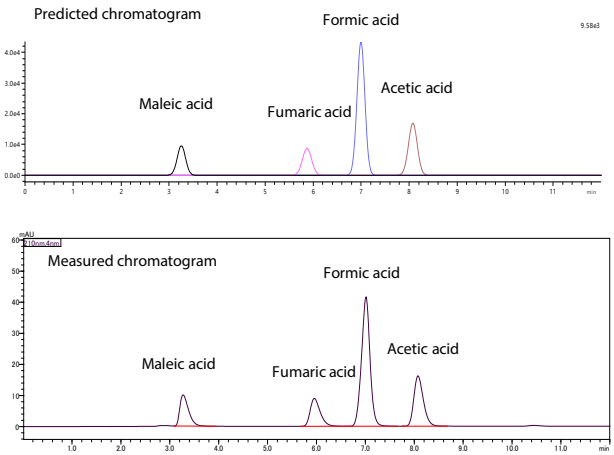

Fig. 6 Predicted and Measured Chromatogram

■ Conclusion

The analytical conditions of the four organic acid components were examined using LabSolutions MD. By using a complex of parameters, it was possible to automatically identify peaks for components whose peak elution order changed. In addition, by drawing the design space, it was possible to visually determine the effect on the resolution of varying various parameters. It was also possible to confirm the optimal analytical conditions.

Using LabSolutions MD makes it possible to optimize analytical conditions based on scientific evidence without depending on the analyst's experience or intuition.

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Application News

High Performance Liquid Chromatograph i-Series LC-2060

High Speed and Simultaneous Analysis for Fat-Soluble Vitamins

Natsuki Iwata and Rie Kato

User Benefits

- Enables simultaneous analysis of fat-soluble vitamins.
- The simplicity of using only methanol as the mobile phase reduces the effort of analytical preparation.
- The photodiode array (PDA) detector allows chromatograms of any wavelength to be obtained with a single analysis.

■ Introduction

Vitamins are nutrients that must be obtained from food because the body cannot synthesize them at all or in sufficient amounts. Vitamins are roughly classified as water-soluble or fat-soluble vitamins, which includes vitamins A, D, E, and K.

Fat-soluble vitamins are often analyzed using normal-phase chromatography, with different analytical conditions specified for each type of vitamin. In this article, vitamins A, D, and E were simultaneously analyzed using reversed-phase chromatography.

This article describes a simultaneous analysis of multiple fat-soluble vitamins.

■ Analysis of Standard Solution

Vitamins A, D, and E were dissolved in a small amount of tetrahydrofuran and then diluted with a mixture of methanol and tetrahydrofuran (97:3) to prepare the specified volume. Fig. 1 shows the chromatograms for the vitamins A, D, and E (50 IU, 100 IU, 0.1 mg/mL) obtained from the standard solution using the analytical conditions indicated in Table 1. A C18 column was used as the analytical column and methanol was used as the mobile phase. The PDA detector built into the integrated HPLC system was used. Using a PDA detector, chromatograms of any wavelength can be obtained after analysis, as long as it is within the specified wavelength range at the time of analysis. For this article, the maximum absorption wavelength of each compound was adopted as the detection wavelength. The system load pressure under these conditions was approximately 40 MPa.

Table 1 Analytical Conditions

System:	LC-2060C 3D
Column:	Shim-pack™ GIST-HP C18*1 (150 mm × 3.0 mm I.D., 2 µm)
Flowrate:	0.7 mL/min
Mobile Phase:	Methanol
Column Temp.:	40 °C
Injection Volume:	4 µL
Vial:	SHIMADZU LabTotal™ for LC 1.5 mL, Glass*2
Detection (PDA):	264 nm (Vitamin D), 280 nm (Vitamin E), 325 nm (Vitamin A)

*1 P/N: 227-30002-05 *2 P/N: 227-34001-01

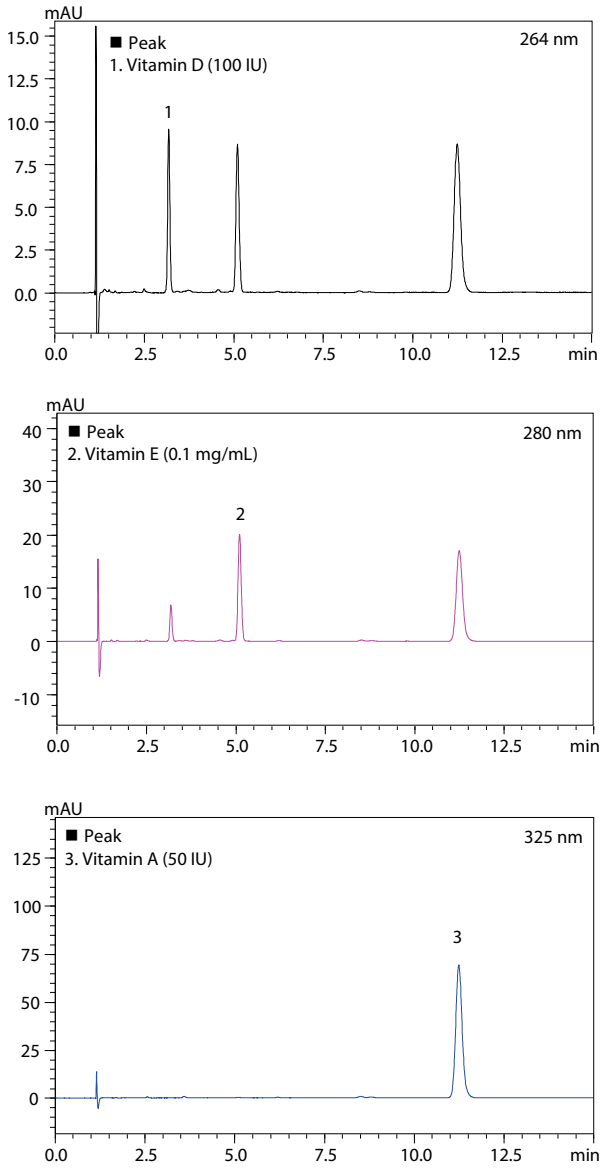

Fig. 1 Chromatograms of Standard Solution

■ Repeatability

Table 2 shows the repeatability (%RSD) of the retention time and the peak area in six repeated analyses of a standard solution of vitamins A, D and E (10 IU, 20 IU, 0.02 mg/mL). The repeatability of the retention time and the peak area was less than 0.7 % for all compounds.

Table 2 Repeatability (%RSD) in Six Repeated Analyses

Compound	Retention time	Peak area
Vitamin A	0.02	0.68
Vitamin D	0.04	0.49
Vitamin E	0.03	0.32

■ Calibration Curves

The calibration curves for the three target compounds were highly linear, with coefficients of determination (r^2) of 0.9999 or greater. Fig. 2 shows the calibration curves and Table 3 shows the concentration ranges of the calibration curves and coefficients of determination for the three target compounds.

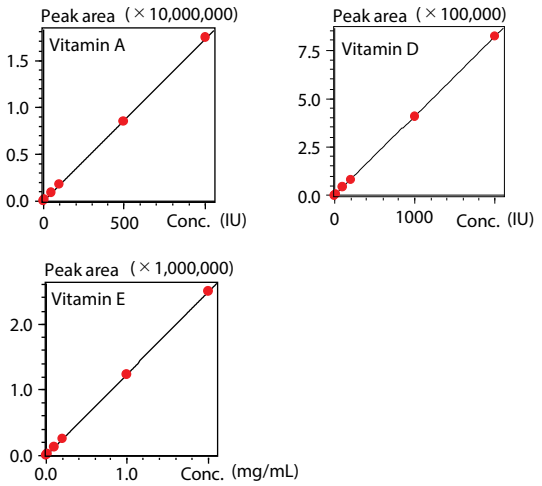

Fig. 2 Calibration Curves

Table 3 Concentration Ranges of Calibration Curves and Coefficients of Determination (r^2)

Compound	Conc. range	r^2
Vitamin A	1-1000 IU	0.9999
Vitamin D	2-2000 IU	0.9999
Vitamin E	0.002-2 mg/mL	0.9999

■ Analysis of Sample Containing Vitamins

0.25 g of the sample was dissolved in a small amount of tetrahydrofuran and then diluted with a 97:3 mixture of methanol/tetrahydrofuran (diluent) to make 10 mL using a volumetric flask. The resulting solution was diluted ten-fold with diluent before HPLC analysis.

The chromatograms of the sample are shown in Fig. 3. Target vitamins A, D, and E were separated and detected from the sample.

■ Conclusion

The combination of using an integrated HPLC system with simple analytical conditions allowed simultaneous analysis of fat-soluble vitamins (A, D, E). It is convenient to use the PDA detector built into the integrated HPLC because it can detect peaks at the optimum wavelength of each compound. Simultaneous analysis of multiple vitamins can improve the efficiency of the analysis process.

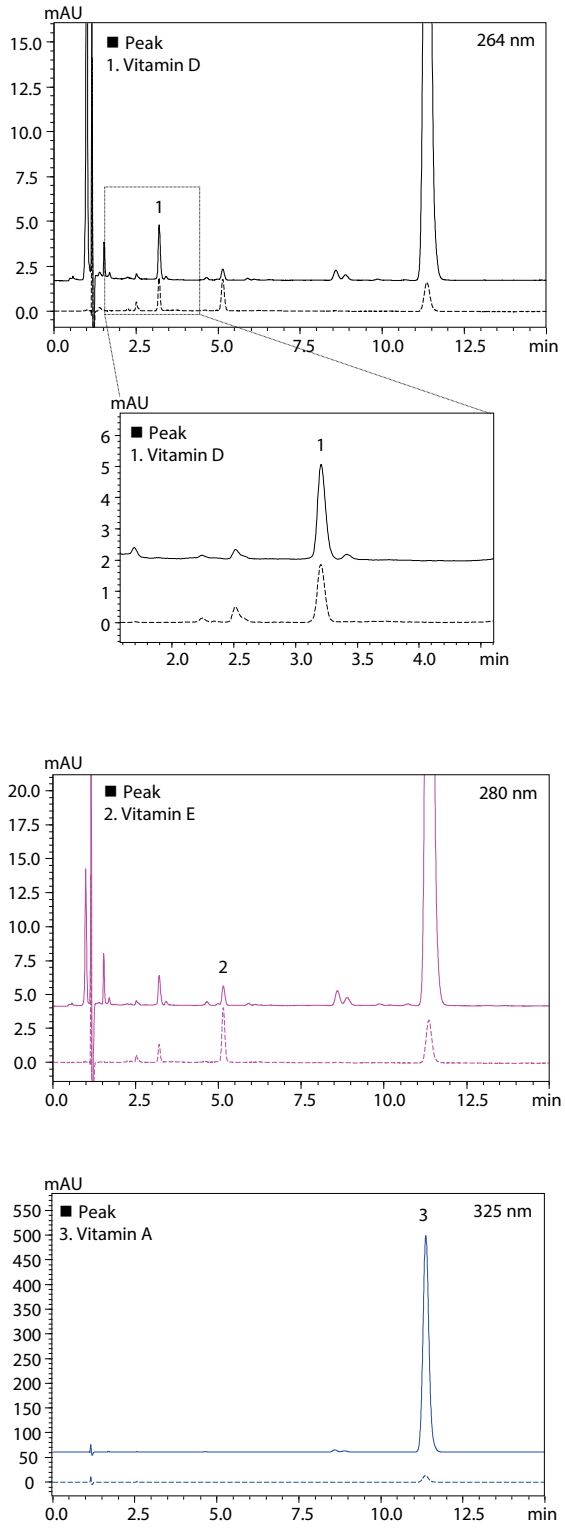

Fig. 3 Chromatograms of the Sample
(Solid Line: Sample, Broken Line: Standard Solution)

01-00413-EN First Edition: Mar. 2023

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Application News

High Performance Liquid Chromatograph LC-2050C 3D

Simultaneous Analysis of Beer Components (Xanthohumol, Humulinones, Iso-α-Acids, α-Acids, and β-Acids) Using an Integrated HPLC System

Mizuki Hayakawa

User Benefits

- Beer components, xanthohumol and isoxanthohumol are considered to provide health benefits. These two components and bitter taste-related components (humlinones, iso-α-acids, α-acids, and β-acids) can be analyzed simultaneously using LC-2050C 3D, an integrated HPLC.
- Improved reliability for beer component quantitation can be obtained using optimized analytical conditions provide reduced effect from contaminants.

■ Introduction

In recent years, beer manufacturers have been developing beer that not only tastes good but also provides health benefits. Xanthohumol is one of the prenylated flavonoids found in hops and attracting attention as being beneficial for human health because of its functions such as antioxidant, antiinflammatory, and antibacterial properties. During wort boiling, xanthohumol is isomerized to isoxanthohumol, which has been reported to have anticancer and antiviral activity. Hops also contain components related to bitterness such as humulinones, iso-α-acids and β-acids. High-speed simultaneous analysis of the six components in only eight minutes was previously reported in Application News 01-00375A.

However, to reduce column damage when connected to a i-Series LC-2050C 3D high performance liquid chromatograph, an analytical column packed with larger particles compared to those in the column used for the previous Application News was employed in this study. Simultaneous analysis beer components (xanthohumol, isoxanthohumol, humulinones, iso-α-acids, α-acids, and β-acids) using optimized analytical conditions including large-particle packed column to reduce the effects of contaminants is described in this article.

■ Analysis of Standard Solution of Xanthohumol, Isoxanthohumol, Humulinones, Iso-α-Acids, α-Acids, and β-Acids

Standard solutions were prepared using the reagents in Table 1 according to the procedure in Fig. 1. The standard solution (containing 10 mg/L xanthohumol, 10 mg/L isoxanthohumol, 20 mg/L humulinones, 20 mg/L iso-α-acids, 20 mg/L α-acids, and 12.5 mg/L β-acids) were analyzed with the conditions listed in Tables 2 and 3 to obtain the chromatograms shown in Fig. 2. Because the reagents used to prepare the standard solution contained multiple homologs (Fig. 4), multiple peaks were detected for the humulinones, iso-α-acids, α-acids, and β-acids. Those related peaks were combined into above mentioned groups to create calibration curves. Detection wavelengths were specified based on references 1), 2), 3), and 4) (Table 2).

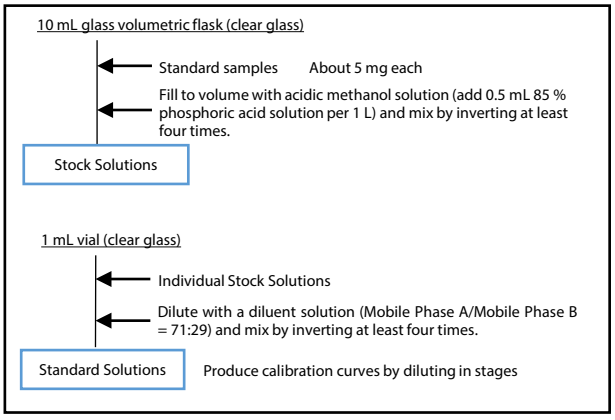


Fig. 1 Standard Solution Preparation

Table 1 Reagents for Preparing Standard Solutions

Reagent	Composition
Xanthohumol	>97.0 %
(2S)-Isoxanthohumol	99.77 %
DCHA-iso,ICS-I4	Total Iso-α-acids 65.2 % (*trans* isomer only)
International Calibration Extract 4	Cohumunone 10.98 % N+adhumulone 31.60 % Total α-acids 42.58 %
	Colupulone 13.02 % N+adlupulone 13.52 % Total β-acids 26.54 %
DCHA-Humulinones, ICS-Hum 1	Humulinones 65.6 %

- Reagent source: Xanthohumol purchased from Tokyo Chemical Industry Co., Ltd., (2S)-isoxanthohumol from MedChemexpress Co., Ltd., "DCHA-iso, ICS-I4," "International Calibration Extract 4," and "DCHA-Humulinones, ICS-Hum1" from ASBC or Labor Veritas
- ICS-I4 contains only the trans isomers.

Table 2 Analytical Conditions

System:	i-Series LC-2050C 3D
Column:	Shim-pack Velox™ Biphenyl (100 mm × 3.0 mm I.D., 2.7 µm) *1
Mobile Phase A*2:	10 mmol/L (Sodium) phosphate buffer (pH2.6) + 0.2 mmol/L EDTA・2Na aq.
Mobile Phase B:	Acetonitrile
Flow Rate:	0.7 mL/min
Column Temp.:	40 °C
Injection Vol.:	20 µL
Detection:	Xanthohumol: 370 nm Isoxanthohumol: 280 nm Iso-α-acids and Humulinones: 270 nm α-acids and β-acids: 314 nm
Vial:	SHIMADZU LabTotal™ for LC 1.5 mL, Glass*3

*1 P/N: 227-32016-03
*2 Mobile phase A: Sodium dihydrogen phosphate dihydrate 5 mmol (1.56 g) and phosphoric acid (85%, 14.7 mol/L) 5 mmol (0.68 mL) and EDTA・2Na 148 mg are dissolved in 2 L deionized water.
*3 P/N: 227-34001-01

Table 3 Time Program

Time (min)	B.conc
00.00	29
17.00	31
17.25	50
29.00	58
29.01	95
32.00	95
32.01	29
35.00	29

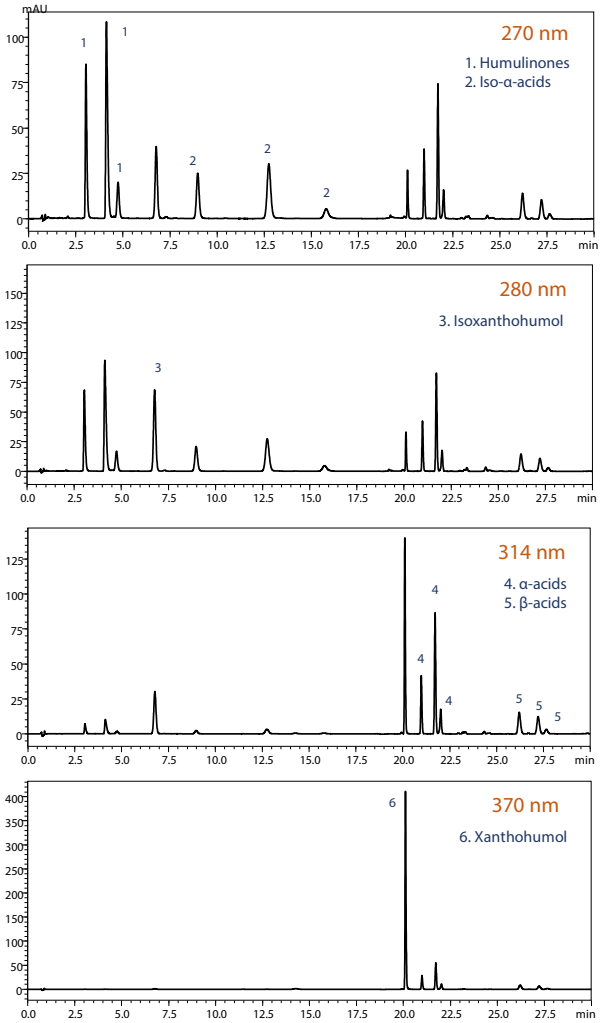
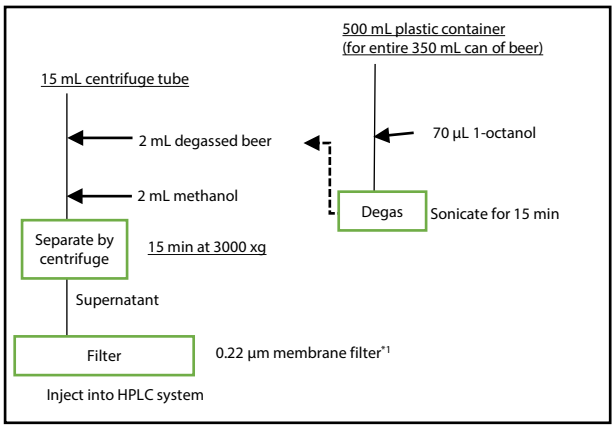


Fig. 2 Chromatograms of Standard Solutions

■ Beer Analysis

The five-level calibration curves created for six types of target components achieved the coefficients of determination over 0.999 and exhibited excellent linearity (Table 4). Three types of beer were pretreated based on the method described in Application News 01-00375A and then analyzed. The pretreatment method is shown in Fig. 3. The chromatograms from real sample analyses are shown in Fig. 5 to 7. The concentrations of the respective components in each beer are shown in Table 5. The concentrations are the totals of related *cis*- and *trans*-isomers because the peaks for presumably the *cis*-isomers of iso-α-acids were detected.

To test reproducibility, the steps after degassing were repeated six times. The relative standard deviation for peak area values of the six components from six times repeated analyses for three samples are listed in Table 6. During beer pretreatment, 1-octanol was added to samples as an antifoaming agent in order to reduce sampling errors caused by beer bubbles. Beer 1 was also used for spike and recovery testing. After the degassing step shown in Fig. 3, samples were spiked with standard solution and then the remaining steps were repeated three times. The spike and recovery test results are listed in Table 7.



*1 P/N: GLCTD-HPTFE1322

Fig. 3 Beer Pretreatment Method

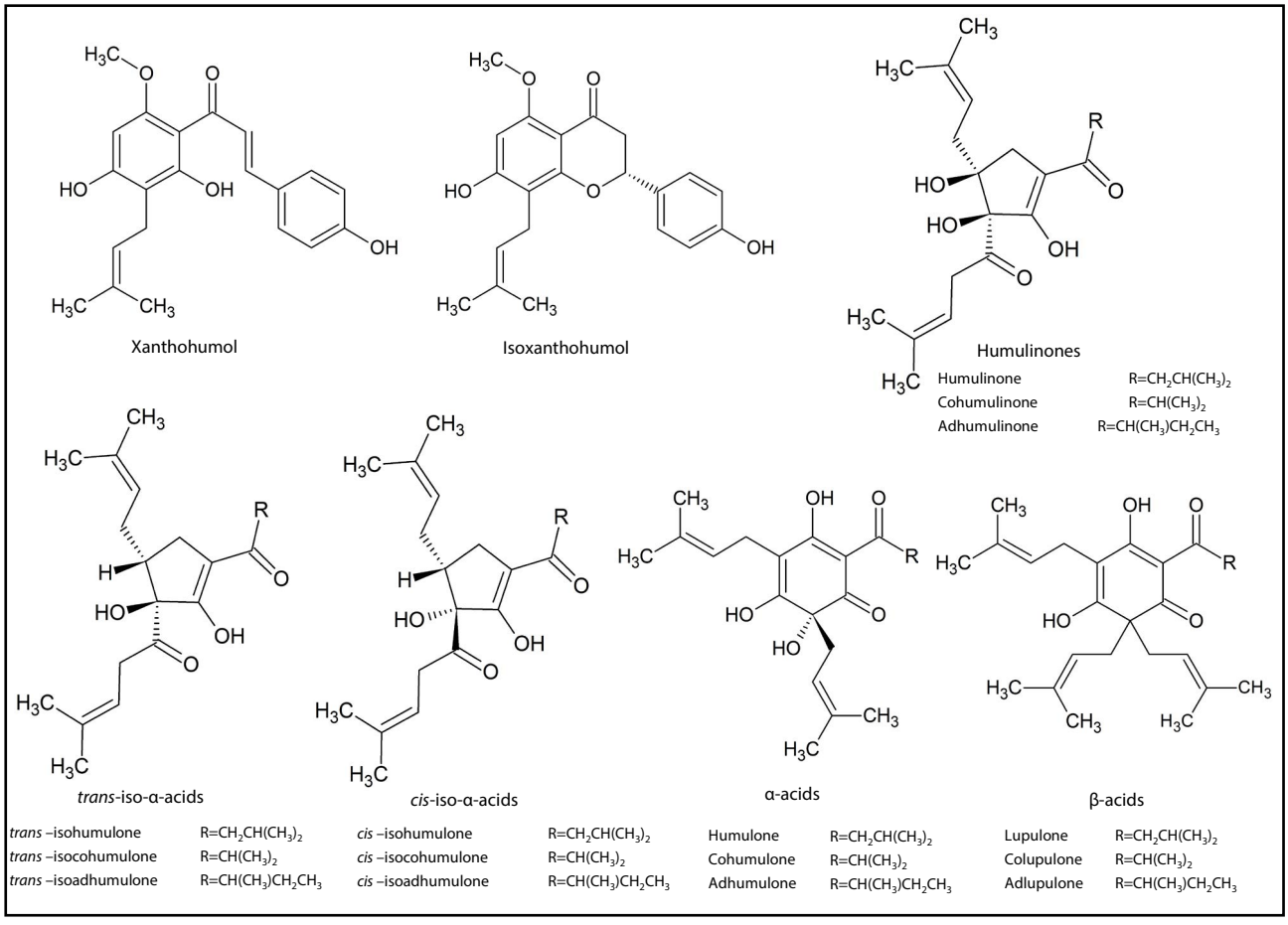


Fig. 4 Structural Formulas of Xanthohumol, Isoxanthohumol, Humulinones, Iso-α-Acid, α-Acid, and β-Acid

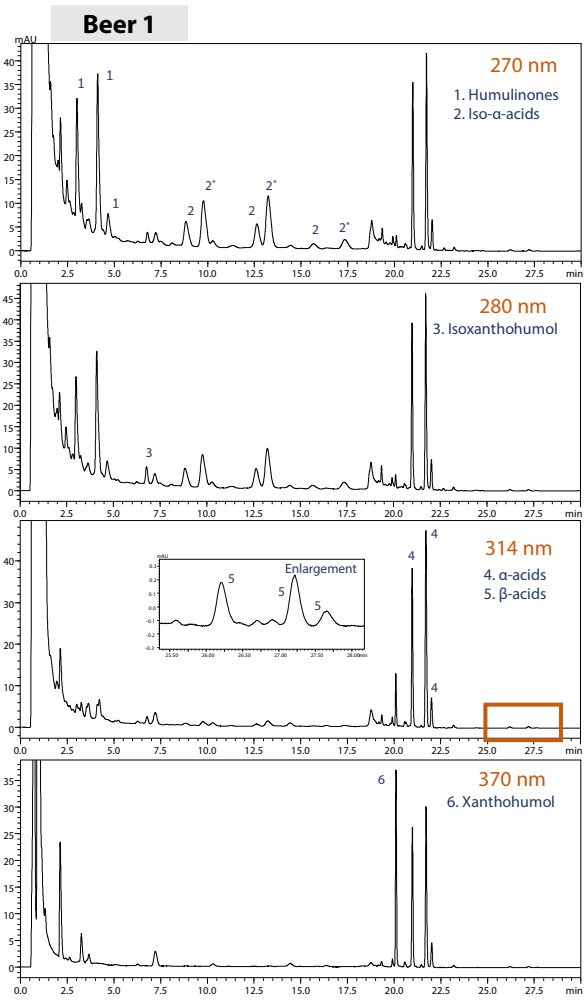


Fig. 5 Chromatograms from Beer 1

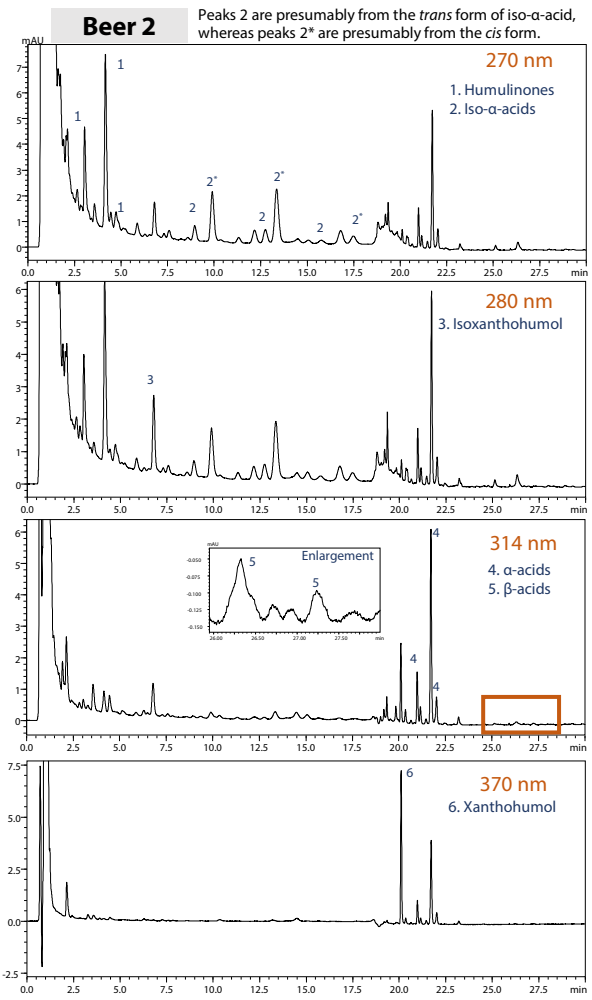


Fig. 6 Chromatograms from Beer 2

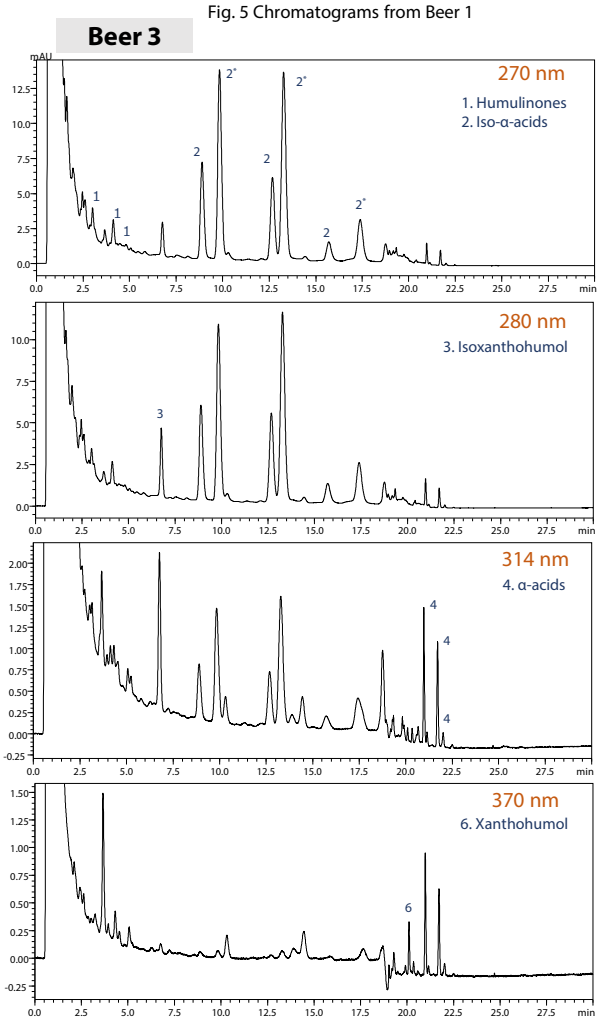


Fig. 7 Chromatograms from Beer 3

Table 4 Calibration Curve Ranges and Coefficients of determination

Compound	Conc. Range(mg/L)	r^2
Xanthohumol	0.016 to 1.000	0.9999
Isoxanthohumol	0.250 to 10.000	0.9998
Humloinones	0.250 to 10.000	1.0000
Iso-α-acids	0.500 to 20.000	0.9998
α-acids	0.500 to 20.000	0.9995
β-acids	0.039 to 1.250	0.9991

Table 5 Concentrations obtained

Unit: mg/L

Sample	Xanthohumol	Isoxanthohumol	Humulinones	Iso-α-acids	α-acids	β-acids
Beer1	1.77	1.07	14.61	29.02	25.16	2.37
Beer2	0.36	0.71	2.18	4.30	3.04	0.29
Beer3	0.01*	1.26	0.84	34.72	1.34	N.D.

* Extrapolated calibration result

Table 6 Reproducibility Test Results (%RSD, n = 6)

	Beer 1	Beer 2	Beer 3
Xanthohumol	3.31	1.33	1.73
Isoxanthohumol	2.90	0.38	1.35
Humlinones	2.72	0.84	2.18
Iso-α-acids	2.71	0.76	1.37
α-acids	3.40	0.62	2.11
β-acids	3.28	0.67	-

Table 7 Spike-Recovery Test Results
(Beer 1, average of n = 3)

Unit: %

	Recovery rate
Xanthohumol	96
Isoxanthohumol	98
Humlinones	92
Iso-α-acids	111
α-acids	97
β-acids	103

■ Summary

This article describes analyzing concentrations of xanthohumol, isoxanthohumol, humulinones, iso-α-acids, α-acids, and β-acids in beer using the i-Series LC-2050C 3D HPLC system. Simultaneous analysis was enabled by improving the separation between target components and contaminants.

Reference Information
1) European Brewery Convention, EBC ANALYTICA, 7.15
2) European Brewery Convention, EBC ANALYTICA, 9.47
3) European Brewery Convention, EBC ANALYTICA, 9.50
4) Vázquez Loureiro P et al. “Determination of Xanthohumol in Hops, Food Supplements and Beers by HPLC”, foods. 2019
5) Dieudonné Nimubona et al. “An approximate shelf life prediction of elaborated lager beer in terms of degradation of its iso-α-acids”. Journal of Food Engineering, 138-143, Nov 2012

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Technical Report

Analysis of Tea Beverages Using Catechin Analysis Kit

Takuya Yoshioka[1], Keiko Yamabe[1], Shinichi Fujisaki[1], Atsushi Ieuji[1], and Keiko Matsumoto[1]

Abstract:

The catechin analysis kit, developed in collaboration with the National Agriculture and Food Research Organization (NARO), provides validated test methods for catechins in tea leaves and green tea drinks from sample pre-treatment to HPLC measurement. You can accurately quantitate the content of 11 catechins (including methylated catechins) and caffeine in tea leaves. This article summarizes the results of the catechins quantitation in several beverages: green tea, black tea, and oolong tea.

Keywords: Catechin, Methylated Catechin, Tea Leaves, Tea Beverages, Catechin Analysis Kit, Nexera™ XR

1. Catechin Analysis Kit

NARO and Shimadzu are researching simple and accurate analytical methods for the analysis and quantitation of functional ingredients in foods and ensuring their public acceptance. The catechin analysis kit was developed in collaboration with NARO and provides validated test methods for catechins in green tea, from sample pre-treatment to measurement by HPLC. The kit includes an HPLC column, an instruction manual that describes a series of steps from sample pre-treatment to HPLC measurement, an LC method file, and a report template. It enables users to quickly start an analysis, regardless of their experience with catechin analysis. In this article, we present examples of the application of the catechin analysis kit to various tea beverages.

2. Analysis of a Standard Sample

The kit can analyze 11 catechins, including 3 methylated catechins and caffeine, for a total of 12 compounds. Fig. 1 shows the chromatogram of standard sample. All 12 compounds were separated in about 24 minutes. The analytical conditions are shown in Table 1. Calibration curves for each compound were prepared within the ranges shown in Table 2, and all the calibration curves gave good linearity with a contribution ratio (r^2) of 0.9999 or more.

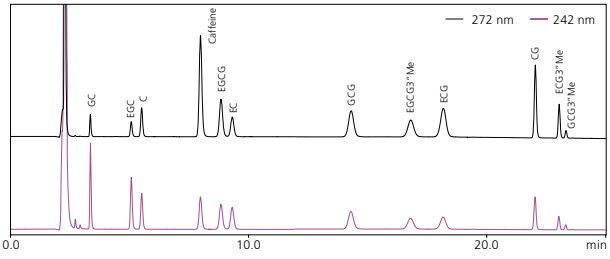

Fig. 1 Chromatogram of a Standard Sample

Table 1 Analytical Conditions

System	: Nexera XR
Column	: Shim-pack™ GISS C18 (150 mm × 4.6 mm I.D., 3 µm)
Mobile phase	: A) 0.2% Phosphoric acid in H_2O B) Methanol
Gradient	: 16%B (0-15 min) – 45%B (26 min) – 90%B (26.01-30 min) – 16%B (30.01-35 min)
Mixer	: MR180 µL II
Flow rate	: 0.85 mL/min
Column temp.	: 55 °C
Injection vol.	: 10 µL
Detection	: PDA 242 nm (GC, EGC), 272 nm (others)

Table 2 Range of Calibration Curve and its Contribution Ratio (r^2)

Compound	Range (mg/L)	Contribution Ratio (r^2)
GC	0.5-19.6	> 0.9999
EGC	0.5-19.3	> 0.9999
C	0.5-9.8	> 0.9999
EGCG	0.9-18.8	> 0.9999
EC	0.5-10.8	> 0.9999
GCG	0.9-18.1	> 0.9999
EGCG3"Me	0.4-8.3	> 0.9999
ECG	0.5-9.8	> 0.9999
CG	0.4-8.8	> 0.9999
ECG3"Me	0.2-4.2	> 0.9999
GCG3"Me	0.2-2.1	> 0.9999
Caffeine	10.0-100.2	> 0.9999

3. Sample Pre-treatment Protocol

Commercially available green, black, and oolong tea were pretreated according to the procedure in Fig. 2 and subjected to HPLC analysis. However, the catechins may be adsorbed depending on the material of the membrane or the housing of the membrane filters, so prior verification of the recovery is required to evaluate the inertness of the filters. The TORAST™ Disc * (hydrophilic PRFE) employed for the current protocol was confirmed to be unaffected and remained fully inert after conditioning the filter with 0.5 mL of the sample. An aqueous solution containing ascorbic acid and EDTA was used as the diluent to prevent sample degradation.

*P/N: GLCTD-HPTFE1345

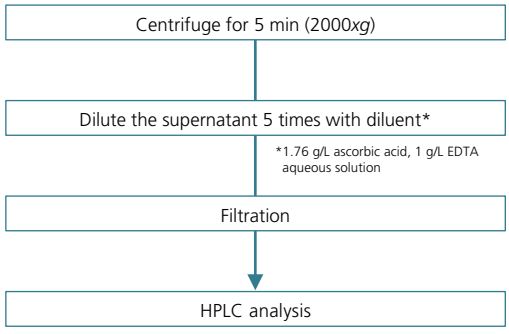

Fig. 2 Pre-treatment Protocol

[1] Analytical & Measuring Instruments Division

4. Analysis of Samples

Chromatograms of green, black, and oolong tea drinks are shown in Fig. 3, and the quantitative results are shown in Table 3. Although none of the samples contained methylated catechins, green tea contained more catechins than black and oolong teas. It is inferred that the catechins in black tea and oolong tea were converted into theaflavins or theasinensins during fermentation. In addition, non-epi-form catechins, which are rarely found in tea leaves, were detected in all tea varieties. It is thought that epi-form catechins were isomerized to non-epi-form during heat sterilization.[1] In this way, this kit enables a variety of scientific insights into catechins.

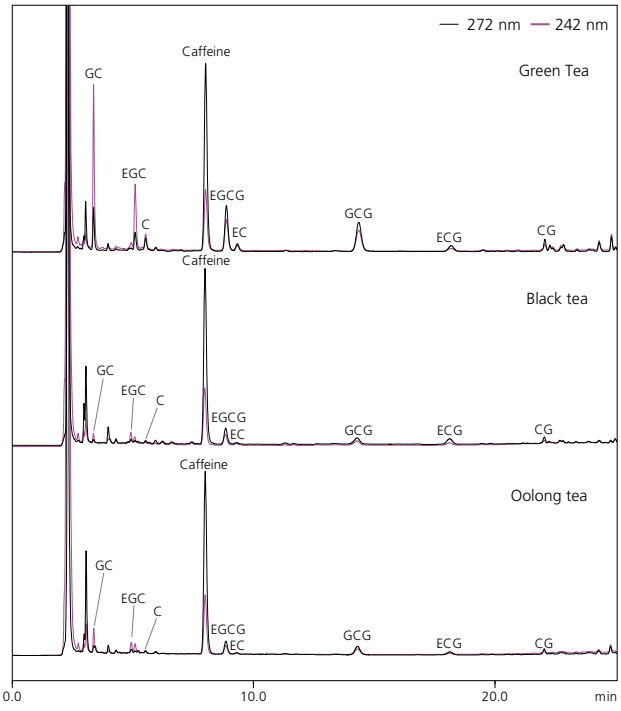

Fig. 3 Chromatograms of Various Teas

Table 3 Catechins Content of Various Teas (mg/L)

Compound	Green Tea	Black Tea	Oolong Tea
GC	87.9	5.0	15.5
EGC	40.7	6.2	9.8
C	25.8	3.5	4.1
EGCG	43.1	21.5	17.0
EC	9.5	4.9	3.2
GCG	44.5	13.2	14.8
EGCG3"Me	N.D.	N.D.	N.D.
ECG	6.6	10.4	4.5
CG	9.1	4.4	3.2
ECG3"Me	N.D.	N.D.	N.D.
GCG3"Me	N.D.	N.D.	N.D.
Total catechins	267.2	69.1	72.1
Caffeine	88.3	98.6	102.1

5. Evaluation of Repeatability and Recovery Rate

For green, black, and oolong teas, we evaluated the repeatability (n = 6) and recovery rate at 5 mg/L (ECG3 "Me and GCG" 3Me spiked 2.5 mg/L and 0.5 mg/L, respectively.). The maximum repeatability (% RSD) was 1.60 % for all samples (Table 4). The recovery rate of all samples ranged from 87.7 % to 107.2 % (Table 5). These results confirmed that this kit can be used to quantitate catechins from various tea species.

Table 4 Repeatability of Various Tea Analyses (n = 6) (% RSD)

Compound	Green Tea	Black Tea	Oolong Tea
GC	0.07	0.27	0.28
EGC	0.93	0.63	0.45
C	0.41	0.53	0.67
EGCG	0.23	0.10	0.74
EC	0.48	1.53	1.60
GCG	0.12	0.52	0.41
EGCG3"Me	N.D.	N.D.	N.D.
ECG	0.27	0.80	0.96
CG	0.26	1.09	0.32
ECG3"Me	N.D.	N.D.	N.D.
GCG3"Me	N.D.	N.D.	N.D.
Caffeine	0.19	0.05	0.05

Table 5 Recovery Rate (%) of Catechin-Spiked Samples (5 mg/L) (n=3)

Compound	Green Tea	Black Tea	Oolong Tea
GC	104.3	99.3	98.5
EGC	101.0	105.5	101.0
C	102.0	99.7	98.6
EGCG	104.2	97.3	97.2
EC	99.2	99.1	96.5
GCG	104.7	100.2	97.1
EGCG3"Me	96.3	98.0	96.2
ECG	99.8	96.8	96.1
CG	99.4	98.8	107.2
ECG3"Me (2.5 mg/L spiked)	98.1	99.5	87.7
GCG3"Me (0.5 mg/L spiked)	99.0	103.6	106.6
Caffeine	103.2	92.6	92.0

6. Summary

This article has shown examples of applying the catechin analysis kit to various tea drinks. This kit can measure catechins in not only green tea but also black and oolong teas. In addition, the method files included with this kit are pre-configured to optimize peak integration and use UV spectrum similarity to prevent misidentification, thus saving analysis time. Using this catechin analysis kit, tea drinks can be easily analyzed regardless of the user's skill level.

< References >

1) Mitsuaki Sano, Function of tea catechins and structural changes during cooking, Journal of the Japanese Society of Cooking, Vol. 40, No. 4, 223 to 230 (2007)

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Application News

High Performance Liquid Chromatograph i-Series LC-2050

Analysis of Carboxymethyl Cellulose Using an Integrated HPLC System

N. Iwata

User Benefits

- Measuring the molecular weight distribution of carboxymethyl cellulose with an eluent of sodium nitrate or sodium sulfate is possible.
- Stable analysis can be achieved with a less corrosive eluent.
- The integrated HPLC system is not dedicated to gel-filtration chromatography (GFC), but also utilized for other purposes.

■ Introduction

Carboxymethyl cellulose (CMC) is used in disintegrating agents for pharmaceuticals, food additives, cosmetics, and other products. CMC is an anionic water-soluble polymer with thickening, water-absorbing, and water-retaining features. Gel-filtration chromatography (GFC) is used to measure molecular weight distribution when evaluating the physical properties and performing product quality control of water-soluble polymers. In general, when analyzing anionic samples by GFC, early elution may occur due to chain enlargement caused by intramolecular ion repulsion, and ion repulsion with packing materials. Depending on the eluent conditions used for GFC, ion repulsion-based electrostatic interactions can make the CMC elution start earlier and disturb an accurate determination of the molecular weight distribution.

This article describes analyses of CMC using an integrated HPLC system, as well as a comparison of different eluents.

■ Comparison of Eluents

Adding salt to the eluent increases the effect of suppressing ion repulsion. However, using halide salts such as sodium chloride in the eluent at high concentrations can corrode stainless steel piping and other flow path components. In such cases, flow paths must be washed after analysis or a metal-free system must be used.

This article compared two less corrosive salts, sodium nitrate and sodium sulfate, when used in the eluent. Analysis was performed on two columns designed to handle different ranges of molecular weights that were connected in series. The sample was commercially available sodium carboxymethyl cellulose (average molecular weight: approx. 90,000). The analytical conditions are shown in Table 1. Fig. 1 shows the comparison of chromatograms for CMC standard solutions (0.1 %, prepared with each eluent). A higher peak was obtained using sodium nitrate in the eluent.

Table 1 Analytical Conditions

System	:	LC-2050C
Column	:	Shodex OHpak SB-803 HQ + SB-805 HQ (300 mm × 8.0 mm I.D. each)
Flow rate	:	1.0 mL/min
Mobile phase (1)	:	100 mmol/L sodium nitrate
Mobile phase (2)	:	100 mmol/L sodium sulfate
Column temp.	:	40 °C
Sample	:	0.1 % CMC solution
Injection volume	:	50 µL
Vial	:	SHIMADZU LabTotal™ for LC 1.5 mL, Glass*1
Detection	:	Refractive index (RID-20A) Cell temp. 40 °C

*1 P/N: 227-34001-01

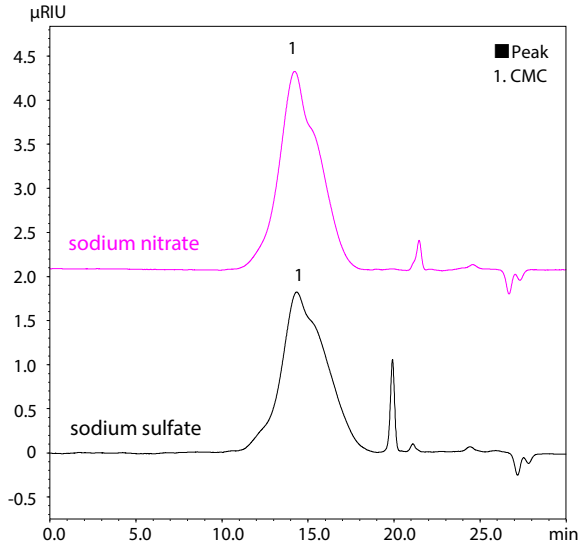


Fig. 1 Comparison of Eluents

■ Calibration Curve

Pullulan reference markers and maltooligosaccharides were used to prepare a calibration curve for molecular weights in the range 342 to 1,600,000.

Fig. 2 shows the calibration curve.

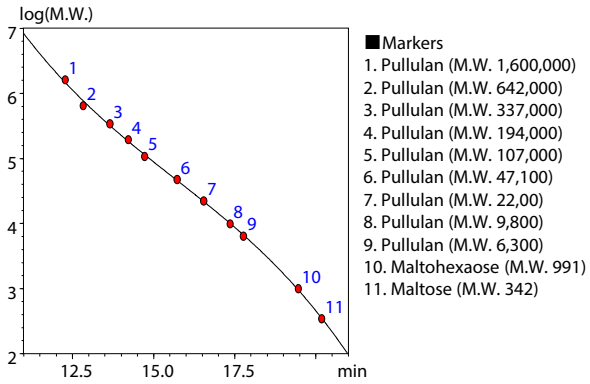


Fig. 2 Calibration Curve

■ Analysis of Carboxymethyl Cellulose

Table 2 shows the analytical conditions used to obtain the chromatogram of CMC standard solution (0.1 %, prepared with the eluent) shown in Fig. 3.

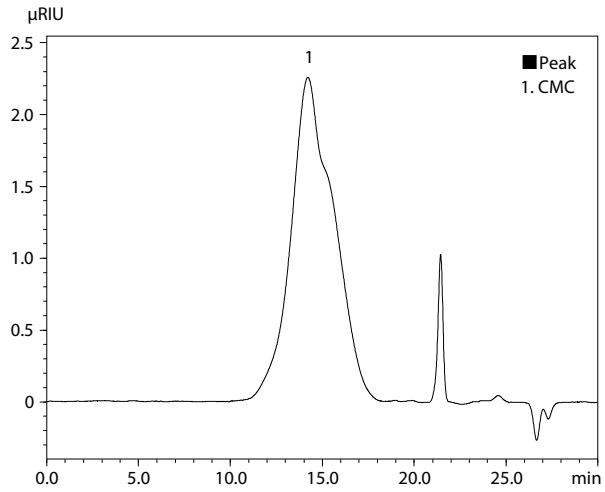


Fig. 3 Chromatogram of CMC Standard Solution (0.1 %)

Table 2 Analytical Conditions

System	:	LC-2050C
Column	:	Shodex OHpak SB-803 HQ + SB-805 HQ (300 mm × 8.0 mm I.D. each)
Flow rate	:	1.0 mL/min
Mobile phase	:	100 mmol/L sodium nitrate
Column temp.	:	40 °C
Sample	:	0.1 % CMC solution
Injection volume	:	50 μL
Vial	:	SHIMADZU LabTotal for LC 1.5 mL, Glass
Detection	:	Refractive index (RID-20A) Cell temp. 40 °C

■ Molecular Weight Distribution and Reproducibility

CMC analysis was performed six times. Table 3 shows the average for the number average molecular weight (Mn), weight average molecular weight (Mw), molecular weight distribution (polydispersity: Mw/Mn), and reproducibility (n = 6). The sample used in this article had a large distribution of molecular weights and was extrapolated on the high molecular weight side. However, good reproducibility of 0.7 % or less was still achieved for Mn, Mw, and Mw/Mn. It should be noted that these results use pullulan-converted molecular weights.

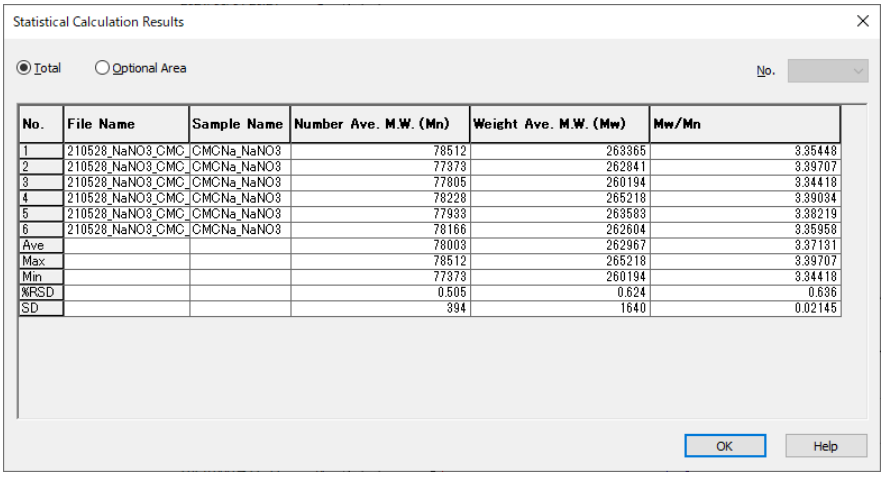
We also offer LabSolutions™ GPC workstation software as an option, which lets the user verify the statistical results of repeated analyses at a glance (Fig. 4).

Table 3 GPC Calculation Results (n = 6)

	Mn	Mw	Mw/Mn
CMC	78,000	263,000	3.37
%RSD	0.51	0.62	0.64

■ Conclusion

The molecular weight distribution of CMC can be measured by the integrated HPLC system coupled with a refractive index detector. Stable analysis can be achieved by using sodium nitrate and other salts that are less corrosive to stainless steel. A single integrated HPLC system can be used for this and other types of analysis, because piping and other flow path components do not need to be replaced.



No.	File Name	Sample Name	Number Ave. M.W. (Mn)	Weight Ave. M.W. (Mw)	Mw/Mn
1	210528_NaNO3_CMC_	CMCNa_NaNO3	78512	263365	3.35448
2	210528_NaNO3_CMC_	CMCNa_NaNO3	77373	262841	3.39707
3	210528_NaNO3_CMC_	CMCNa_NaNO3	77805	260194	3.34418
4	210528_NaNO3_CMC_	CMCNa_NaNO3	78228	265218	3.39034
5	210528_NaNO3_CMC_	CMCNa_NaNO3	77933	263583	3.38219
6	210528_NaNO3_CMC_	CMCNa_NaNO3	78166	262604	3.35958
Ave			78003	262967	3.37131
Max			78512	265218	3.39707
Min			77373	260194	3.34418
%RSD			0.505	0.624	0.636
SD			394	1640	0.02145

Fig. 4 Window Showing the Statistical Results

01-00207-EN First Edition: Sep. 2021

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i-Series LC-2050C 3D, High Performance Liquid Chromatograph

Application News

Simultaneous Analysis of Dipotassium Glycyrrhizinate and Tranexamic Acid

Miho Akagi, Hiromasa Iboshi

User Benefits

- Simultaneous analysis of dipotassium glycyrrhizate and tranexamic acid in cosmetics can be performed with simple pretreatment.
- Photodiode array (PDA) detector provides reliable identification performance for even heavily contaminated samples using UV-visible absorption spectral information.

■ Introduction

Glycyrrhizic acid is a medicinal compound found in Chinese crude medicines such as licorice and is generally known to have pharmacological effects such as anti-allergy, anti-inflammation, and detoxification. Dipotassium glycyrrhizate (GK2), a salt from glycyrrhizic acid is widely used for products such as cosmetics, shampoos, and toothpastes, in addition to over-the-counter drugs. Tranexamic acid (TA), an artificially synthesized amino acid, is generally known for its anti-inflammatory, hemostatic, and other pharmacological effects. Additionally, it is also used in cosmetics as an active whitening agent.

In general, it is difficult to retain TA on C18 columns due to its high polarity, and it often show tailing peak shape due to its basicity. On the other hand, GK2 is a compound that is relatively well retained on C18 columns. Therefore, this article introduces of TA and GK2 in cosmetics employing sodium perchlorate as a component of mobile phase for simultaneous analysis of these two compounds.

■ Analysis of Mixed Standard Solution

Fig. 1 shows the chromatogram of a mixed standard solution of GK2 and TA (GK2: 200 mg/L, TA: 4,000 mg/L, prepared in ultrapure water). Table 1 shows its analytical conditions. TA was sufficiently retained with suppressed peak tailing by adding sodium perchlorate to the mobile phase. Sodium perchlorate provides rapid stabilization of baseline condition compared to typical ion-pair reagents such as sodium alkyl sulfonate, and can be used with the mobile phase containing large portion of organic solvent.

Fig. 2 shows the UV spectra obtained by analyzing standard solution. In addition to retention time, UV spectrum obtained with PDA detector is effective for the peak identification especially in the analysis of complicated sample such as cosmetics that have many unknown compounds.

Table 1 Analytical Conditions

System	:	i-Series LC-2050C 3D
Column	:	Shim-pack™ VP-ODS, 5 µm*1 (150 mm×4.6 mm I.D., 5 µm)
Flow rate	:	1.0 mL/min
Mobile phase	:	A) 100 mM $NaClO_4$ in 10 mmol/L (Sodium) phosphate buffer (pH 2.6) B) 100 mM $NaClO_4$ in acetonitrile
Time Program	:	2%B (0 min)→90%B (9.00 -12.00 min) →2%B (12.01-15.00 min)
Mixer	:	40 µL
Column temp.	:	40 ℃
Injection volume	:	5 µL
Vial	:	SHIMADZU LabTotal™ for LC 1.5 mL, Glass*2
Detection (PDA)	:	250 nm (GK2), 220 nm (TA)

*1 P/N: 228-34937-91
*2 P/N: 227-34001-01

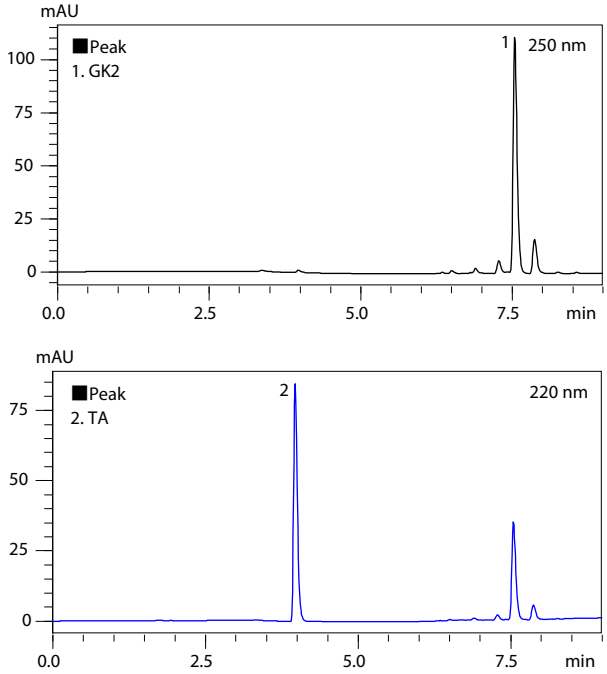

Fig. 1 Chromatograms of mixed standard solution

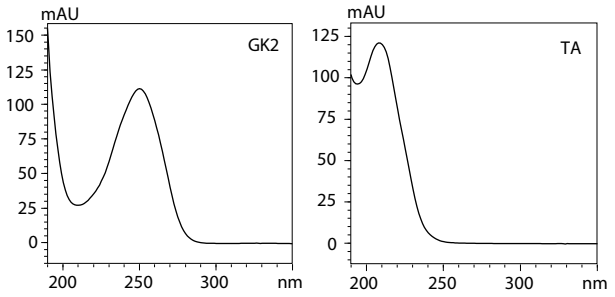

Fig. 2 UV spectra of separated standard compounds

■ Repeatability

The repeatabilities of retention times and peak areas for the two compounds of interest were confirmed by five times repeated analyses. Table 2 and Table 3 show the repeatabilities of retention times and peak areas in terms of relative standard deviations (%RSD) respectively. Good repeatabilities were obtained for both.

Table 2 Evaluation of retention time repeatability (n=5)

Compound	Average of retention time (min)	%RSD
GK2	7.5	0.03
TA	4.0	0.06

Table 3 Evaluation of peak area repeatability (n=5)

Compound	Average of peak area	%RSD
GK2	509,962	0.3
TA	350,576	0.1

■ Calibration Curve

Fig. 3 shows the calibration curves for the two compounds of interest. Good linearities were obtained for both with the coefficients of determination of $r^2 = 0.9999$ or higher.

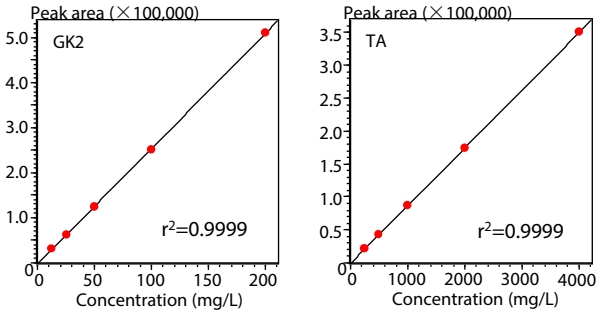

Fig. 3 Calibration curves of GK2 and TA

■ Analysis of Cosmetics

One mL of commercially available lotion was diluted ten times with ultrapure water, filtered through 0.45 µm membrane filter, then subjected to HPLC (Fig. 4).

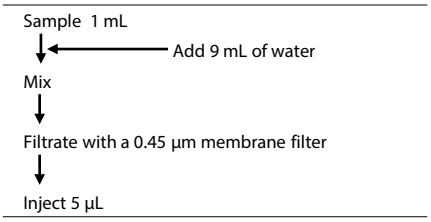

Fig. 4 Pretreatment procedures for lotion

Fig. 5 shows the chromatogram of the lotion sample. Fig. 6 shows UV- spectra at the retention times of GK2 and TA. Table 4 shows the similarities when compared to the UV- spectra of the standard compounds. Comparing the retention times and the related UV spectra simultaneously, it was confirmed that the respective peaks are GK2 and TA, and that the unknown contaminations were also separated.

Table 4 Obtained results

Compound	Similarity
GK2	0.97
TA	0.99

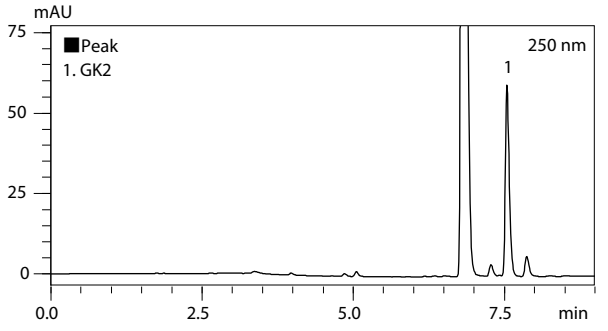

Fig. 5 Chromatograms of lotion

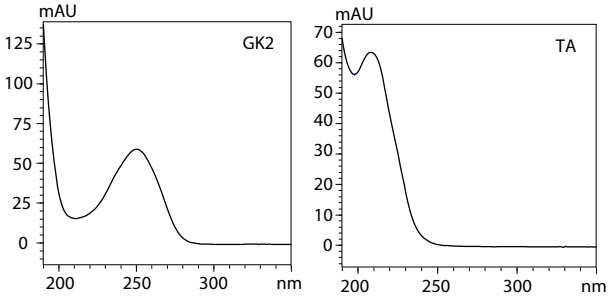

Fig. 6 UV spectra of GK2 and TA in lotion

The obtained concentrations of GK2 and TA in the lotion after pretreatment and their recovery rates are shown in Table 5. The recovery rates were calculated through three times repeated analyses of the lotion sample added with GK2 and TA to make the increases of 100 mg/L and 2,000 mg/L in concentration respectively.

Table 5 Obtained results

Compound	Average of concentration (mg/L)	%RSD	Average of recovery (%)
GK2	106.8	0.6	98.1
TA	1,970	0.5	97.7

■ Conclusion

Simultaneous analysis of GK2 and TA, the active ingredients in cosmetics, was successfully performed. By adding sodium perchlorate to the mobile phase, TA was able to be retained on the C18 column, resulting in simultaneous analysis of these two compounds. In addition to the retention times, the UV spectra from PDA detector enabled reliable identifications of the target compounds in cosmetics.

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Application News

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

Method Scouting for Simultaneous Analysis of Twelve Tar Dyes

Yiting Zhou

User Benefits

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

■ Introduction

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

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

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

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

■ Scouting for Analytical Conditions of Simultaneous Analysis

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

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

Table 1 Analytical Conditions

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

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

Fig. 1 Appearance of i-Series Method Scouting System

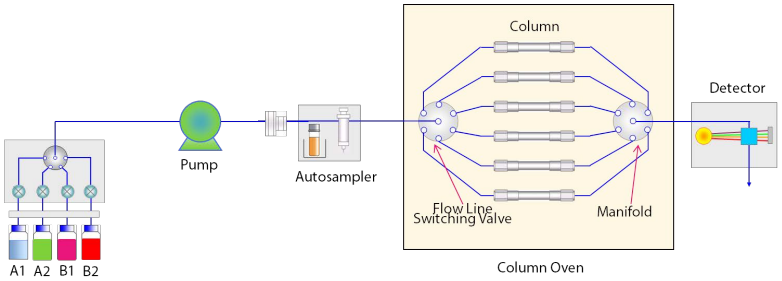

Fig. 2 Flow Path Diagram of i-Series Method Scouting System

Table 2 Compound Names of Twelve Tar Dyes

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

■ Settings of Method Scouting Solution

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

■ Result of Method Scouting

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

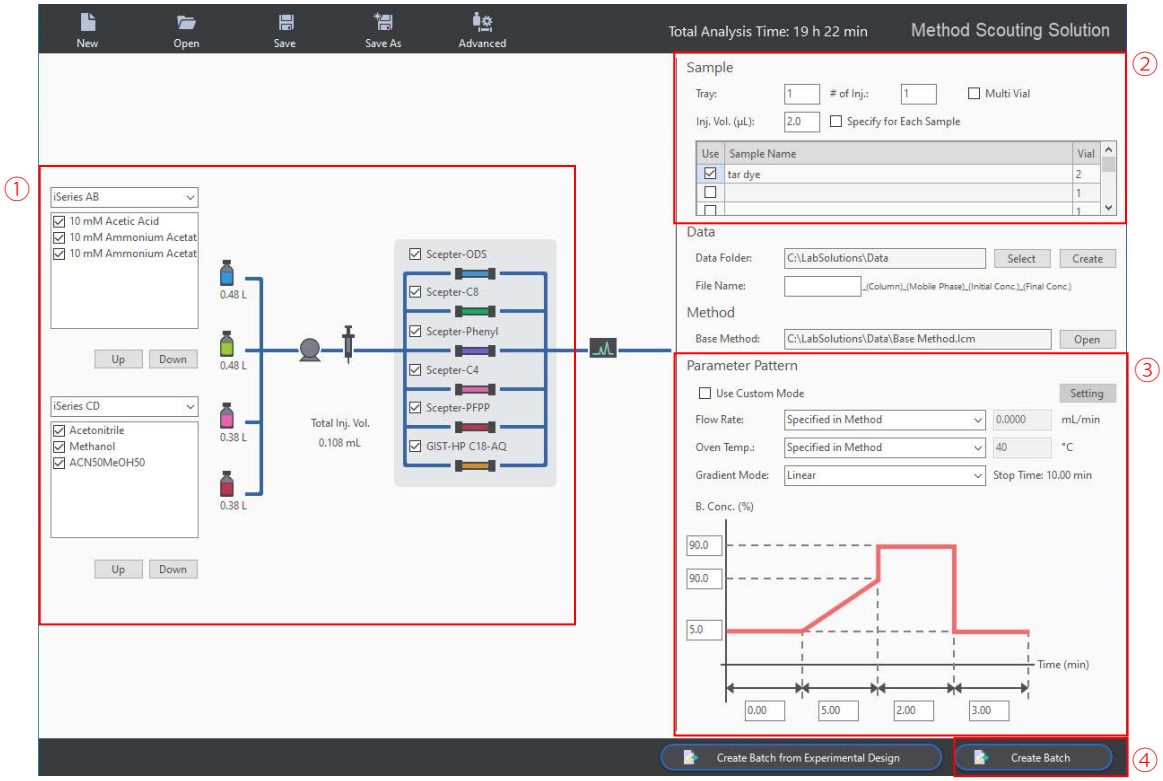

Fig. 3 Main Window of Method Scouting Solution

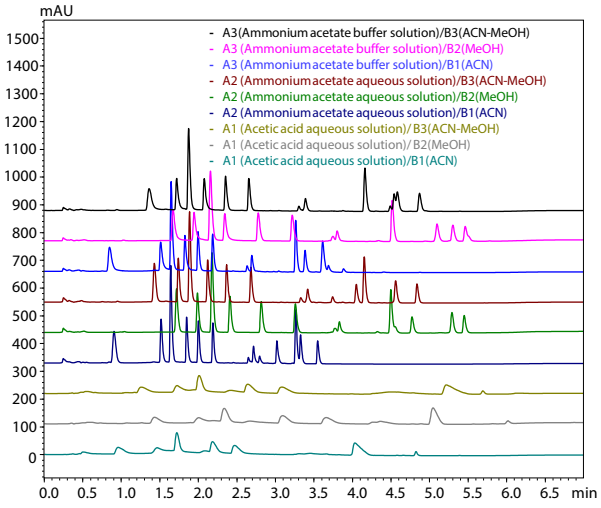

Fig. 4 Chromatograms of Shim-pack Scepter C18-120 (1)

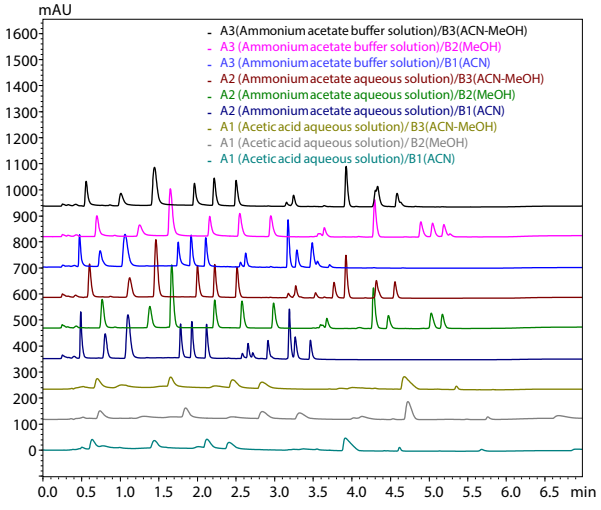

Fig. 5 Chromatograms of Shim-pack Scepter C8-120 (2)

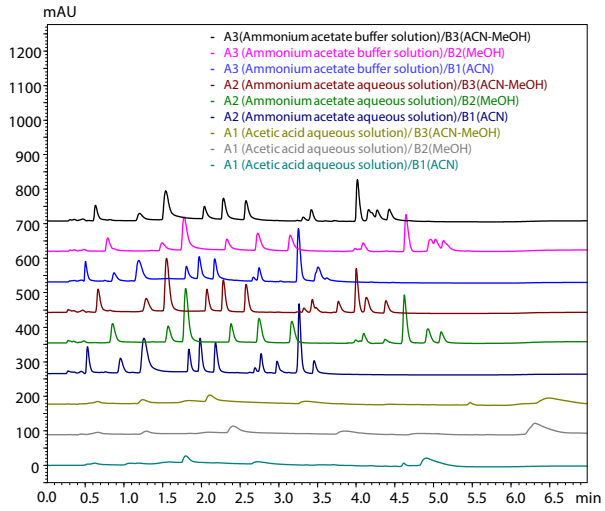

Fig. 6 Chromatograms of Shim-pack Scepter Phenyl-120 (3)

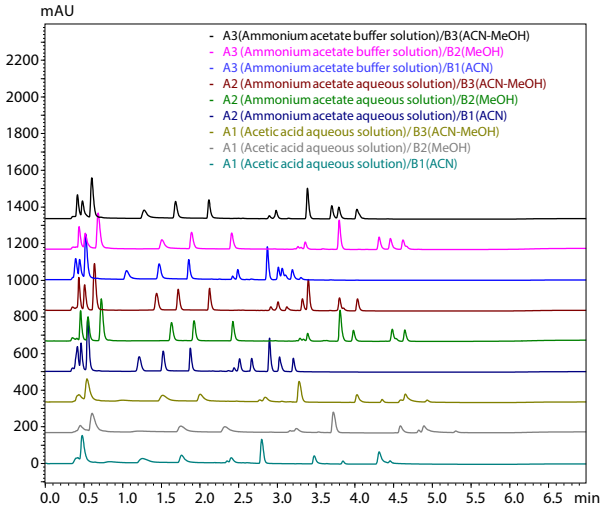

Fig. 7 Chromatograms of Shim-pack Scepter C4-300 (4)

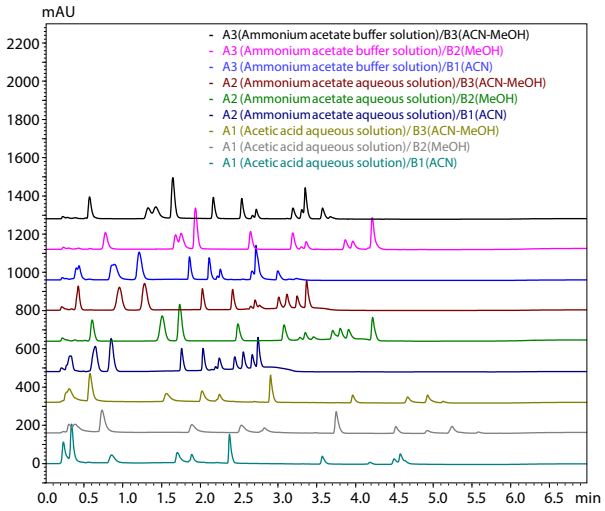

Fig. 8 Chromatograms of Shim-pack Scepter PFPP-120 (5)

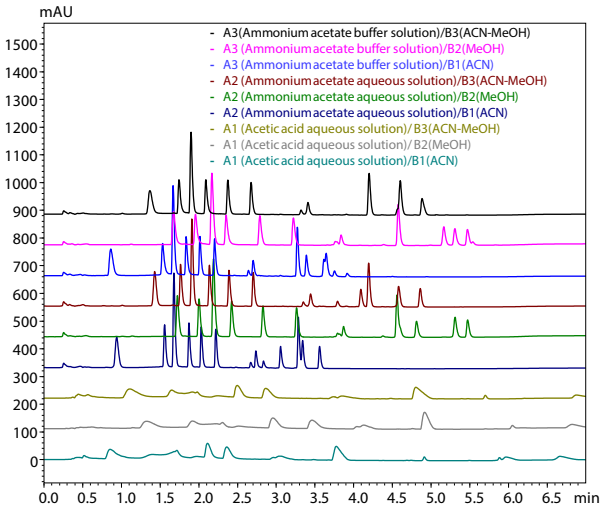

Fig. 9 Chromatograms of Shim-pack GIST C18-AQ HP (6)

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

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

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

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

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

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

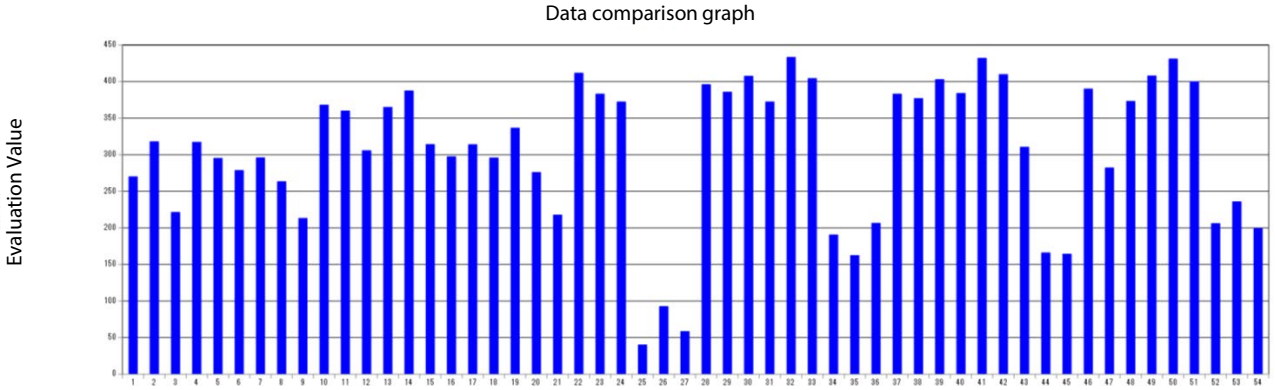

Fig. 10 Result of Evaluation Values obtained using Multi-data Report

Evaluation Report

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

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

■ Optimal Conditions for Simultaneous Analysis of Twelve Tar Dyes

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

Table 3 Analytical Conditions with Highest Evaluation Value

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

■ Conclusion

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

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

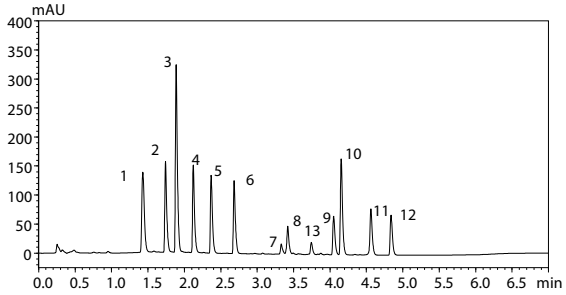

Fig. 12 Chromatogram Obtained with the Optimal Analytical Conditions (50 mg/L each)
See Table 4 for the peak numbers. 13: Impurity of R3 (Erythrosine)

Table 4 Peak Parameters Obtained with Optimal Analytical Conditions

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

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Functional Food Analysis / i-series LC-2050C

Application News

Determination of Functional Component in Agricultural Product: β-cryptoxanthin in Mandarin Orange by HPLC Method

Zhe Sun, Zhaoqi Zhan

User Benefits

- Quantitative determination of functional component of β-cryptoxanthin (BCR) in fresh mandarin orange samples using an i-series LC-2050C with a high sensitivity UV detector and an excellent ODS column for separation.
- High throughput, accurate and reliable method to measure the functional nutrient in fresh fruits.

■ Introduction

Satsuma Mandarin is listed as a functional food in Japan, which can be recognized by Functional Food Claims (FFC), a new regulatory system of health claim introduced in Japan in 2015. The Japanese Agricultural Standards (JAS) are established to support the FFC new functional food system [1]. β-cryptoxanthin can be found in fruits such as mandarin orange, papaya, and mango. β-cryptoxanthin has several beneficial effects on human health, such as acting as an antioxidant on scavenging free radicals from our body for preventing cancer, being a precursor to vitamin A on improving human health vision, reducing the signs of ageing, etc. In this application news, a HPLC method associated with the JAS 0003 reference method [2] is described for quantitation of BCR in fresh mandarin orange. The sample preparation and HPLC analysis were performed in accordance with the JAS method 0003.

■ Experimental

Reagents and standard

β-cryptoxanthin standard, reagents, chemicals, and apparatus used in sample preparation were prepared in reference to JAS 0003 monograph [2]. The high purity standard BCR was purchased from a local chemical supplier. Reagents and chemicals such as pyrogallol, ascorbyl palmitate, potassium hydroxide (KOH), sodium chloride and sodium sulfate are analytical grade. Solvents including n-hexane, ethyl acetate, petroleum ether, ethanol, methanol, chloroform and 2-propanol are HPLC grade.

HPLC analytical conditions

The analysis was carried out using Shimadzu i-series LC-2050C system. LabSolutions workstation was used for data acquisition and data analysis. The system configuration, HPLC column and detailed parameters are compiled into Table 1.

Table 1. Analytical conditions of β-cryptoxanthin by HPLC

Column	Shim-pack™ GIS-HP C18, 150 × 3.0 mm, 3 µm
Flow rate	1.0 mL/min
Mobile phase	methanol-chloroform mixture (96/4, v/v) with 0.12 mM ascorbyl palmitate
Elution mode	Isocratic elution
Oven Temp.	40°C
Detection	455 nm
Injection volume	10 µL

■ Results and Discussion

Sample preparation

Fresh mandarin orange samples were obtained from the local market for this testing. The samples were stored under cold conditions kept at 5°C. Prepare all mandarin samples in 1 day to avoid the change in BCR concentration. Perform steps 2 to 5 on each sample first. One whole mandarin is for 1 analysis.

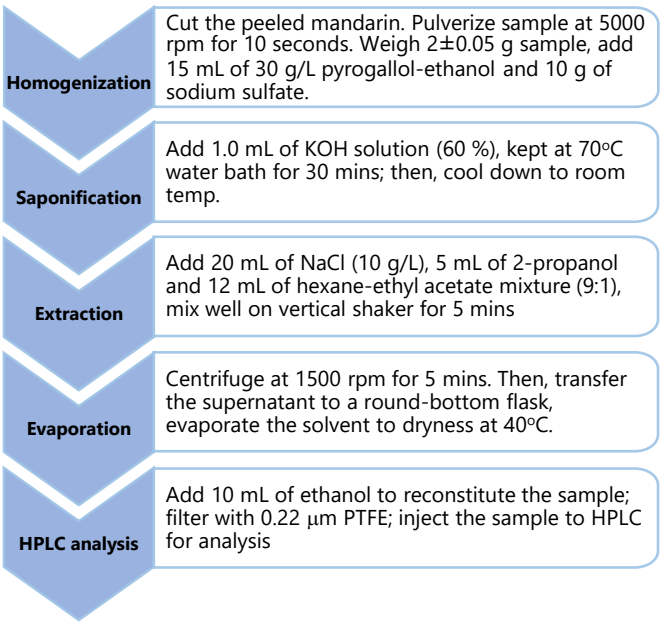

Figure 1. Sample preparation procedure for BCR analysis in mandarin orange by HPLC (JAS 0003 monograph).

The JAS 0003 monograph describes in details the procedure of sample preparation as outlined in Figure 1, which includes four steps before the sample was injected to LC-2050C for analysis.

Calibration curve

Figure 2 shows the HPLC result of the BCR standard (level L1). The retention time of BCR was 3.31 min. A linear calibration curve was set up using a calibration series from L1 to L4 (0.25, 0.5, 1 and 2 µg/mL) of BCR dissolved in ethanol from a BCR stock solution of 10 µg/mL. The concentration of BCR of the stock solution prepared or received must be measured by UV-VIS absorbance measurement to obtain the accurate value (see section 4.23.2 [2]). After this correction, the actual concentrations of L1~L4 calibration series obtained were 0.247~1.98 µg/mL. The correlation coefficient of the linear calibration curve for the above range was 0.999, which met the requirement (r > 0.995) stated in JAS 0003.

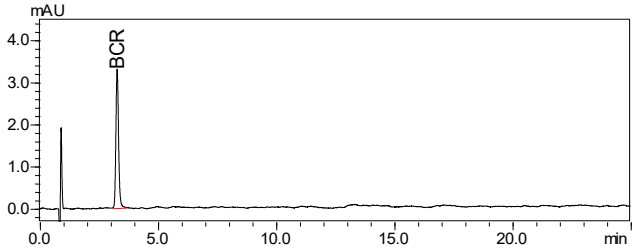

Figure 2. Chromatogram of BCR standard of 0.25 µg /mL in neat solvent.

Quantitation of β-cryptoxanthin in mandarin orange

The contents of BCR (mg/kg) in the mandarin orange samples were determined by the established HPLC method. Two mandarin samples were prepared and measured. The results of BCR content in mandarin can be calculated from the concentrations of BCR measured using a formula as shown below. The average results of BCR in the mandarin orange samples after calculation was ~ 12.3 mg/kg, which fell in the BCR mean content range shown in the reference data (see Table A.1, in the Annex A).

$$W(\mathrm{mg/kg}) = \frac{C \times V \times d1}{M \times d2}$$

Where

W = Amount of BCR in mandarin samples (mg/kg)
C = Concentration of BCR in the samples (µg/mL)
V = Volume of volumetric flask used (5 mL)
M = Weight of testing samples (g)
d1 = Constant volume at time of extraction (50 mL)
d2 = Saponification fraction volume (10 mL)

Identification

Identification of BCR peak in extract samples (Figure 3) relies on matching the retention time (RT) with BCR standard. The RTs of BCR in the samples matched perfectly (shift less than 0.1 min) with standard without obvious interference.

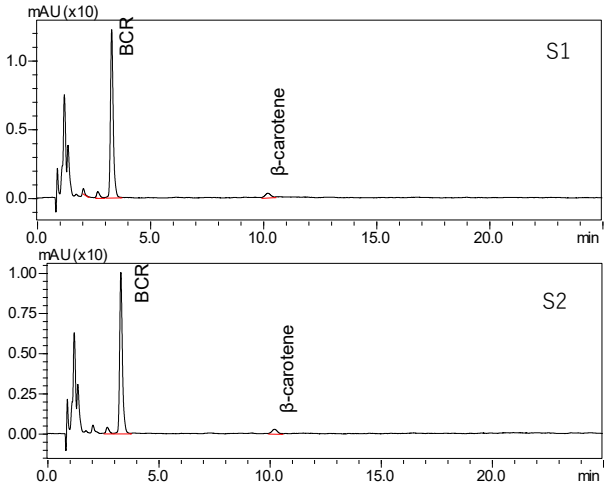

Figure 3. Representative chromatogram profiles of BCR mandarin extracts, S1 (top) and S2 (bottom).

Precision

Interlaboratory tests are required essentially to verify the precision of analysis results. Details of the interlaboratory tests are described in Annex A in JAS 0003 monograph. Repeatability limit ($r = 2.8 \times S_r$) and reproducibility limit ($R = 2.8 \times S_R$) were used to evaluate the precision of the analyses in laboratories intended to provide testing of BCR in mandarin orange following JAS method. The procedure shown in this application news was considered as a practice of interlaboratory tests. The results (data not shown) were compared with that obtained by an accredited laboratory to verify the precision.

■ Conclusion

This work demonstrates the procedure and quantitation results of β-cryptoxanthin in fresh mandarin orange analyzed by a HPLC method associated with JAS0003 monograph. The process from sample preparation to HPLC analysis was performed as a practice for interlaboratory tests as described in Annex A of JAS0003.

■ References

1. Development of functional agricultural products and use of a new health claim system in Japan, Trends in Food Science & Technology (69) 2017, 324-332.
2. Japanese Agricultural Standards Method JAS 0003: 2019

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Technical Report

Simple Method Transfer using i-Series (LC-2050/LC-2060)

Daiki Fujimura[1], Keiko Matsumoto[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. Therefore, as a solution for such compatibility problems, Shimadzu offers new i-Series integrated LC systems (LC-2050/LC-2060) that are compatible with a wide variety of systems and system volumes.

This Technical Report describes an example of using an i-Series system to transfer a method by correcting for the system gradient delay volume. It also describes an actual example of using the analytical condition transfer and optimization (ACTO) function to seamlessly transfer a method.

Keywords: 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 time, 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.

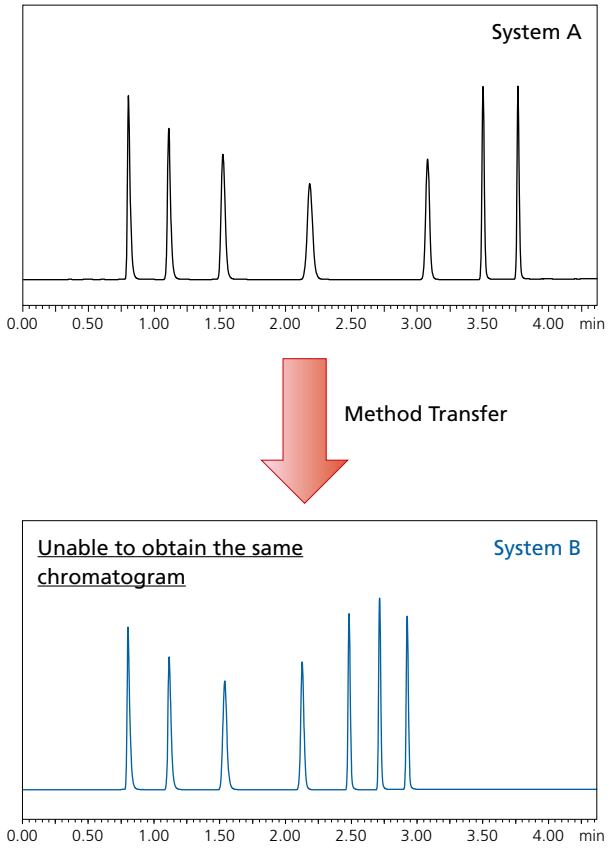

Fig. 1 Problems in Method Transfer

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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. Fig. 3 shows how the gradient in a system with a larger system volume (lower chromatogram) starts later than in a system with a smaller system volume (upper chromatogram). 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.

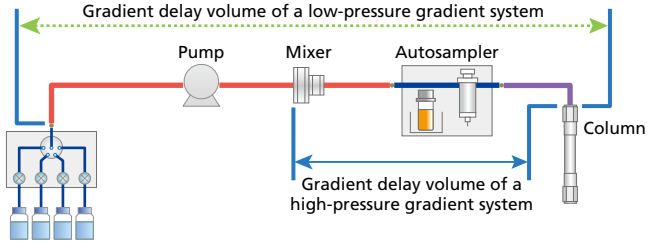


Fig. 2 Gradient Delay Volumes (System Volumes)

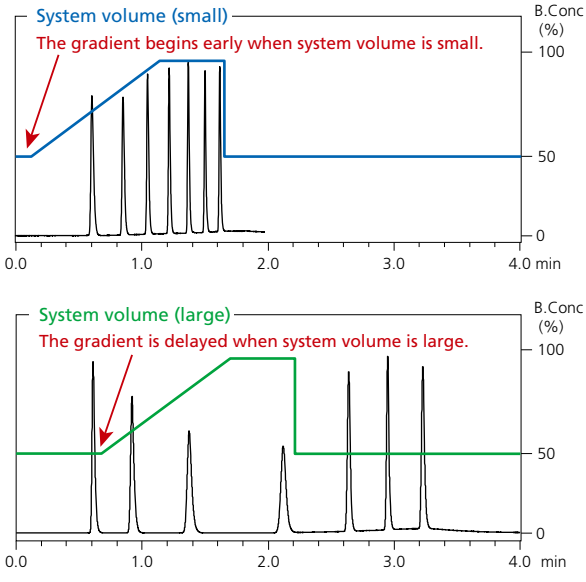


Fig. 3 Gradient Delay Volumes (System Volumes)

3. Correction of Gradient Delay

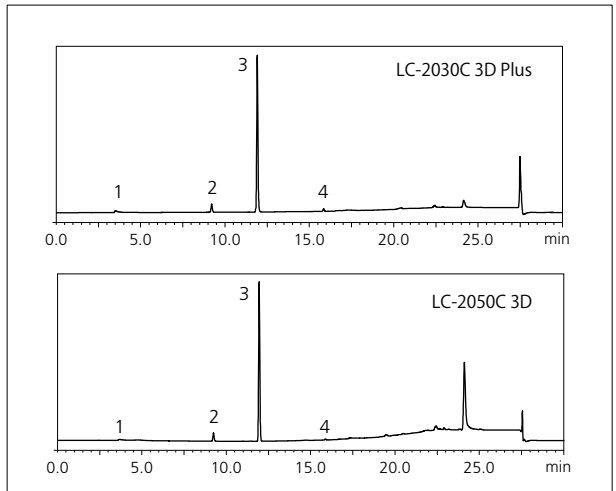
We have discussed differences in chromatograms caused by method transfer and the origin of these differences. Next, we describe an example analysis and method transfer using multiple LC systems that have different system volumes.

3-1. Example of Method Transfer with Corrected System Delay Volume

In this example, a Shimadzu LC-2050 integrated LC system was used to match the delay volume to a variety of systems and then compare chromatograms from performing the identical analysis with both systems.

First, chromatograms were compared with a previous Shimadzu LC-2030 Plus model. The results are shown in Fig. 4. That comparison confirms that given the same analytical conditions, the LC-2030 Plus, which was designed to have an equivalent system volume as the LC-2050, produces an identical chromatogram. Next, chromatograms were compared with an other vendors' LC system and a first-generation Shimadzu LC-2010C HT integrated LC system. The results are shown in Fig. 5. In this example, the system volumes of the other vendors' LC system (System A) and the LC-2050 were equivalent, so given identical analytical conditions, they produced equivalent results. However, the LC-2010C HT system has a larger system volume than the LC-2050, so a delay volume compatibility kit was added to the LC-2050 to match the system volume of both systems. That resulted in almost the same chromatogram pattern, just as in the previous case.

Thus, when transferring methods, it is important to match the system volumes so that the gradient starts at the same time.



■ Peaks
1. Salicylic acid, 2. Salicylamide, 3. Ethenzamide, 4. Methyl salicylate

■ Analytical Conditions

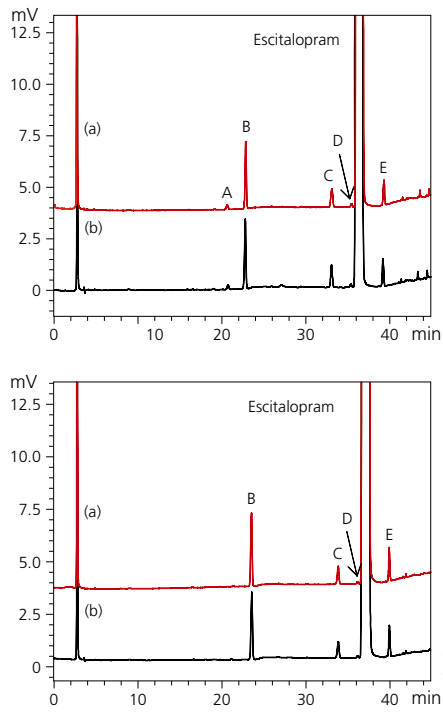
Column : Shim-pack™ GIST (150 mm L. × 4.6 mm I.D., 5 µm)

Mobile phase A	: Water
Mobile phase B	: Methanol
Initial concentration	: A/B = 90/10
Flow rate	: 1.0 mL/min
Temp.	: 40 °C
Detector	: Photodiode array absorbance detector
Injection volume	: 5 µL

Time program:

Time	Unit	Func	Value
2.00	Pump	B.Conc	10
20.0	Pump	B.Conc	95
25.0	Pump	B.Conc	95
25.1	Pump	B.Conc	10
30.0	Controller		Stop

Fig. 4 Example of Method Transfer between LC-2030 Plus and LC-2050 Systems



■ Analytical Conditions

Column	: Shim-pack VP-ODS (250 mm L. × 4.6 mm, 5 µm)
Mobile phase A	: Acetonitrile / 25 mM phosphate buffer (pH 3.0) = 1/9 (v/v)
Mobile phase B	: Acetonitrile / 25 mM phosphate buffer (pH 3.0) = 13/7 (v/v)
Initial concentration	: A/B = 95/5
Flow rate	: 1.0 mL/min (0–45 min), 2.0 mL/min (45–60 min)
Temp.	: 45 °C
Detection	: UV 237 nm
Injection volume	: 20 µL

Time program:

Time	Unit	Func	Value
0.00	Pump	B.Conc	5
35.00	Pump	B.Conc	35
45.00	Pump	B.Conc	100
60.00	Pump	B.Conc	100
60.01	Pump	B.Conc	5
68.00	Controller	Stop	

Fig. 5 Example of Method Transfer by Correction of Delay Volume of System

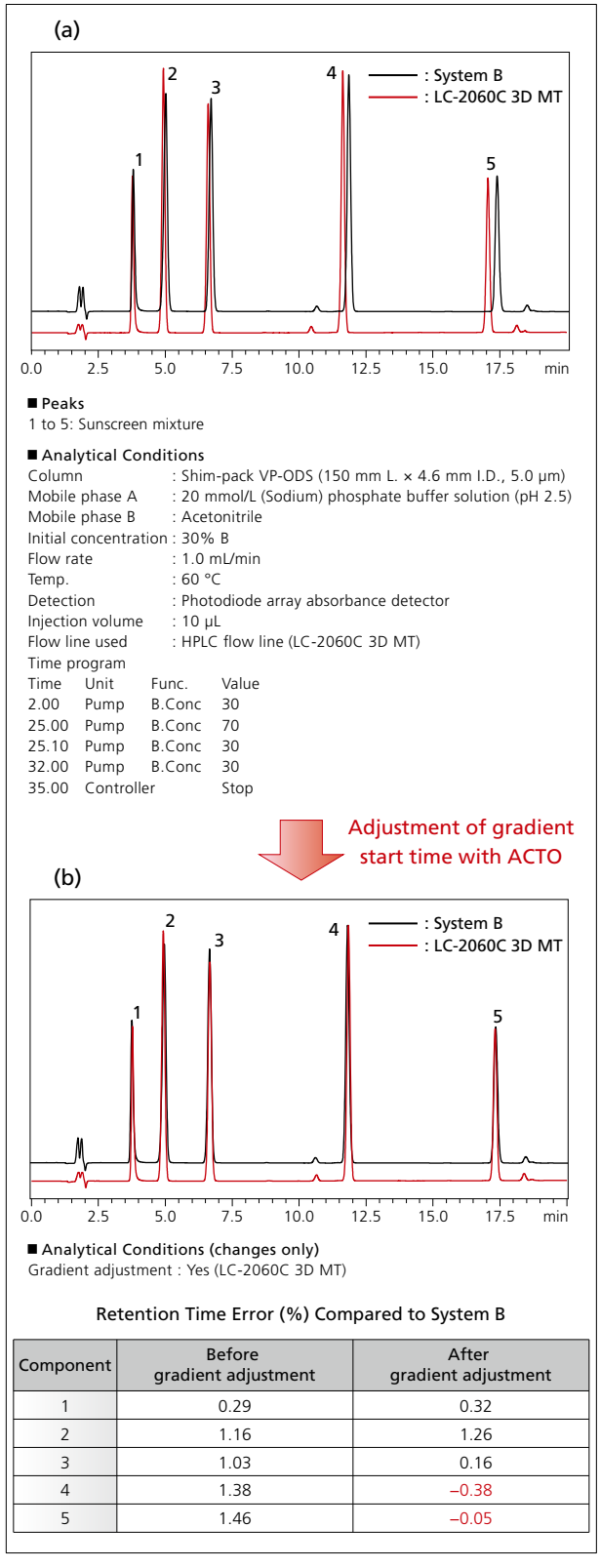
3-2. Method Transfer using ACTO Function

In this example, software functionality was used to match system delay volumes for method transfer, rather than by changing mixers or tubing. Fig. 6 (a) was obtained by analyzing a sunscreen mixture sample with a Shimadzu LC-2060C 3D MT system and a different other vendors' LC system (System B). Of the two flow lines available in the LC-2060C 3D MT system, the HPLC flow line was used.

Though the same method was used for both analyses, the peaks after 10 minutes do not match. 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. 6 (b) and the inset table in Fig. 6, 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. Note that adjusting the initial hold time is permitted by the respective pharmacopoeias, is not considered to be changing the method, and 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 flow line (LC-2060C 3D 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

■ Analytical Conditions (changes only)
Gradient adjustment : Yes (LC-2060C 3D MT)

Retention Time Error (%) Compared to System B

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. 6 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 (LC-2050/LC-2060) 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. 7 i-Series Integrated LC System

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.

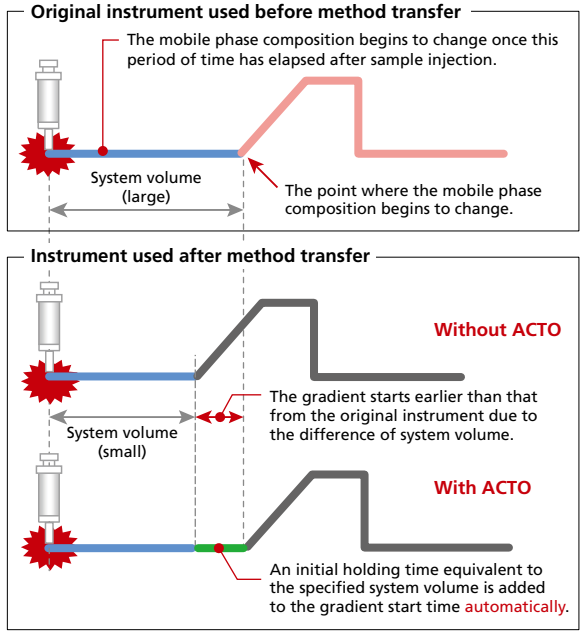


Fig. 8 Adjusting the Gradient Start Time

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Technical Report

Incorporating Analytical Intelligence into the Integrated i-Series—New Analytical Workflow Automation

Chihiro Hosoi[1], Daiki Fujimura[1], Keiko Matsumoto[1]

Abstract:

Significant progress has been made in automating analytical operations as more efficient and flexible work styles are required. The integrated i-Series contains all the functions required for LC analysis in a compact unit. These functions have been optimized for ease of operation and maintenance. In addition to the exceptional instrument performance offered in previous models, the new i-Series (LC-2050/LC-2060) models also feature Analytical Intelligence functionality for supporting automation of analytical operations and ensuring acquisition of highly reliable data. Analytical Intelligence is a new concept for analytical instruments offered by Shimadzu. Analytical Intelligence consists of systems and software that simulate expert operators automatically determining whether or not conditions and results are good or bad, providing feedback to users, and solving common problems. It increases data reliability by compensating for any differences between users in their instrument knowledge or experience. This Technical Report bulletin describes the new Analytical Intelligence functionality included in the new i-Series.

Keywords: Analytical Intelligence, i-Series, auto-startup, FlowPilot, mobile phase monitor, i-PeakFinder™, i-PDeA II

1. Background

As users demand higher operating efficiencies and more flexibility in working practices, what is considered the ideal for LC analytical processes is beginning to change significantly. Users want an environment where equivalent analytical operations and data analysis can be performed to obtain identical results, even by users not located in the laboratory or users unfamiliar with operating the system.

Analytical and testing operations often require fundamental knowledge about analytical chemistry and experience-based expertise. Experienced analysts have a good understanding of the principles underlying analytical techniques and systems and are able to apply their expertise gained from past experience to avoid problems and obtain highly reliable data. In contrast, it is difficult for analysts with minimal experience to predict potential risks in advance and analyze samples with corresponding countermeasures implemented. In addition, during data analysis, it is much more likely that an expert analyst will discover hidden problems in the data.

Overall operating efficiency taking into consideration data reliability and instrument uptime rate, etc., is dependent not only on analysis cycles, throughput, and other factors that can be resolved with instruments and software, but is also greatly dependent on the knowledge and skill level of users. Furthermore, whereas improving the knowledge and skill level of users requires a time-consuming process of training personnel, the number of expert analysts available in the analytical workplace is dwindling and the proportion of analysts with minimal experience is increasing. This trend is a major issue currently being faced by the analysis and testing industries.

ANALYTICAL INTELLIGENCE

- Automated support functions utilizing digital technologies, such as M2M, IoT, and Artificial Intelligence (AI), that enable higher productivity and maximum reliability.
- Allows a system to monitor and diagnose itself, handle any issues during data acquisition without user input, and automatically behave as if it were operated by an expert.
- Supports the acquisition of high quality, reproducible data regardless of an operator's skill level for both routine and demanding applications.

2. Analytical Intelligence in Integrated LC System i-Series

ANALYTICAL INTELLIGENCE

That issue cannot be resolved by only improving work efficiency achieved through improvements in the basic performance of instruments or operability of software. It can only be truly solved if highly reliable results can be acquired at any time by any users, regardless of their knowledge or skill.

Analytical Intelligence is a new concept for analytical instruments offered by Shimadzu. It automates the expertise of expert analysts who avoid common pitfalls to ensure that equally high quality data can be acquired by anyone. As shown in Fig. 1, Analytical Intelligence reduces the risk of system problems by automating mobile phase volume checks and column equilibration.

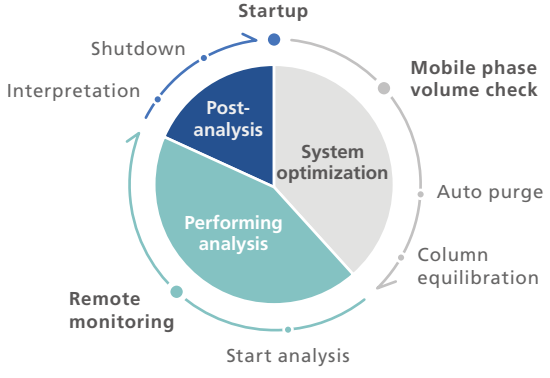

Fig. 1 Workflow of LC Analysis

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2. Integrated LC System i-Series

The integrated LC System i-Series contains all the functions required for LC analysis in a compact unit. These functions have been optimized for ease of operation and maintenance with an intuitive touch panel (See Fig. 2). LC-2050/LC-2060 models feature robust hardware performance with a solid reputation plus new Analytical Intelligence functionality for supporting analysis.

Fig. 2 Integrated LC System i-Series (LC-2050/LC-2060)

3. FlowPilot Enabling Fully Automated Workflow for Analysis

FlowPilot enables fully automated workflow for analytical operations from startup and system suitability test (SST) to analysis and shutdown.

3-1. Intelligent Start-up with FlowPilot

When starting up the system and equilibrating the column, it is well known that pressure shock can affect column performance by reducing column lifetime and leading to channeling, which results in peak-splitting in the corresponding chromatogram. Expert analysts will gradually increase the flowrate as the column temperature is controlled to prevent exposing the column to any excessive pressure loads.

When the i-Series auto-startup function starts up the system at the specified date and time, the FlowPilot function starts equilibrating the column by gradually increasing the mobile phase flow rate as the column temperature increases. That means the system automatically replaces the manual operations of expert analysts to avoid column damage and finishes preparing the system (Fig. 3). The FlowPilot status during execution can even be confirmed on the touch panel (Fig. 4).

3-2. Automation of Entire Analytical Procedures

The FlowPilot function can be coupled with the warm-up function and scheduled depending on the user requirements. The system can also be evaluated automatically using the automatic SST function. Scheduled shutdown automatically turns off the system and switches it to power-saving mode when all analytical operations are complete.

The combination of these functions allows the user to fully automate an entire analytical cycle: Shutdown → Start-up → SST → Analysis → Results report → Shutdown (Fig. 5).

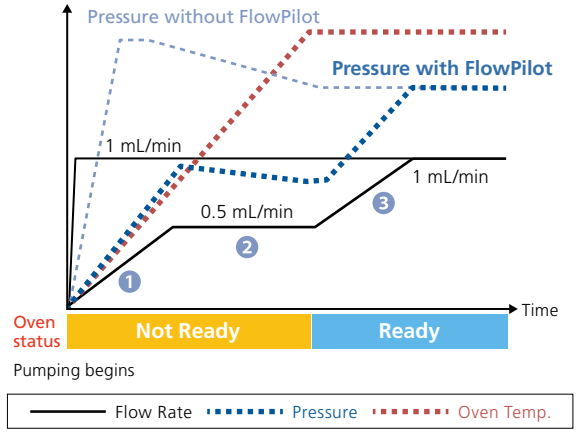

Fig. 3 Diagram of System Pressure Profile during Start-up with the FlowPilot Function

Fig. 4 Touch Panel when FlowPilot Function is used

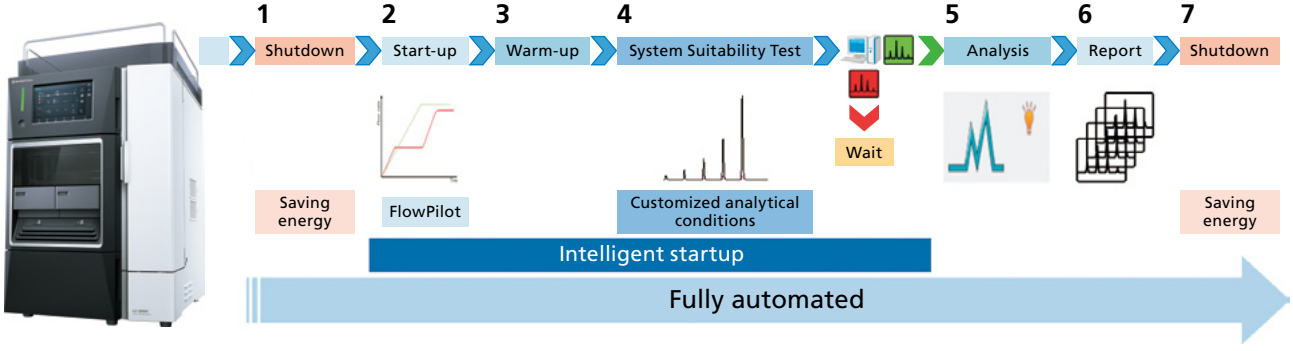

Fig. 5 Workflow Diagram showing the Fully-automated Operation of LC Analysis

3-3. Fully-automated SST

SST are used to verify that the chromatography system is adequate for the intended analysis. The tests are based on the concept that the equipment, electronics, analytical operations, and samples analyzed constitute an integrated system that can be evaluated as such. SST is mandatory in USP, FDA, and EP standards to check and ensure the ongoing performance of analytical systems. Nevertheless, several different parameters can be evaluated depending on the system and the analytical conditions. For this reason, there is a growing demand for a degree of flexibility in the set-up of SST parameters and possibilities for their customization in modern LC systems.

SST parameters are embedded in the analytical method file. This means that users can easily create an SST with specific analytical conditions, in which selected parameters are evaluated (e.g. number of theoretical plates, tailing factor, resolution, capacity factor *k*). After creating the SST, it is possible to choose when to run the SST during a batch analysis (at the beginning, after analysis of some samples or at the end of the batch).

Once the SST is complete, a "pass" or "fail" result is issued depending on the previously-selected criteria, and this result will then trigger specific actions based on user preferences (Refer to Technical Report C190-E227).

4. Mobile Phase Monitor Prevents Mobile Phase Depletion

Mobile phase levels must be appropriately managed because running out of mobile phase during an analysis not only causes the analysis to fail, but can also damage expensive analytical columns. A mobile phase monitor can be used to measure the amount of mobile phase remaining in real time, and is operated in combination with LabSolutions™ or dedicated software to check the mobile phase level.

Previously, LabSolutions could be used to calculate an estimated value of the remaining mobile phase level based on the volume consumed, but the MPM-40 unit (Fig. 6) is configured with a weight sensor and controller that can be used to calculate the level based on the actual mobile phase weight. The MPM-40 sends the current quantity inside the mobile phase bottle to a computer or smartphone in real time via a LAN connection. Dedicated MPMChecker™ software then graphically displays the remaining level (Fig. 7). When the remaining quantity of mobile phase decreases to the specified level, a warning (orange) or error (red) signal is emitted to notify the user. It also stops the LC system if specified criteria are satisfied. (Refer to Technical Report C190-E226)

Two types of bottle holders are available for holding either 1-liter bottle or large 2 to 5-liter bottles.

Fig. 6 MPM-40

Fig. 7 MPMChecker™

5. Accurate Peak Detection without Manual Peak Integration Automatic Peak Integration Using i-PeakFinder

Given increasingly fast analysis capabilities and shorter data acquisition times, if manual operations are required for integrating chromatogram peaks, then the data processing step becomes a bottleneck that prevents truly improving operational efficiency. Therefore, automating the peak integration process is essential. However, for chromatograms with a large number of peaks from contaminant components and target components, automating the peak integration process while eliminating the effects of baseline fluctuations and unseparated peaks can require complicated steps, such as configuring detailed settings for a peak integration program. Also, manual peak integration processes are prone to causing differences between individual operators, which reduces the consistency of quantitation values.

i-PeakFinder, which is one of the peak integration algorithms available in LabSolutions, uses a completely automated integration function to accurately detect peaks, as shown below, without the need to specify special parameter settings (Fig. 8).

- Shoulder peaks can be detected very accurately.
- Baseline processing can be changed easily.
- Reliable peak tracking enables improved reproducibility.
- Peaks can be integrated correctly even with variability from baseline drift.

With applicable parameter settings available for a wide range of complex chromatogram patterns, i-PeakFinder can output highly accurate peak integration results even when processing large quantities of data at the same time. i-PeakFinder is part of the standard functionality included with LabSolutions software, so it can be used for chromatograms obtained with non–i-Series LC systems as well. (Refer to Technical Reports C191-E044, C190-E243)

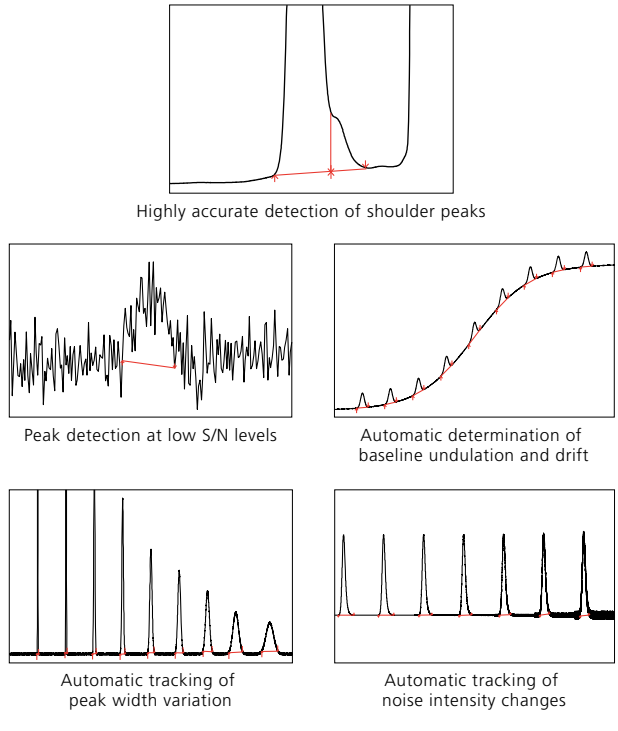

Fig. 8 Example of Automatic Peak Integration Using i-PeakFinder

6. Separating Unseparated Peaks Using a PDA Detector Detecting Overlapping Peaks with i-PDeA II

If there are other peaks present near target component peaks, such as when analyzing multiple components simultaneously, checking for synthesis, or analyzing samples with many contaminant components, to ensure quantitative accuracy, it is important to check for any peaks eluted together with target peaks and overlapping in the chromatogram. However, in reality, checking for such peaks is quite difficult unless a mass spectrometer or any other instrument with high selectivity is used for detection. Also, if unseparated peaks are discovered, it usually requires reassessing peak separation in the column.

i-Series models equipped with a PDA unit (LC-2050C 3D or LC-2060C 3D) can use a unique data analysis technique in Intelligent Peak Deconvolution Analysis II (i-PDeA II) functionality to isolate target peaks from unseparated peaks. The i-PDeA II data analysis technique extracts target peaks from unseparated peaks by analyzing photodiode array (PDA) detector data using the chemometric multivariate curve resolution alternating least squares (MCR-ALS) method. The technology uses a PDA detector to identify overlapping peaks that were not adequately separated by the column and either separates those peaks in the chromatogram or determines the UV spectrum of each peak. Consequently, it can be used to check for impurity peaks hidden by key component peaks, extract chromatograms for individual components (Fig. 9 (b)), or confirm peak purity (Fig. 9 (c)). (Refer to Technical Report C191-E042)

7. Conclusions

Based on a completely new concept, Analytical Intelligence consists of various supporting functionality that was developed for the purpose of promoting higher efficiency through workflow improvements, while also ensuring the reliability of data from instrumental analysis. Automatic operation of the system which simulates operation by an expert analyst reduces the risk of system problems. Consistent data analysis results are provided by automating the operations that tend to result in variability between individual operators and by automatically identifying overlapping peaks that are easily overlooked.

LC-2050/LC-2060 have various functions of Analytical Intelligence in addition to their excellent basic performances, and contribute to maximizing system utilization rates and improving operating efficiency by always acquiring data that is consistently highly reliable and by avoiding system problems, regardless of knowledge and skill level of users.

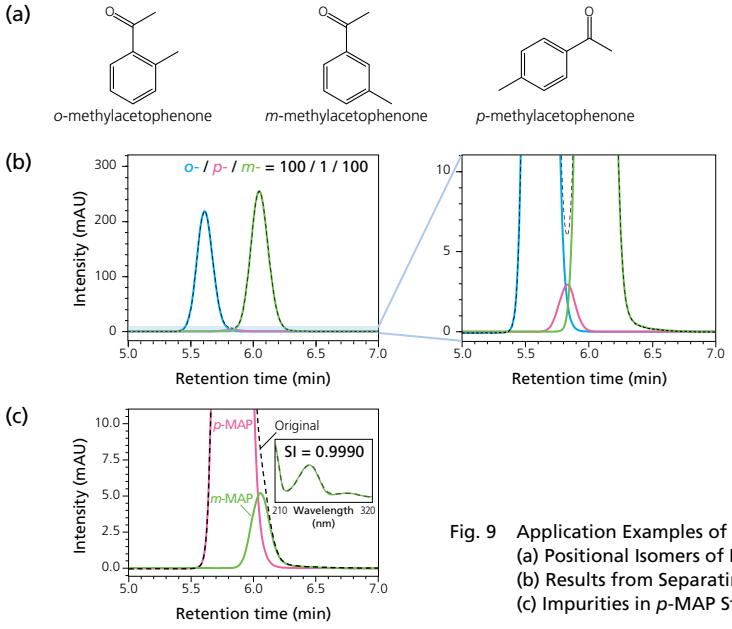

Fig. 9 Application Examples of Peak Separation Algorithm using i-PDeA II
(a) Positional Isomers of Methylacetophenone
(b) Results from Separating a Mixture Sample of *o*-MAP, *m*-MAP, and *p*-MAP
(c) Impurities in *p*-MAP Standard Sample

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Technical Report

Support of Remote Analysis Work with Integrated LC System i-Series

Hiroshi Ohashi[1], Daiki Fujimura[1], Keiko Matsumoto[1]

Abstract:

A variety of workplaces are currently facing the challenge of further improving efficiency through more extensive adoption of new working styles, such as working from home or teleworking. Due to dramatic advances in digital and networking technologies in recent years, it is now possible to specify instrumental analysis settings, to perform analyses, and to perform data processing of acquired data, all remotely. In addition, Shimadzu i-Series integrated LC systems include "Analytical Intelligence" functionality that can remotely perform a wide variety of operations that were previously performed in person, such as startup or column equilibration. Such functionality will provide a powerful boost to the transition to remotely performed analytical processes.

Keywords: automatic startup, remote operation and monitoring, LabSolutions™ Direct, LabSolutions CS

1. A New Style of Analytical Operations

With the demand for higher operational efficiency and more flexibility in working style, there have been significant changes in what is considered ideal for LC analytical processes. Environments are increasingly required in which even operators not physically situated in the laboratory or inexperienced operators can obtain identical results by executing equivalent analytical operations and data processing. While ensuring the same outstanding performance as previous systems, i-Series integrated LC systems (LC-2050/LC-2060) consistently provide highly reliable analytical results by addressing the increasing need for diversity in analytical work with respect to personnel, workplace, and working style.

i-Series can automatically and remotely perform all the steps involved in an analysis, including system startup, column equilibration, start of analysis, and system shutdown, which previously

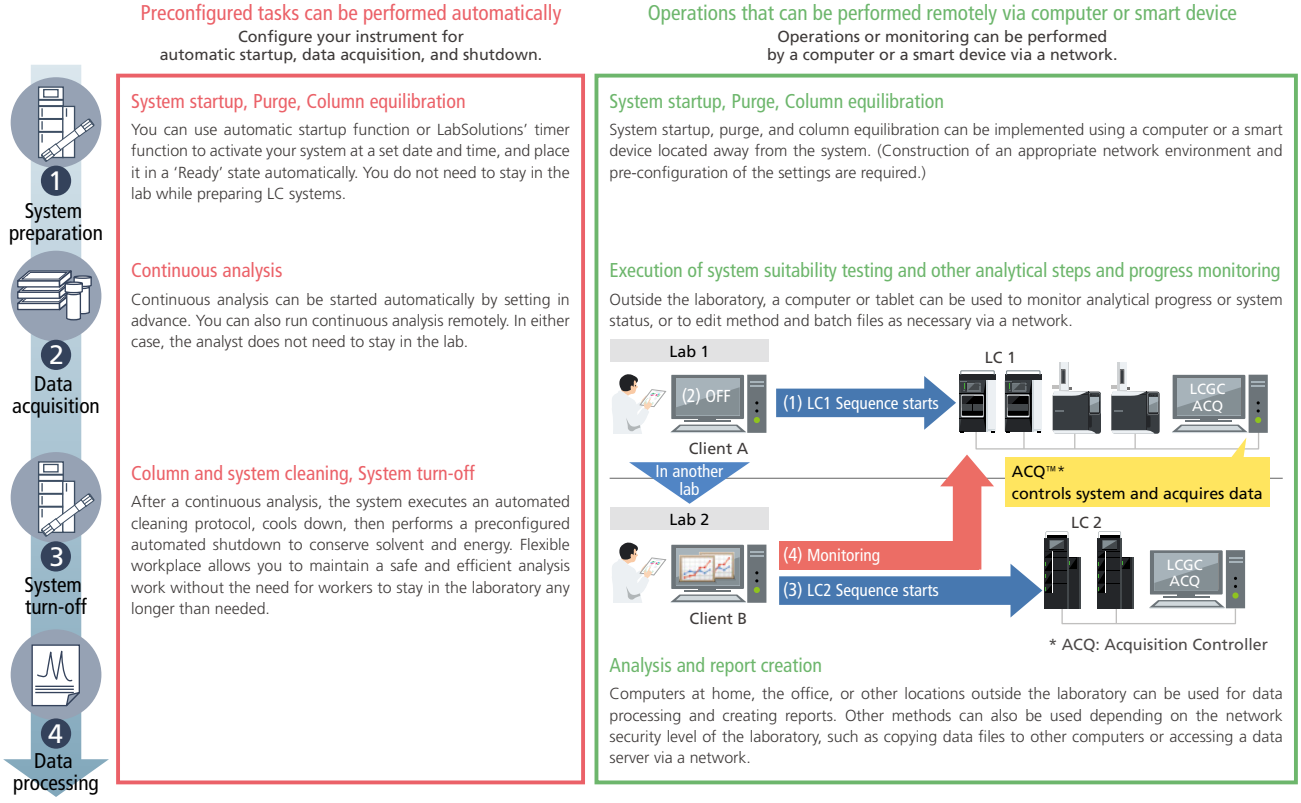


Fig. 1 New Analysis Workflow Provided by i-Series

1 Analytical & Measuring Instruments Division

required the physical presence of an operator. Remote operation and monitoring can be performed with either a computer or a smart device.

Using dedicated software, it is even possible to perform data processing and create reports from outside the laboratory, assuming the software and data files are accessible. The optimal platform can be selected based on the laboratory network security level required.

2. Solutions Supporting Remote Operability

There are two basic types of Shimadzu LabSolutions workstation software available, either standalone or networked. The solutions for the remote operability of analytical processes are shown below for a standalone system and a networked system, respectively.

Standalone LabSolutions LC/GC	Networked LabSolutions CS
• System is controlled via connected computers. • Data is saved on individual computers.	• Client terminals are used to control the system, view the data, and perform data processing. • Data is managed centrally on a server.

2-1. Standalone System (LabSolutions Direct)

LabSolutions Direct is a remote access tool of LabSolutions LC/GC (Fig. 2) for remotely controlling or monitoring HPLC systems via a web browser on a smart device or computer. With this software, method files or batch files are easily selected and specified for use in analysis. Analysis can be started after confirming column equilibration by monitoring the system status, detector baseline, or other factors. Chromatograms can be monitored during analysis, and analysis reports in PDF format can be viewed after analysis.

2-2. Networked System (LabSolutions CS)

With LabSolutions CS, which is designed for networked systems with a server and client terminals, system control and data processing can be performed simultaneously from any client terminal. Since it is centrally managed in a database on LabSolutions server, the data can be viewed from any client terminal.

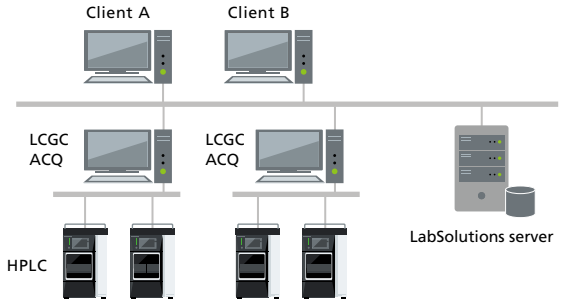
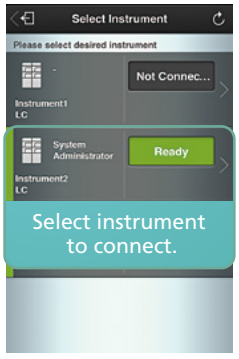


Fig. 3 LabSolutions CS System Configuration

1

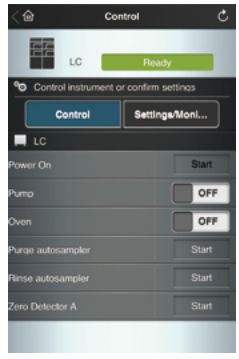


After login, select the instrument to be used.

2

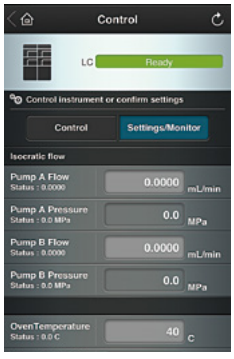
Download the selected method.

3



LC system can be power on. In addition, the autosampler can be rinsed and the mobile phase and rinsing solution can be purged.

4



Pump pressure values and column oven temperature can be monitored to check that the instrument has stabilized. The flow rate and oven temperature can be changed.

5

Select the batch file and execute data acquisition.

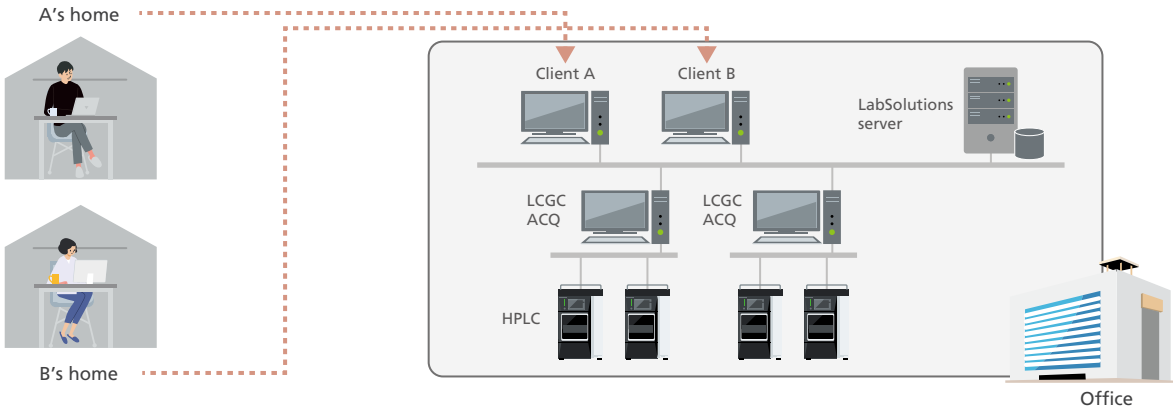
6

During data acquisition, chromatograms, pump pressure values, etc. can be monitored.

Fig. 2 LabSolutions Direct Operating Window

2-2-1. Remote Desktop Connection

With this method, the connection to LabSolutions CS client computers uses remote desktop functionality (Fig. 4). Although instruments and data can be accessed from any terminal, appropriate security measures are required for connections from home or other locations outside the network.



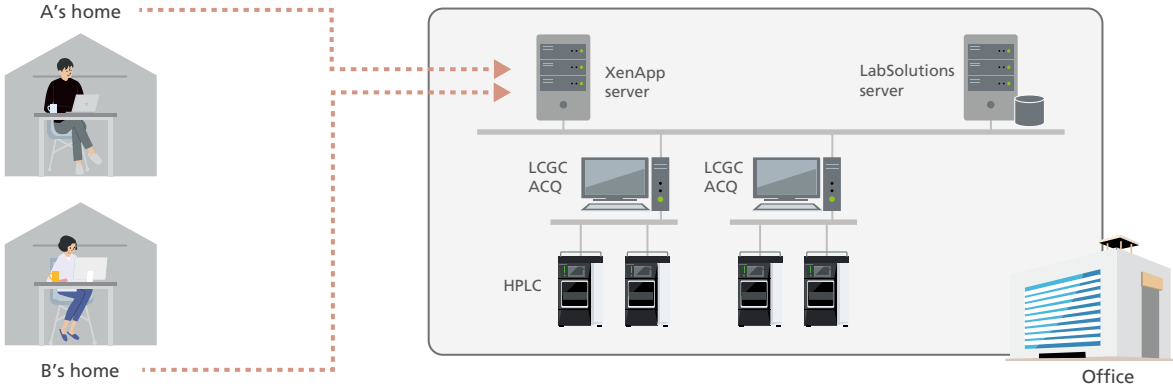
- ✓ It is not necessary to install software on business PC.
- ✓ Startup, method editing, monitoring, data processing and report creation for all LC, GC and LCMS instruments.
- ✓ All the results are stored in the LabSolutions server.
- ✗ Since multiple PCs are connected from the outside, it takes time and effort for security measures.

*1: It is assumed that you can connect with a VPN.
*2: For equipment except for chromatography system, connect to the corresponding ACQ via remote desktop.

Fig. 4 LabSolutions CS Case #1: Remote Desktop Connection

2-2-2. Citrix XenApp Connection

With this method, the connection uses a combination of LabSolutions CS and Citrix XenApp virtual platform software (Fig. 5). The Citrix XenApp system transmits only screenshots of the applications being run on a server to computers used for operations. Because it only enables remote operation of LabSolutions applications and uses proprietary data compression technology, it requires less network capacity and exposes the network to less risk than remote desktop connections.



- ✓ It is not necessary to install software on business PC.
- ✓ Startup, method editing, monitoring, data processing and report creation for all LC, GC and LCMS instruments.
- ✓ All the results are stored in the LabSolutions server.
- ✓ Regarding LC, GC and LCMS instruments, only XenApp server can be connected externally.

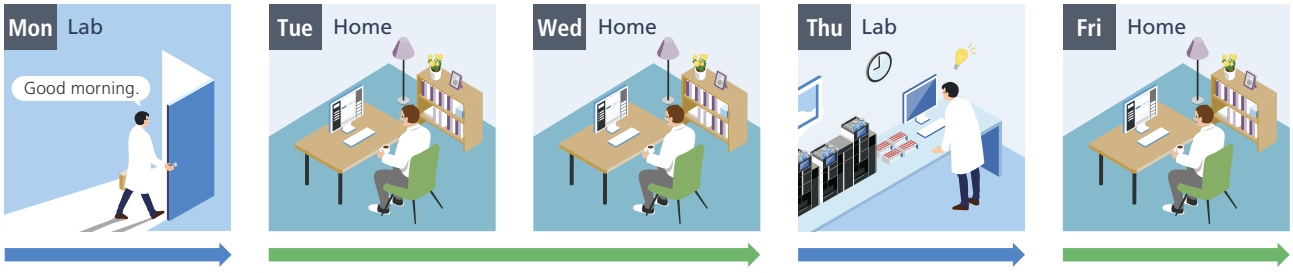
*1: It is assumed that you can connect with a VPN
*2: For equipment except for chromatography system, connect to the corresponding ACQ via remote desktop.

Fig. 5 LabSolutions CS Case #2: Citrix XenApp Connection

3. Supporting a New Style of Analytical Operations

Analytical work using conventional LC systems involves commuting to work each day, starting up the system, and equilibrating the column, and then acquiring and analyzing the data. All the steps must be performed at the laboratory, which makes it difficult to work remotely from home or elsewhere. Accordingly, using i-Series integrated LC systems in combination with LabSolutions Direct or LabSolutions CS can improve operational efficiency and minimize travel to the office/laboratory (Fig. 6).

A new style of analytical operations can be achieved by combining the technology for remote and automated operability built into i-Series integrated LC systems with the networking technology described in this article.



Preparation of analysis

- Preparation of standard solutions and QC samples
- Sample pretreatment and installation
- Preparation and installation of mobile phase
- Column installation
- Start of analysis

Monitor sequence running

Data processing

- Monitoring analysis progress
- Data processing
- System turn-off

Preparation of analysis

Monitor sequence running

Data processing

Fig. 6 Example of Work Style Using an i-Series and LabSolutions CS

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ANALYTICAL INTELLIGENCE

Maximum Reliability and Stability

— Fundamental functions assure analysis results —

Advanced
i-Series

Auto Shutdown Function Reduces Power Consumption

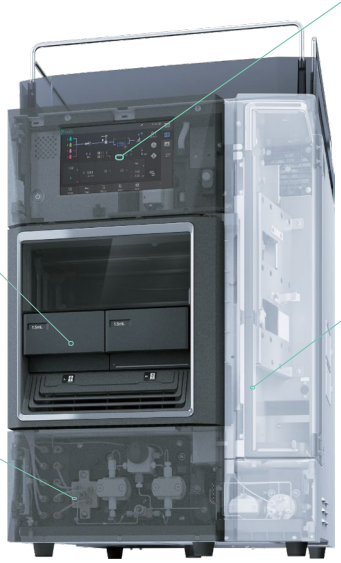
After analysis is complete, the auto shutdown function minimizes power consumption in standby mode and can reduce power consumption by at least 95% compared to normal standby mode.

Refined Usability

Control panel with a color LCD touch panel allows anyone to operate the instrument, regardless of experience level. The LCD displays chromatogram in real time.

Supports High-Speed Multi-Analyte Processing

A 14-second injection cycle maximizes the number of samples that can be processed. Moreover, a total of 1536 samples can be accommodated in right and left sample racks.



Large Capacity Column Oven with Ultra Wide Temperature Range

The forced-air circulation method is used to support temperatures up to 90 °C. A standard system fits either three 300 mm long columns or six 100 mm long columns.

Quaternary Solvent Delivery Unit

A 10 µL micro plunger ensures accurate quaternary gradient delivery.

Use of Multiple Detectors Expands Application Range

The i-Series can be equipped as standard with either a UV/VIS or photodiode array (PDA) detector. It can be expanded with a fluorescence detector, differential refractive index detector, the compact LCMS-2050 mass spectrometer, or other detectors.

ELSD-LTⅢ

LCMS-2050

SPD-M40

RF-20AXS