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Application News

Nexera™ Organic Acid Analysis System High Performance Liquid Chromatograph

Nexera Organic Acid Analysis System and its Application

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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.

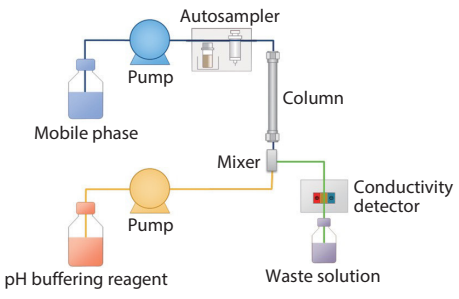
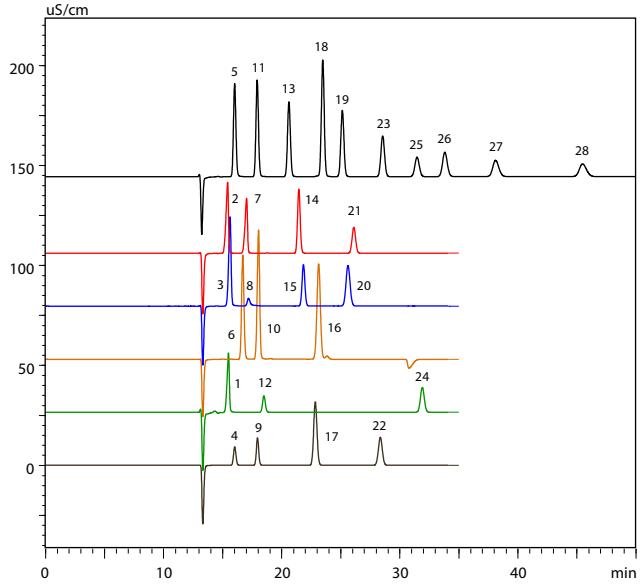


Fig. 1 Flow Path Diagram of Nexera Organic Acid Analysis System



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

(500 mg/L each)

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

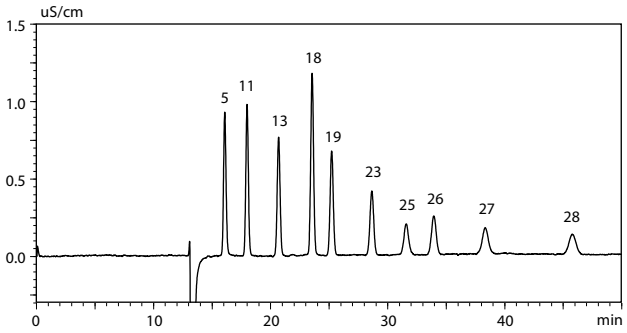
Table 1 Analysis Conditions

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

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



5. Citric acid
11. Malic acid
13. Succinic acid
18. Formic acid
19. Acetic acid
23. Propionic acid
25. Isobutyric acid
26. Butyric acid
27. Isovaleric acid
28. Valeric acid

(10 mg/L each)

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^2)
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

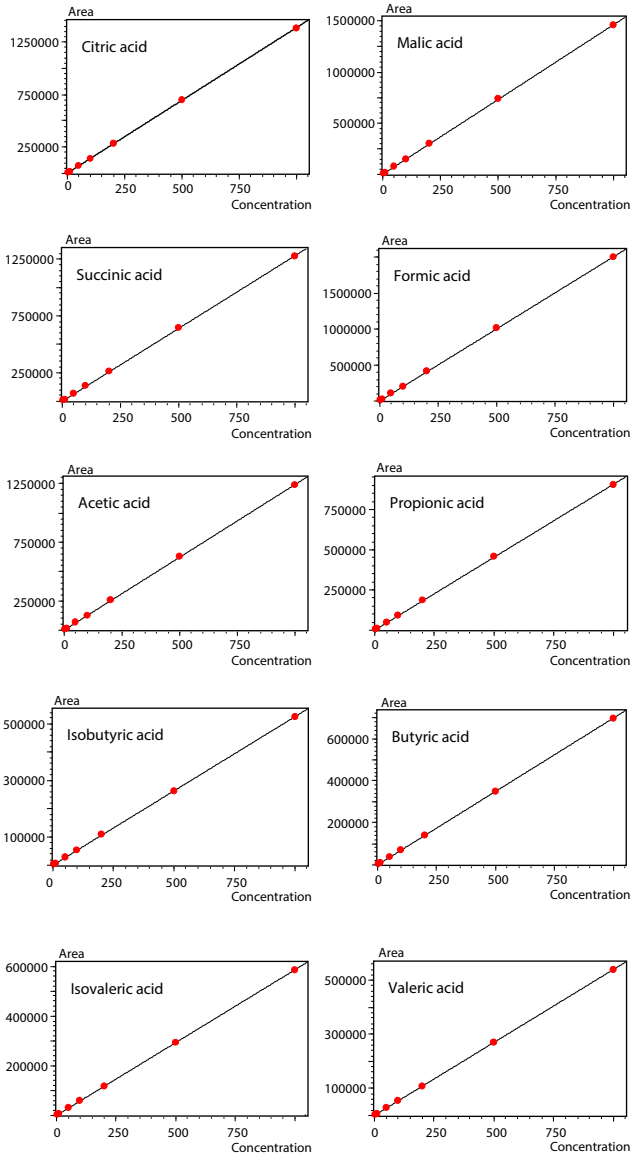
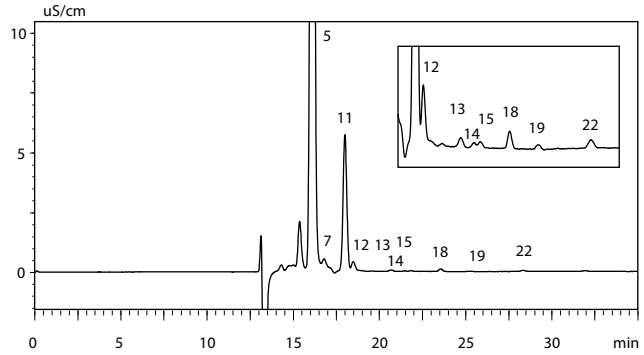


Fig. 4 Calibration Curves of 10 Components of Organic Acid Standard Sample

■ 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.

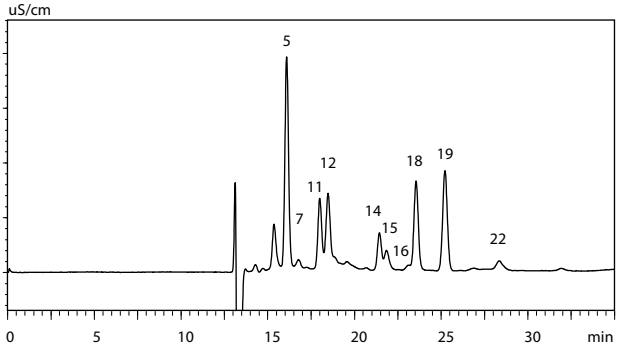


5. Citric acid
7. Pyruvic acid
11. Malic acid
12. Kinic acid
13. Succinic acid
14. Glycolic acid
15. Lactic acid
18. Formic acid
19. Acetic acid
22. Pyroglutamic acid

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.

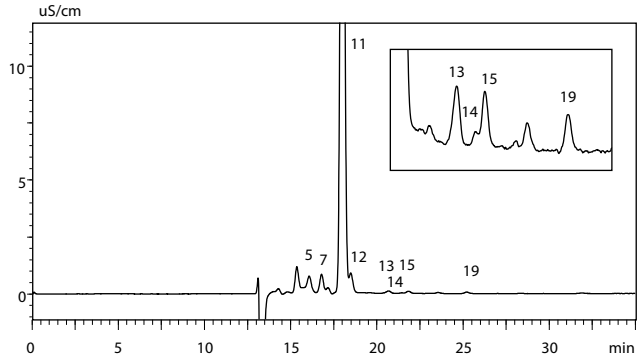


5. Citric acid
7. Pyruvic acid
11. Malic acid
12. Kinic acid
14. Glycolic acid
15. Lactic acid
16. Fumaric acid
18. Formic acid
19. Acetic acid
22. Pyroglutamic acid

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.

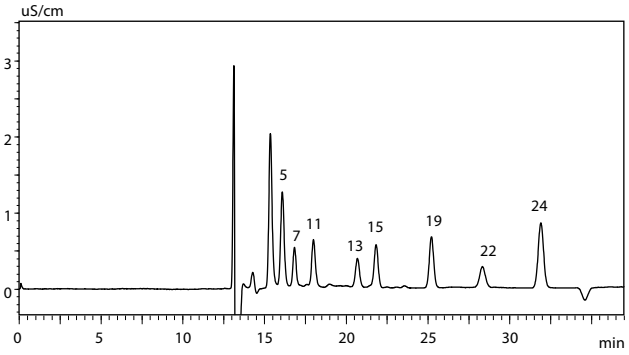


5. Citric acid
7. Pyruvic acid
11. Malic acid
12. Kinic acid
13. Succinic acid
14. Glycolic acid
15. Lactic acid
19. Acetic acid

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.

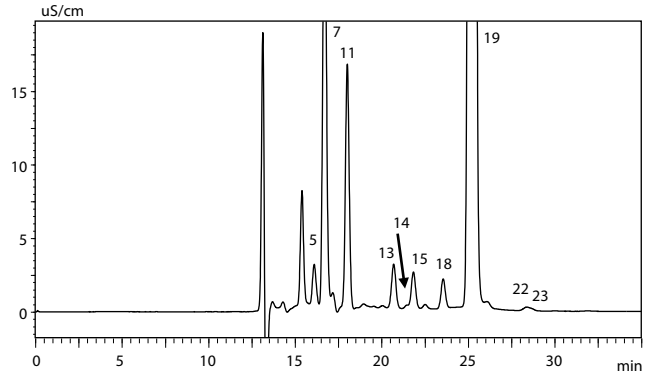


5. Citric acid
7. Pyruvic acid
11. Malic acid
13. Succinic acid
15. Lactic acid
19. Acetic acid
22. Pyroglutamic acid
24. Carbonic acid

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.

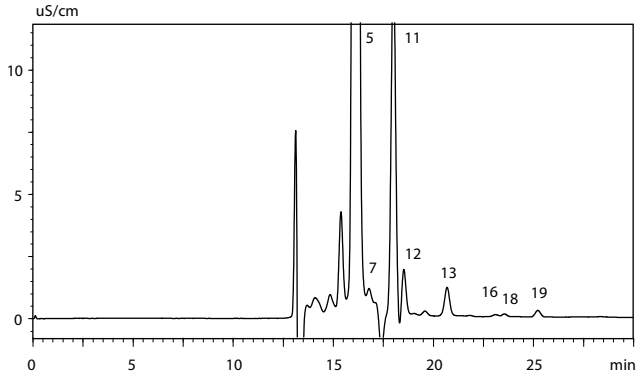


5. Citric acid
7. Pyruvic acid
11. Malic acid
13. Succinic acid
14. Glycolic acid
15. Lactic acid
18. Formic acid
19. Acetic acid
22. Pyroglutamic acid
23. Propionic acid

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.

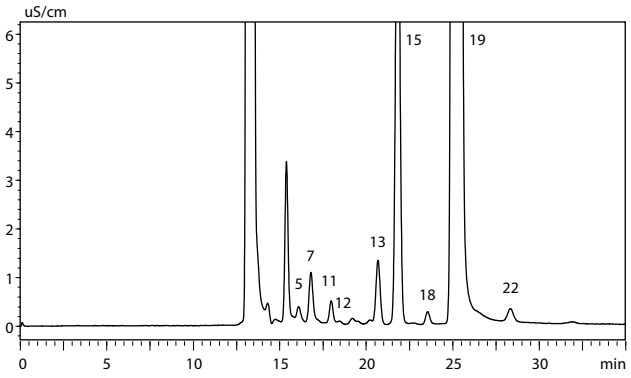


5. Citric acid
7. Pyruvic acid
11. Malic acid
12. Kinic acid
13. Succinic acid
16. Fumaric acid
18. Formic acid
19. Acetic acid

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.

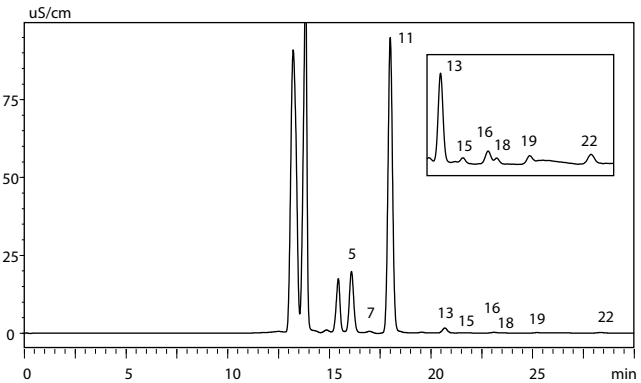


5. Citric acid
7. Pyruvic acid
11. Malic acid
12. Kinic acid
13. Succinic acid
15. Lactic acid
18. Formic acid
19. Acetic acid
22. Pyroglutamic acid

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.

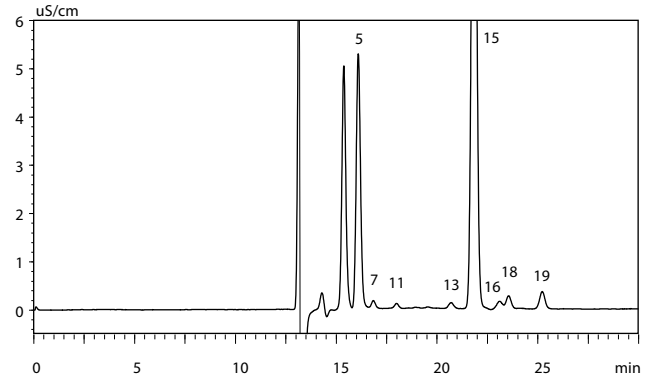


5. Citric acid
7. Pyruvic acid
11. Malic acid
13. Succinic acid
15. Lactic acid
16. Fumaric acid
18. Formic acid
19. Acetic acid
22. Pyroglutamic acid

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.

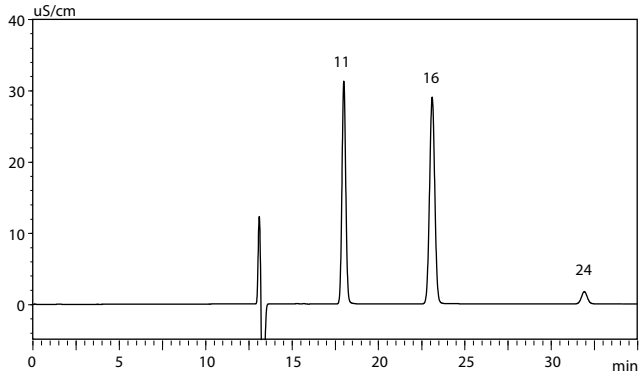


5. Citric acid
7. Pyruvic acid
11. Malic acid
13. Succinic acid
15. Lactic acid
16. Fumaric acid
18. Formic acid
19. Acetic acid

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.

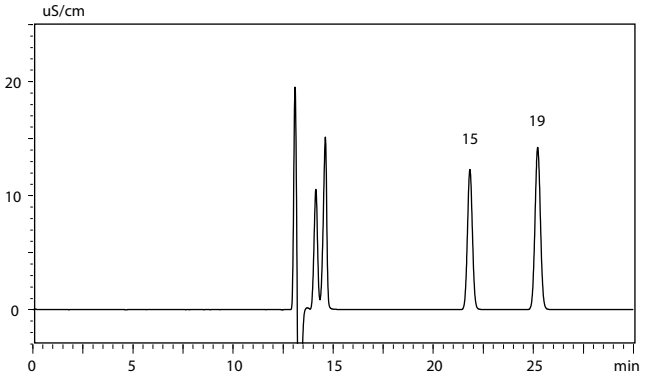


11. Malic acid
16. Fumaric acid
24. Carbonic acid

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.

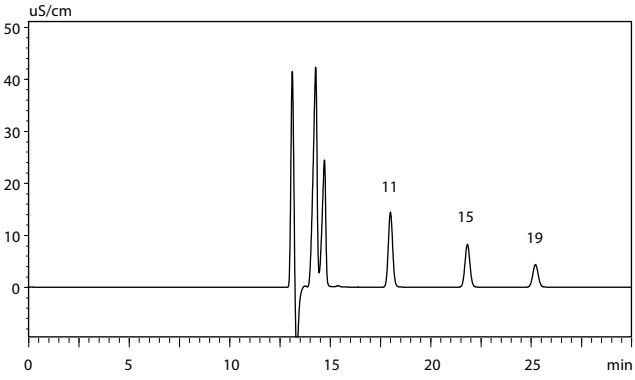


15. Lactic acid
19. Acetic acid

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.

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Analytical & Measuring Instruments Division
Global Application Development Center

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