

## Nexera Organic Acid Analysis System and its Application

A. Tanabe, R. Kato

### User Benefits

- ◆ Excellent selectivity and high sensitivity are obtained in analysis of organic acids.
- ◆ Stable analyses can be conducted easily by using the Mobile Phase Reagents Kit for Organic Acid Analysis, which eliminates the time and trouble of reagents formulation.
- ◆ The Nexera Organic Acid Analysis System can also be applied to analyses of short-chain fatty acids and lower fatty acids in various fields.

### Introduction

Organic acids are highly hydrophilic compounds which are difficult to retain in the ODS columns generally used in HPLC. Regarding detection, organic acids have absorption in the short wavelength region in Ultra Violet absorbance detection, and are easily affected by interferences from contaminants, so ingenuity is required in the detection method in order to perform analyses with high sensitivity and high selectivity.

The Nexera organic acid analysis system employs the "post-column pH buffering electric conductivity detection method" in which organic acids are separated using ion exclusion chromatography and then mixed with a pH buffering reagent to enhance detection sensitivity. It is optimized for organic acid analysis.

Moreover, results with excellent repeatability can be obtained easily by using a Shimadzu mobile phase and reagent kit for organic acid analysis, which includes the mobile phase and the pH buffering reagent.

This article introduces applications and retention indexes obtained by the Nexera Organic Acid Analysis system.

### Analysis of Standard Samples

Fig. 1 shows the flow path diagram of this system. Fig. 2 shows a chromatogram of 28 components of an organic acid standard sample using a Shim-pack™ SCR-102H ion exclusion column, and Table 1 shows the analysis conditions. With the exceptions of Tables 5 and 6, all analyses in this article were conducted under the conditions in Table 1.

In the ion exclusion mode, fine adjustment of separation is possible by changing the temperature or the mobile phase concentration when separation of the target compound or separation from contaminants is inadequate. For more information, see retention indexes.

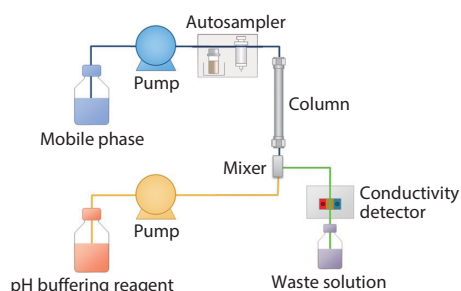
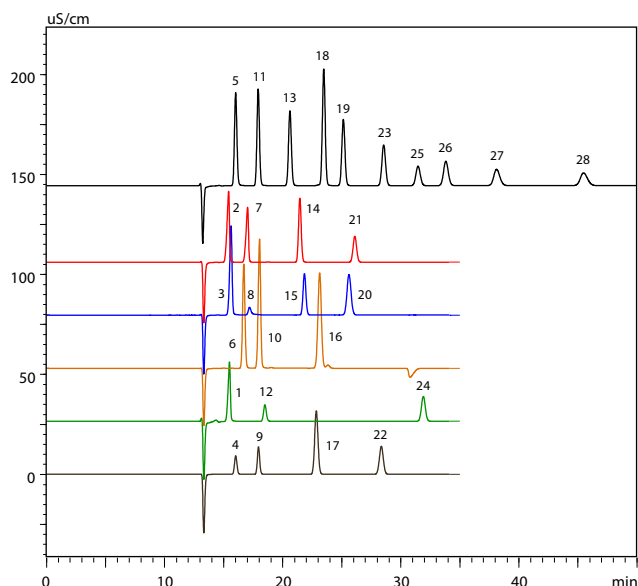


Fig. 1 Flow Path Diagram of Nexera Organic Acid Analysis System



- |                      |                         |                       |
|----------------------|-------------------------|-----------------------|
| 1. Phosphoric acid   | 11. Malic acid          | 21. Levulinic acid    |
| 2. Maleic acid       | 12. Kinic (quinic) acid | 22. Pyroglutamic acid |
| 3. Ketoglutaric acid | 13. Succinic acid       | 23. Propionic acid    |
| 4. Glucuronic acid   | 14. Glycolic acid       | 24. Carbonic acid     |
| 5. Citric acid       | 15. Lactic acid         | 25. Isobutyric acid   |
| 6. Tartaric acid     | 16. Fumaric acid        | 26. Butyric acid      |
| 7. Pyruvic acid      | 17. Glutaric acid       | 27. Isovaleric acid   |
| 8. Gluconic acid     | 18. Formic acid         | 28. Valeric acid      |
| 9. Glyoxylic acid    | 19. Acetic acid         |                       |
| 10. Malonic acid     | 20. Adipic acid         | (500 mg/L each)       |

Fig. 2 Chromatogram of 28 Components of Organic Acid Standard Sample

Table 1 Analysis Conditions

System	: Nexera Organic Acid Analysis System
Column	: Shim-pack SCR-102H (300 mm × 8.0 mm I.D., 7 μm) <sup>*1</sup> × 2 : Guard column SCR-102H (50 mm × 6.0 mm I.D.) <sup>*2</sup>
Mobile Phase	: 5 mmol/L <i>p</i> -toluensulfonic acid (Reagents kit for Organic Acid Analysis System <sup>*3</sup> )
Flow Rate	: 0.8 mL/min
pH Buffering Solution	: 5 mmol/L <i>p</i> -toluensulfonic acid, 20 mmol/L Bis-Tris <sup>*4</sup> , : 0.1 mmol/L EDTA <sup>*5</sup> (Reagents kit for Organic Acid Analysis System <sup>*3</sup> )
Flow Rate	: 0.8 mL/min
Mixer	: Organic Acid Analysis Plumbing Kit (MR) <sup>*6</sup>
Column Temp.	: 45 °C
Injection Vol.	: 20 μL
Vial	: SHIMADZU LabTotal · for LC 1.5 mL, Glass <sup>*7</sup>
Detection	: Conductivity

<sup>\*1</sup> P/N : 228-17893-91, <sup>\*2</sup> P/N : 228-17924-91, <sup>\*3</sup> P/N : 228-61465-91,  
<sup>\*4</sup> Bis-(2-hydroxyethyl)iminotris(hydroxymethyl)methane,  
<sup>\*5</sup> ethylenediaminetetraacetic acid,  
<sup>\*6</sup> P/N : 228-77532-41, <sup>\*7</sup> P/N : 228-15652-92

### ■ Repeatability

Fig. 3 shows the chromatogram of 10 components of organic acid standard sample (10 mg/L each), and Table 2 shows the repeatability of the retention times and area values for 6 repeated analyses.

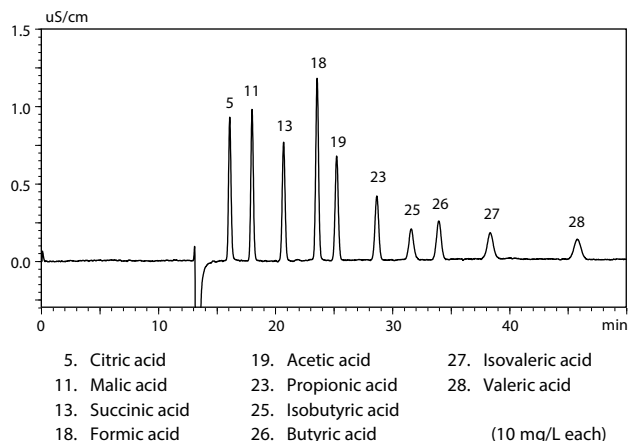


Fig. 3 Chromatogram of 10 Components of Organic Acid Standard Sample

Table 2 Retention Times and Area Repeatability of 10 Components of Organic Acid Standard Sample (10 mg/L, n=6)

	Average Retention time (min)	Retention time (%RSD)	Average Area	Area (%RSD)
Citric acid	16.10	0.013	13974	0.74
Malic acid	18.01	0.015	14940	0.68
Succinic acid	20.70	0.011	13220	0.62
Formic acid	23.57	0.015	20161	0.43
Acetic acid	25.24	0.013	12773	0.68
Propionic acid	28.67	0.016	9167	0.43
Isobutyric acid	31.61	0.027	5277	0.85
Butyric acid	33.97	0.017	7066	0.88
Isovaleric acid	38.36	0.030	5650	0.94
Valeric acid	45.78	0.063	5172	0.90

### ■ Lower Limit of Quantification

Table 3 shows the S/N ratio of each organic acid component calculated from the peak heights and noise values in Fig. 3, together with the lower limit of quantification (LOQ) calculated as the concentration at which S/N ratio was 10.

Table 3 S/N Ratios and Lower Limit of Quantification for 10 Components of Organic Acid Standard Sample (10 mg/L Each)

	S/N	LOQ (mg/L)
Citric acid	179	0.56
Malic acid	189	0.54
Succinic acid	148	0.69
Formic acid	226	0.44
Acetic acid	130	0.79
Propionic acid	79.8	1.27
Isobutyric acid	37.5	2.70
Butyric acid	48.1	2.09
Isovaleric acid	31.7	3.04
Valeric acid	24.4	3.96

### ■ Coefficient of Determination

Table 4 and Fig. 4 show the calibration curves and coefficients of determination of the 10 components of the organic acid standard sample in the concentration range of 5 to 1000 mg/L. Satisfactory linearity was obtained, as the coefficient of determination was 0.9999 or higher in all cases.

Table 4 Calibration Curve Range and Coefficient of Determination of 10 Components of Organic Acid Standard Sample

	Calibration Curve Range (mg/L)	Contribution Rate (r <sup>2</sup> )
Citric acid	5 - 1000	0.99997
Malic acid		0.99997
Succinic acid		0.99993
Formic acid		0.99998
Acetic acid		0.99998
Propionic acid		0.99998
Isobutyric acid		0.99999
Butyric acid		0.99999
Isovaleric acid		0.99999
Valeric acid		0.99999

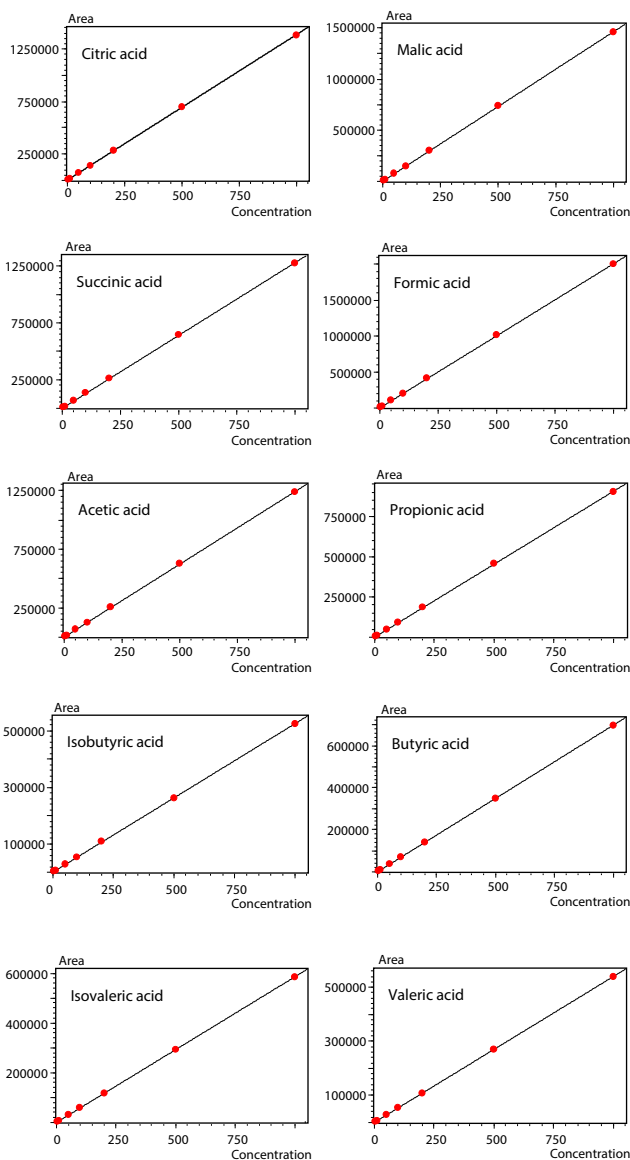


Fig. 4 Calibration Curves of 10 Components of Organic Acid Standard Sample

## ■ Applications

### • Analysis of Orange Juice

Orange juice (100 % fruit juice) was diluted 10 times with ultrapure water and filtered with a 0.45 µm membrane filter. Fig. 5 shows the chromatogram.

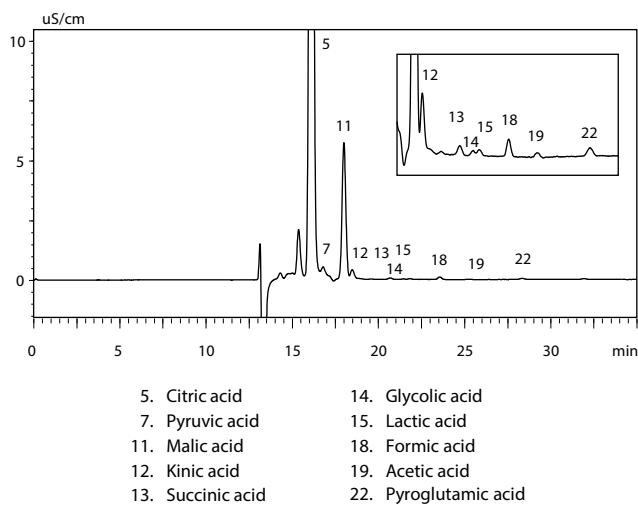


Fig. 5 Chromatogram of Orange Juice

### • Analysis of Coffee

Coffee (Arabica variety) was diluted 5 times with ultrapure water and filtered with a 0.45 µm membrane filter. Fig. 7 shows the chromatogram.

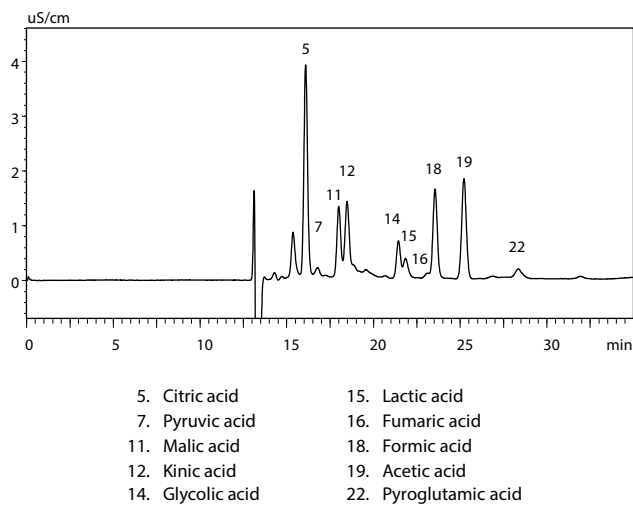


Fig. 7 Chromatogram of Coffee

### • Analysis of Apple Juice

Apple juice (100 % fruit juice) was diluted 10 times with ultrapure water and filtered with a 0.45 µm membrane filter. Fig. 6 shows the chromatogram.

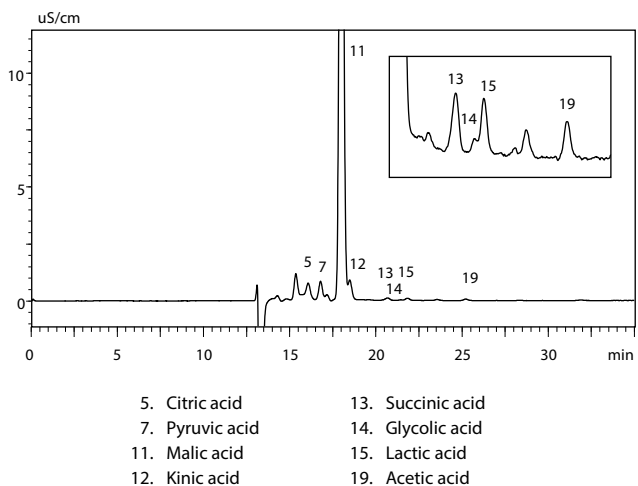


Fig. 6 Chromatogram of Apple Juice

### • Analysis of Beer

Canned beer was shaken to remove the carbonic acid, and diluted 10 times with ultrapure water, followed by filtration with a 0.45 µm membrane filter. Fig. 8 shows the chromatogram.

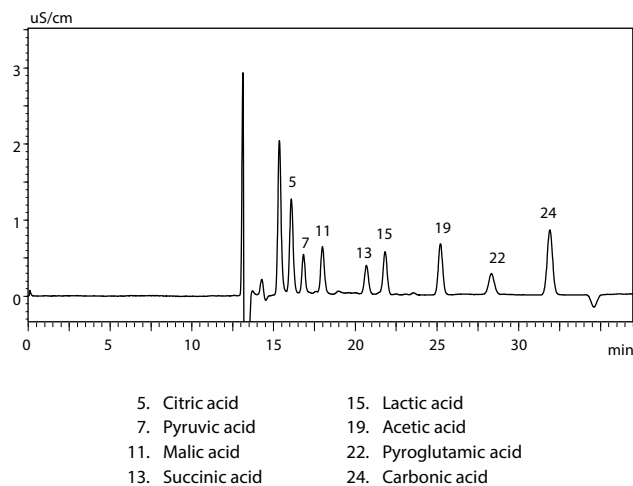
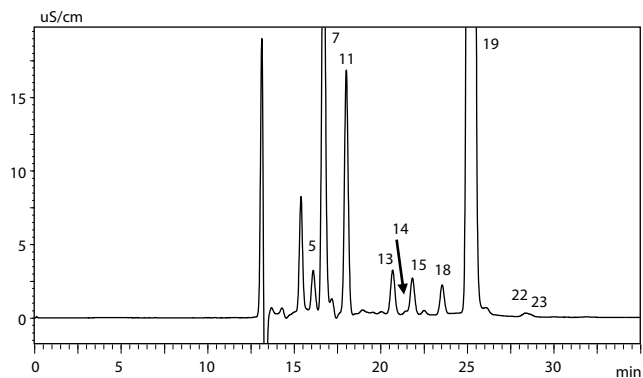


Fig. 8 Chromatogram of Beer

### • Analysis of Balsamic Vinegar

Balsamic vinegar was diluted 10 times with ultrapure water, followed by filtration with a 0.45 µm membrane filter. Fig. 9 shows the chromatogram.

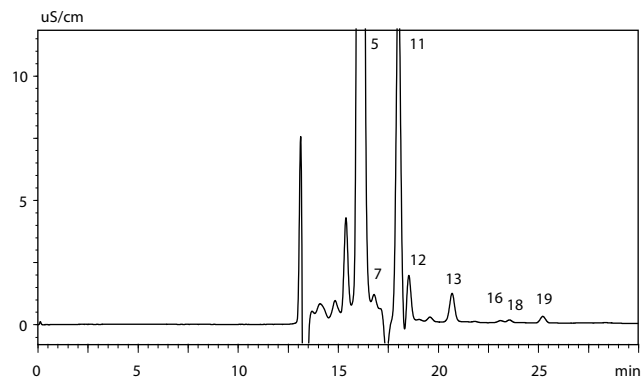


- |                   |                       |
|-------------------|-----------------------|
| 5. Citric acid    | 15. Lactic acid       |
| 7. Pyruvic acid   | 18. Formic acid       |
| 11. Malic acid    | 19. Acetic acid       |
| 13. Succinic acid | 22. Pyroglutamic acid |
| 14. Glycolic acid | 23. Propionic acid    |

Fig. 9 Chromatogram of Balsamic Vinegar

### • Analysis of Blueberries

The blueberry sample was prepared for analysis by lightly crushing 8.6 g of frozen blueberries, followed by addition of 5 mL of ultrapure water and homogenizing for 2 min. The sample was then centrifugally separated (14000 rpm, 5 min), after which the supernatant was filtered with a 0.45 µm membrane filter and analyzed. Fig. 11 shows the chromatogram.

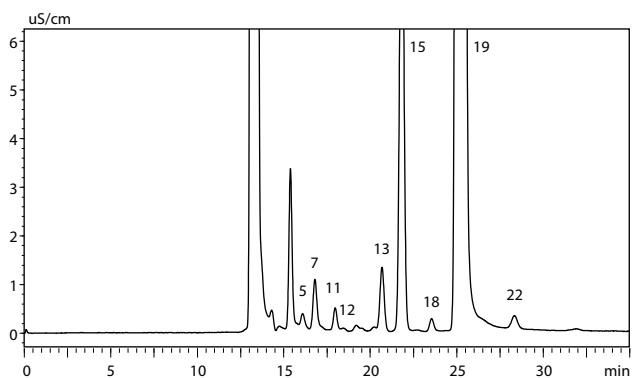


- |                 |                   |
|-----------------|-------------------|
| 5. Citric acid  | 13. Succinic acid |
| 7. Pyruvic acid | 16. Fumaric acid  |
| 11. Malic acid  | 18. Formic acid   |
| 12. Kinic acid  | 19. Acetic acid   |

Fig. 11 Chromatogram of Blueberry Extract

### • Analysis of Chili Pepper Sauce

Chili pepper sauce was diluted 10 times with ultrapure water, followed by filtration with a 0.45 µm membrane filter. Fig. 10 shows the chromatogram.

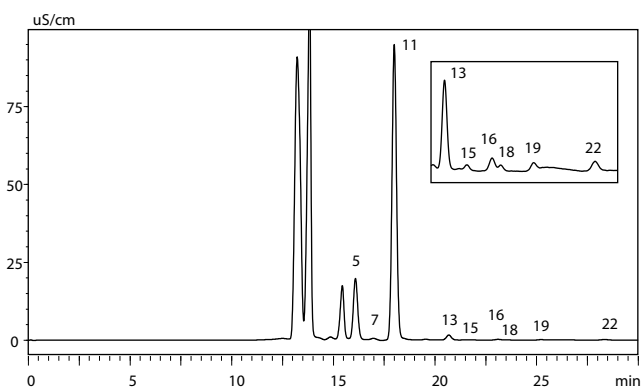


- |                   |                       |
|-------------------|-----------------------|
| 5. Citric acid    | 15. Lactic acid       |
| 7. Pyruvic acid   | 18. Formic acid       |
| 11. Malic acid    | 19. Acetic acid       |
| 12. Kinic acid    | 22. Pyroglutamic acid |
| 13. Succinic acid |                       |

Fig. 10 Chromatogram of Chili Pepper Sauce

### • Analysis of Spinach

The spinach sample was prepared for analysis by shredding 3.4 g of frozen spinach, followed by addition of 5 mL of ultrapure water and homogenizing for 2 min. The sample was then centrifugally separated (14000 rpm, 5 min), after which the supernatant was filtered with a 0.45 µm membrane filter and analyzed. Fig. 12 shows the chromatogram.

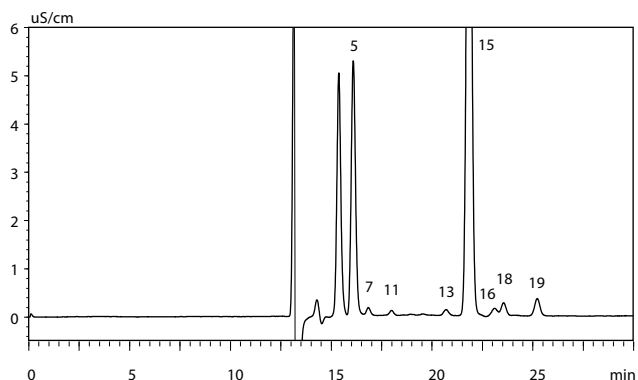


- |                   |                       |
|-------------------|-----------------------|
| 5. Citric acid    | 16. Fumaric acid      |
| 7. Pyruvic acid   | 18. Formic acid       |
| 11. Malic acid    | 19. Acetic acid       |
| 13. Succinic acid | 22. Pyroglutamic acid |
| 15. Lactic acid   |                       |

Fig. 12 Chromatogram of Spinach Extract

### • Analysis of Yogurt

0.5 g of yogurt (from milk) was dissolved in 10 mL of ultrapure water, followed by deproteinization with an ultrafiltration cartridge (molecular weight cutoff 10000). Fig. 13 shows the chromatogram.

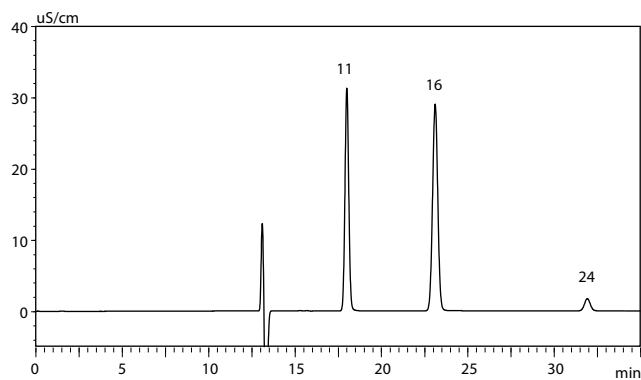


- |                   |                  |
|-------------------|------------------|
| 5. Citric acid    | 15. Lactic acid  |
| 7. Pyruvic acid   | 16. Fumaric acid |
| 11. Malic acid    | 18. Formic acid  |
| 13. Succinic acid | 19. Acetic acid  |

Fig. 13 Chromatogram of Yogurt

### • Analysis of Bathwater Additive

1 g of bathwater additive (bath salts) was dissolved in 10 mL of ultrapure water, followed by filtration with a 0.45 µm membrane filter and 50 times dilution with ultrapure water. Fig. 15 shows the chromatogram.

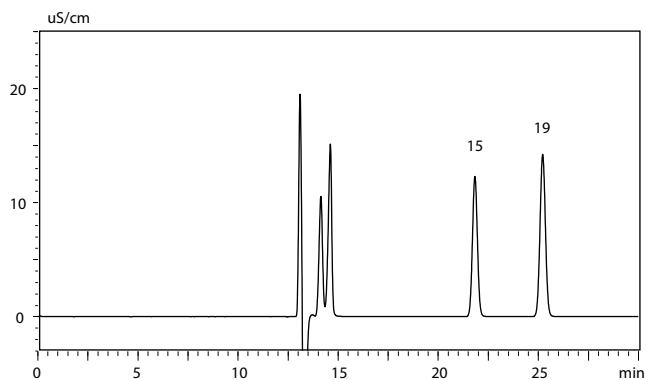


- |                |                  |                   |
|----------------|------------------|-------------------|
| 11. Malic acid | 16. Fumaric acid | 24. Carbonic acid |
|----------------|------------------|-------------------|

Fig. 15 Chromatogram of Bathwater Additive

### • Analysis of Plating Solution A

Plating Solution A was diluted 100 times with ultrapure water, followed by filtration with a 0.45 µm membrane filter. Fig. 14 shows the chromatogram.

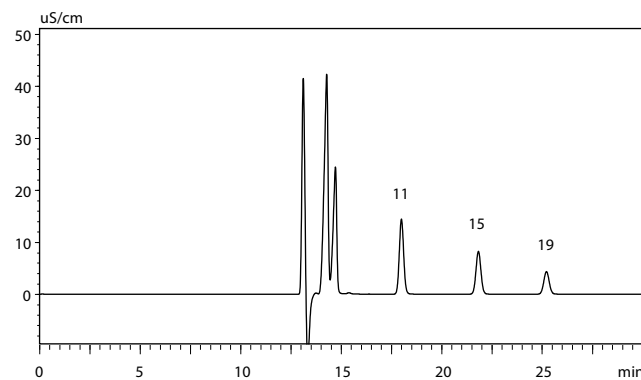


- |                 |                 |
|-----------------|-----------------|
| 15. Lactic acid | 19. Acetic acid |
|-----------------|-----------------|

Fig. 14 Chromatogram of Plating Solution A

### • Analysis of Plating Solution B

Plating Solution B was diluted 100 times with ultrapure water, followed by filtration with a 0.45 µm membrane filter. Fig. 16 shows the chromatogram.



- |                |                 |                 |
|----------------|-----------------|-----------------|
| 11. Malic acid | 15. Lactic acid | 19. Acetic acid |
|----------------|-----------------|-----------------|

Fig. 16 Chromatogram of Plating Solution B

## ■ Retention Index <Mobile Phase Reagents Kit for Organic Acid Analysis (Without Dilution): 5 mmol/L *p*-toluenesulfonic acid>

Table 5 shows the standard retention times of the 28 components of the organic acid standard sample at the four temperature levels of 35, 40, 45, and 50 °C.

Table 5 Standard Retention Times of 28 Components of Organic Acid Standard Sample at Various Temperatures (Undiluted Mobile Phase Reagents Kit; Unit: Minutes)

Component	35 °C	40 °C	45 °C	50 °C
1 Phosphoric acid	15.32	15.40	15.50	15.59
2 Maleic acid	15.54	15.48	15.43	15.38
3 Ketoglutaric acid	15.79	15.71	15.64	15.57
4 Glucuronic acid	16.02	16.03	16.03	16.02
5 Citric acid	16.26	16.18	16.12	16.04
6 Tartaric acid	16.86	16.79	16.71	16.64
7 Pyruvic acid	17.10	17.07	17.03	17.00
8 Gluconic acid	17.22	17.21	17.20	17.19
9 Glyoxylic acid	17.99	17.98	17.96	17.93
10 Malonic acid	18.25	18.15	18.05	17.95
11 Malic acid	18.23	18.11	18.02	17.92
12 Kinic acid	18.63	18.56	18.50	18.43
13 Succinic acid	21.16	20.92	20.71	20.50
14 Glycolic acid	21.66	21.56	21.46	21.36
15 Lactic acid	21.94	21.90	21.85	21.80
16 Fumaric acid	24.35	23.72	23.14	22.62
17 Glutaric acid	23.79	23.11	22.85	22.43
18 Formic acid	23.83	23.69	23.58	23.45
19 Acetic acid	25.55	25.38	25.24	25.06
20 Adipic acid	27.15	26.35	25.62	24.94
21 Levulinic acid	27.10	26.60	26.11	25.65
22 Pyroglutamic acid	29.61	28.96	28.36	27.82
23 Propionic acid	29.20	28.91	28.65	28.36
24 Carbonic acid	31.78	31.85	31.92	31.90
25 Isobutyric acid	32.28	31.91	31.56	31.15
26 n-Butyric acid	34.97	34.43	33.91	33.36
27 Isovaleric acid	39.71	38.96	38.21	37.41
28 n-Valeric acid	48.46	47.01	45.59	44.16

## ■ Retention Index <2 Times Dilution of Mobile Phase Reagents Kit for Organic Acid Analysis: 2.5 mmol/L *p*-toluenesulfonic acid>

Table 6 shows the standard retention times at 35 °C and 45 °C when the acid concentration was reduced by 2 times dilution of the Mobile Phase Reagents Kit for Organic Acid Analysis with ultrapure water.

Table 6 Standard Retention Times of 28 Components of Organic Acid Standard Sample at Various Temperatures (For 2 Times Dilution of Mobile Phase Reagents Kit; Unit: Minutes)

Component	35 °C	45 °C
1 Phosphoric acid	14.66	14.79
2 Maleic acid	14.70	14.61
3 Ketoglutaric acid	14.96	14.84
4 Glucuronic acid	15.73	15.74
5 Citric acid	15.86	15.73
6 Tartaric acid	16.35	16.22
7 Pyruvic acid	16.10	16.00
8 Gluconic acid	17.06	17.04
9 Glyoxylic acid	17.59	17.55
10 Malonic acid	17.43	17.26
11 Malic acid	17.87	17.68
12 Kinic acid	18.31	18.19
13 Succinic acid	21.03	20.60
14 Glycolic acid	21.40	21.21
15 Lactic acid	21.69	21.61
16 Fumaric acid	22.92	21.90
17 Glutaric acid	23.70	22.77
18 Formic acid	23.44	23.22
19 Acetic acid	25.49	25.19
20 Adipic acid	27.06	25.54
21 Levulinic acid	27.05	26.06
22 Pyroglutamic acid	28.231	27.17
23 Propionic acid	29.147	28.61
24 Carbonic acid	31.7	31.92
25 Isobutyric acid	32.24	31.52
26 n-Butyric acid	34.92	33.87
27 Isovaleric acid	39.68	38.17
28 n-Valeric acid	48.47	45.56

## ■ Conclusion

This article introduced examples of application analyses, and the retention indexes using the Nexera Organic Acid Analysis System. Analyses with high repeatability can be conducted easily by using the Mobile Phase Reagents Kit for Organic Acid Analysis. In addition to the food and chemical fields, use in a wide variety of fields is also expected in the future, including the environment, pharmaceuticals, and the life sciences.

Nexera, and Shim-pack are trademarks of Shimadzu Corporation or its affiliated companies in Japan and/or other countries.



Shimadzu Corporation  
Analytical & Measuring Instruments Division  
Global Application Development Center

www.shimadzu.com/an/

### For Research Use Only. Not for use in diagnostic procedures.

This publication may contain references to products that are not available in your country. Please contact us to check the availability of these products in your country.

The content of this publication shall not be reproduced, altered or sold for any commercial purpose without the written approval of Shimadzu. See <http://www.shimadzu.com/about/trademarks/index.html> for details.

Third party trademarks and trade names may be used in this publication to refer to either the entities or their products/services, whether or not they are used with trademark symbol "TM" or "®".

Shimadzu disclaims any proprietary interest in trademarks and trade names other than its own.

The information contained herein is provided to you "as is" without warranty of any kind including without limitation warranties as to its accuracy or completeness. Shimadzu does not assume any responsibility or liability for any damage, whether direct or indirect, relating to the use of this publication. This publication is based upon the information available to Shimadzu on or before the date of publication, and subject to change without notice.