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Technical Report

High-Speed Analysis of Organic Acids Using Shim-pack Fast-OA and pH-Buffered Electrical Conductivity Detection

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Abstract:

Organic acids are analyzed in a wide variety of fields, not only in food products, but also as counterions in pharmaceuticals, and ingredients in chemical products. Because post-column pH-buffered electrical conductivity detection is capable of analyzing organic acids selectively and with high sensitivity, this method is used to analyze organic acids in samples that contain many contaminants. However, shortening the analysis time when monitoring fermentation or analyzing intestinal flora has been problematic. Additionally, in research on the generation of alternative energies from biomass materials, the quantities of organic acids generated as metabolic products must be analyzed quickly in order to flexibly control the activity of microorganisms. This report describes techniques for achieving the high-speed analysis of organic acids with high selectivity, using the Shimadzu Shim-pack Fast-OA high-speed organic acid analytical column, in combination with the post-column pH-buffered electrical conductivity detection method.

Keywords: organic acid analysis, high-speed, post-column pH-buffered electrical conductivity detection, mobile phase reagent set for organic acid analysis

1. Analyzing Organic Acids by Detecting Electrical Conductivity

Organic acids absorb short wavelengths due to the carboxyl group they contain, which means that analysis is easily affected by contaminants, and that the detection method must be modified to achieve high sensitivity and specificity.

Shimadzu's unique post-column pH-buffered electrical conductivity detection method involves the successive addition of a pH-buffering reagent after column separation, in order to adjust the pH level to close to neutral. This not only reduces background noise, but also dissociates organic acids from the substance being analyzed. Consequently, electrical conductivity detection can then detect these organic acids with high sensitivity and selectivity.

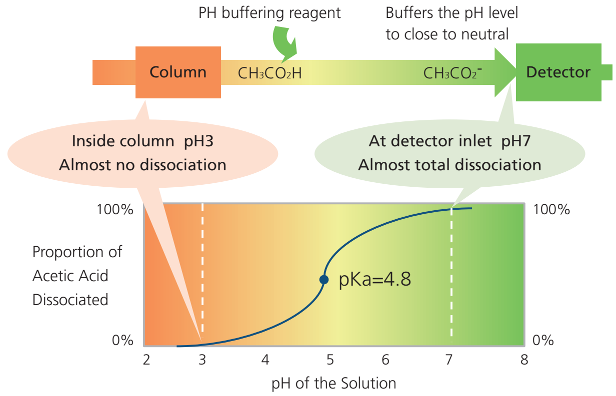
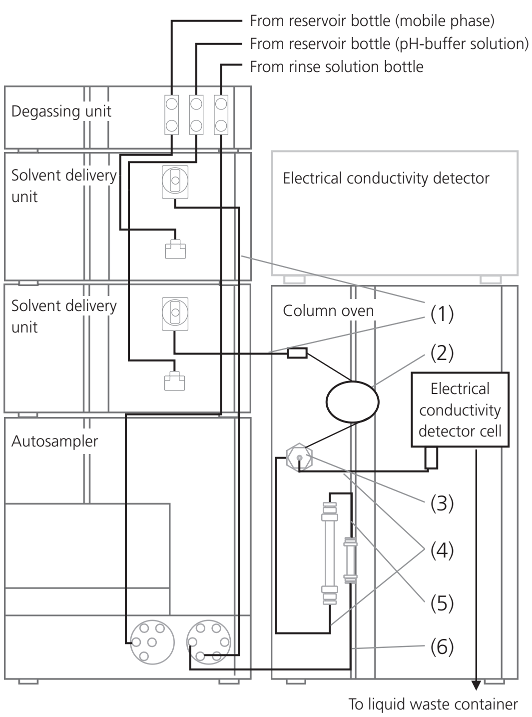


Fig. 1 Status of Organic Acids in the PH-Buffering Method

The post-column pH-buffered electrical conductivity detection system consists of flow channels for post-column buffering, and a CDD-10AVP electrical conductivity detector. A schematic of the tubing is shown in Fig. 2. Each organic acid component separated by the column is mixed with a pH-buffer solution in the mixer, and is then delivered in a dissociated state to the electrical conductivity detector, where it is detected with high sensitivity.



	Flow Channel Components
1	0.3 mm I.D. × 600 mm stainless steel tubing
2	0.1 mm I.D. × 2000 mm stainless steel tubing
3	MR-100 High-efficiency microreactor mixer
4	0.13 mm I.D. PEEK tubing (100 mm Length) *1
5	Stainless steel tubing with guard column
6	0.1 mm I.D. × 600 mm stainless steel tubing

*1: Before use, cut this from a commercially available 2-meter coil using a tube cutter.

Fig. 2 Tubing Diagram

1 Analytical & Measuring Instruments Division

2. Retention Time Index for Organic Acid High-Speed Analytical Columns

Shim-pack Fast-OA columns are packed with an ion-exclusion polymer, and utilizing an acidic mobile phase, can separate mixture solution based on the pKa value of each sample component. The analytical conditions for this column are shown in Table 1, and the chromatograms of 25 organic acids are shown in Fig. 3. Because the column design is optimized for the high-speed analysis of organic acids, even short-chain fatty acids such as valeric acid are eluted within 10 minutes.

Reference retention time values for each organic acid component when the Shim-pack Fast-OA column is used are shown in Table 2 and Fig. 4. In the ion exclusion mode, the degree of separation depends on the column temperature and the mobile phase concentration. If separation of target components is inadequate, the table below can presumably be used as a reference for improving separation by changing the column temperature. However, it would be difficult to separate all the components in the table simultaneously.

Table 1 Analytical Conditions

Column	: Shim-pack Fast-OA (100 mm L. × 7.8 mm I.D., 5 µm)
Guard column	: Shim-pack Fast-OA (G) (10 mm L. × 4.0 mm I.D.)
Mobile phase	: 5 mmol/L p-toluenesulfonic acid
Flow rate	: 0.8 mL/min
pH buffering solution	: 5 mmol/L p-toluenesulfonic acid 20 mmol/L Bis- Tris 0. 1 mmol/L EDTA
Flow rate	: 0.8 mL/min
Detection	: Conductivity detector (CDD-10AVP)
Injection volume	: 10 µL

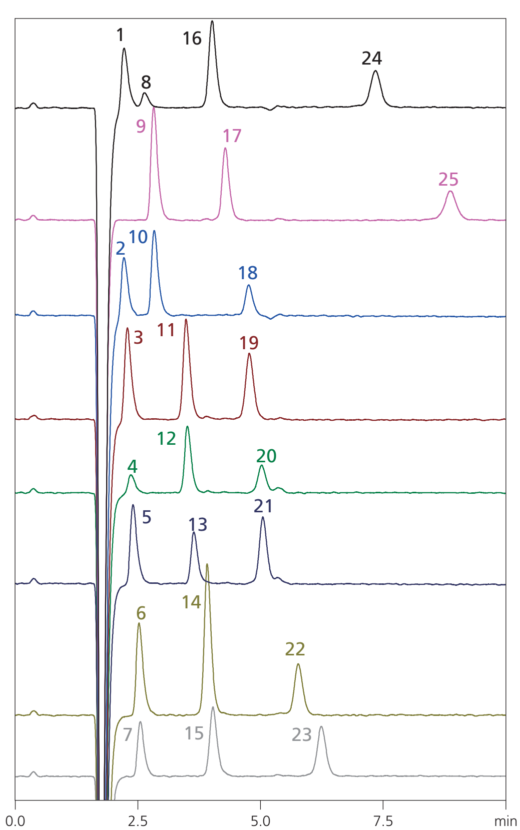


Fig. 3 Chromatogram of Standard Sample at 45 °C

Note 1: There is a dip in the baseline near the 1.8-minute point due to the sample solvent (water).
Note 2: Peak numbers and corresponding organic acids are as indicated in Table2

Table 2 Reference Retention Time Values for Organic Acids (with One Column)

	Organic acids	35°C	40°C	45°C	50°C
1	Phosphoric acid	2.209	2.228	2.250	2.272
2	Maleic acid	2.260	2.252	2.244	2.237
3	Alpha-ketoglutaric acid	2.333	2.321	2.310	2.299
4	Glucuronic acid	2.377	2.380	2.384	2.385
5	Citric acid	2.444	2.432	2.421	2.410
6	Tartaric acid	2.565	2.553	2.543	2.532
7	Pyruvic acid	2.577	2.573	2.570	2.566
8	Gluconic acid	2.654	2.657	2.659	2.660
9	Malonic acid	2.874	2.857	2.841	2.826
10	Malic acid	2.883	2.866	2.850	2.835
11	Succinic acid	3.573	3.531	3.490	3.451
12	Glycolic acid	3.545	3.531	3.519	3.505
13	Lactic acid	3.657	3.654	3.649	3.642
14	Formic acid	3.951	3.934	3.918	3.900
15	Glutaric acid	4.221	4.121	4.031	3.946
16	Fumaric acid	4.246	4.124	4.015	3.914
17	Acetic acid	4.329	4.307	4.284	4.260
18	Levulinic acid	4.950	4.850	4.754	4.662
19	Adipic acid	5.083	4.916	4.763	4.622
20	Pyroglutamic acid	5.238	5.116	5.012	4.916
21	Propionic acid	5.125	5.084	5.041	4.995
22	Isobutyric acid	5.879	5.823	5.763	5.696
23	Butyric acid	6.400	6.316	6.227	6.134
24	Isovaleric acid	7.594	7.464	7.328	7.182
25	Valeric acid	9.355	9.099	8.840	8.584

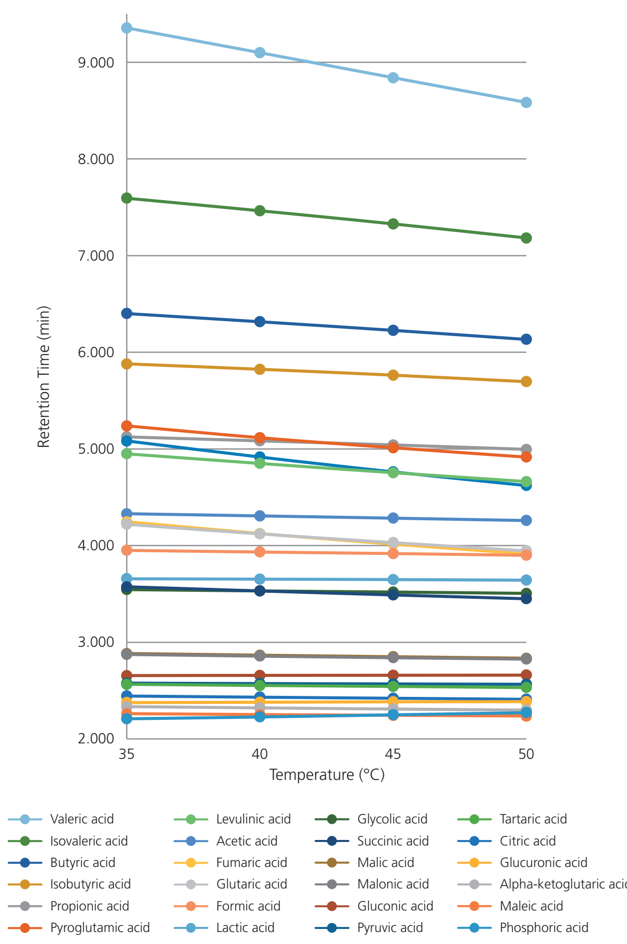


Fig. 4 Changes in Organic Acid Retention Times with Temperature

3. Improving the Separation by Connecting Multiple Columns in Series

If separation with one column is inadequate, up to three Shim-pack Fast-OA columns can be connected in series to improve the separation. Table 3 indicates reference retention time values when two connected columns are used, and Table 4 indicates reference retention time values when three connected columns are used.

As an example, there is almost no difference between the retention times for lactic acid and formic acid, which makes it difficult to achieve full separation with only one column. Chromatograms for up to three connected columns are shown in Fig. 5. These illustrate that connecting the columns can improve the separation.

Table 3 Reference Retention Time Values for Organic Acids (with Two Columns)

	Organic acids	35°C	40°C	45°C	50°C
1	Phosphoric acid	4.017	4.051	4.090	4.126
2	Maleic acid	4.150	4.118	4.086	4.062
3	Alpha-ketoglutaric acid	4.292	4.260	4.219	4.178
4	Glucuronic acid	4.415	4.409	4.403	4.400
5	Citric acid	4.531	4.489	4.449	4.412
6	Tartaric acid	4.783	4.743	4.704	4.666
7	Pyruvic acid	4.821	4.796	4.772	4.755
8	Gluconic acid	4.988	4.986	4.973	4.957
9	Malonic acid	5.452	5.400	5.347	5.305
10	Malic acid	5.455	5.402	5.350	5.303
11	Succinic acid	6.906	6.796	6.688	6.585
12	Glycolic acid	6.860	6.813	6.764	6.724
13	Lactic acid	7.089	7.072	7.040	7.003
14	Formic acid	7.710	7.657	7.599	7.549
15	Glutaric acid	8.253	8.019	7.798	7.601
16	Fumaric acid	8.271	7.996	7.738	7.508
17	Acetic acid	8.503	8.435	8.361	8.293
18	Levulinic acid	9.787	9.556	9.320	9.096
19	Adipic acid	10.068	9.687	9.328	9.009
20	Pyroglutamic acid	10.345	10.076	9.822	9.601
21	Propionic acid	10.154	10.043	9.923	9.799
22	Isobutyric acid	11.738	11.589	11.423	11.257
23	Butyric acid	12.807	12.608	12.380	12.147
24	Isovaleric acid	15.296	14.991	14.640	14.310
25	Valeric acid	18.927	18.335	17.723	17.136

Table 4 Reference Retention Time Values for Organic Acids (with Three Columns)

	Organic acids	35°C	40°C	45°C	50°C
1	Phosphoric acid	5.852	5.908	5.969	6.037
2	Maleic acid	6.050	6.014	5.978	5.948
3	Alpha-ketoglutaric acid	6.268	6.210	6.174	6.124
4	Glucuronic acid	6.435	6.432	6.435	6.437
5	Citric acid	6.624	6.574	6.522	6.477
6	Tartaric acid	6.996	6.946	6.897	6.856
7	Pyruvic acid	7.047	7.022	6.997	6.976
8	Gluconic acid	7.303	7.289	7.292	7.282
9	Malonic acid	7.979	7.911	7.848	7.792
10	Malic acid	8.000	7.934	7.867	7.808
11	Succinic acid	10.168	10.016	9.868	9.734
12	Glycolic acid	10.085	10.031	9.974	9.922
13	Lactic acid	10.437	10.406	10.383	10.345
14	Formic acid	11.349	11.278	11.210	11.148
15	Glutaric acid	12.184	11.855	11.543	11.263
16	Fumaric acid	12.192	11.802	11.438	11.118
17	Acetic acid	12.540	12.455	12.363	12.272
18	Levulinic acid	14.478	14.135	13.814	13.501
19	Adipic acid	14.896	14.344	13.834	13.372
20	Pyroglutamic acid	15.290	14.912	14.558	14.244
21	Propionic acid	15.016	14.866	14.705	14.545
22	Isobutyric acid	17.383	17.181	16.959	16.730
23	Butyric acid	18.993	18.693	18.392	18.069
24	Isovaleric acid	22.707	22.261	21.792	21.314
25	Valeric acid	28.180	27.323	26.455	25.607

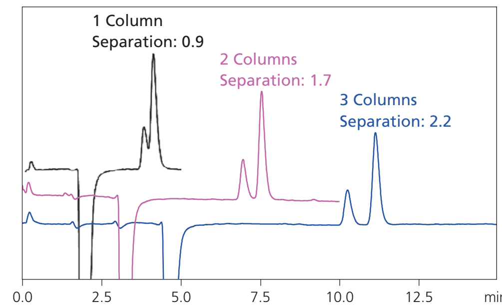


Fig. 5 Improvement in Separation by Connecting Multiple Columns

4. Example of a Standard Sample Analysis

The types of organic acids targeted will differ depending on the type of sample analyzed. Separation patterns for organic acids typically analyzed in various fields are shown in Figs. 6 to 9.

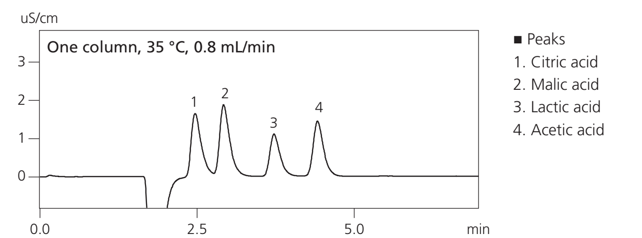


Fig. 6 Four Organic Acid Components Analyzed in the Food Industry

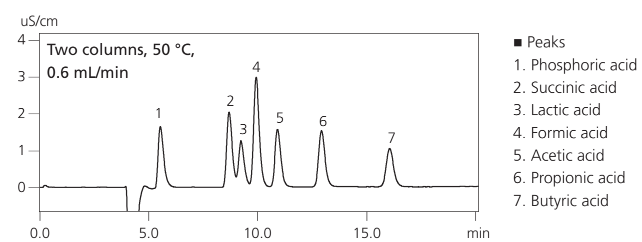


Fig. 7 Seven Organic Acid Components Analyzed in Intestinal Flora Research

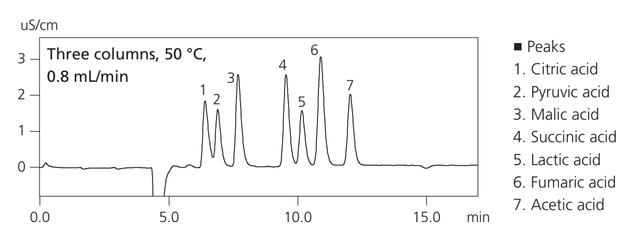


Fig. 8 Seven Organic Acid Components Analyzed in Culture Media Analysis

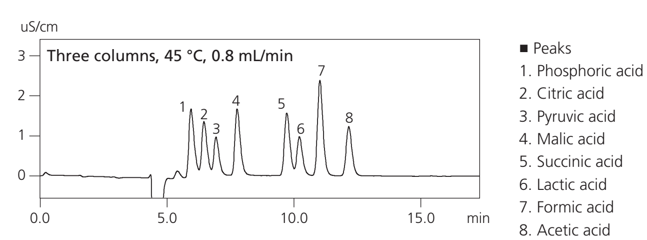


Fig. 9 Eight Organic Acid Components

5. Analyzing Actual Samples

When used in combination with a Shim-pack Fast-OA high-speed organic acid analytical column, the post-column pH-buffered electrical conductivity detection method is ideal for applications that require the high-speed analysis of samples containing a large number of contaminants, such as in fermentation monitoring.

The results from an analysis of organic acids added to a culture solution are shown in Fig. 10 and the analytical conditions are shown in Table 5. Because this method can shorten analysis cycle times to 20 minutes or less, it can not only reduce mobile phase consumption and improve operating efficiency, but can also be used to control the status of cultures, or ensure a given number of repeated analyses.

Table 6 shows the calibration curve for the 7 organic acids. The calibration curve is shown in Fig. 11. A good linearity of R^2 = 0.999 or more was obtained for each component.

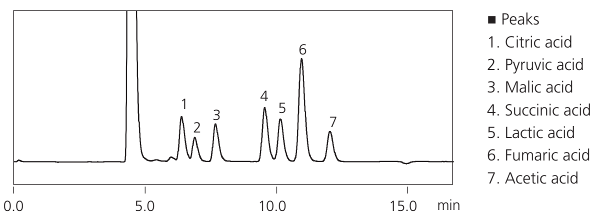


Fig. 10 Chromatogram of a Culture Solution

Table 5 Analytical Conditions

Column	: Shim-pack Fast-OA 3 columns in series (100 mm L. × 7.8 mm I.D., 5 µm)
Guard column	: Shim-pack Fast-OA (G) (10 mm L. × 4.0 mm I.D.)
Mobile phase	: 5 mmol/L p-toluenesulfonic acid
Flow rate	: 0.8 mL/min
pH buffering solution	: 5 mmol/L p-toluenesulfonic acid 20 mmol/L Bis- Tris 0. 1 mmol/L EDTA
Flow rate	: 0.8 mL/min
Column Temperature	: 50 °C
Detection	: Conductivity detector (CDD-10Avp)
Injection volume	: 10 µL

Table 6 Calibration Curve Concentration Range and Contribution Rate for Target Components

Compound	Calibration Curve Range (mg/L)	Contribution Rate (R^2)
Citric acid	5-100	0.99944
Pyruvic acid	5-100	0.99999
Malic acid	1-100	0.99997
Succinic acid	1-100	0.99997
Lactic acid	1-100	0.99992
Fumaric acid	1-100	0.99994
Acetic acid	1-100	0.99995

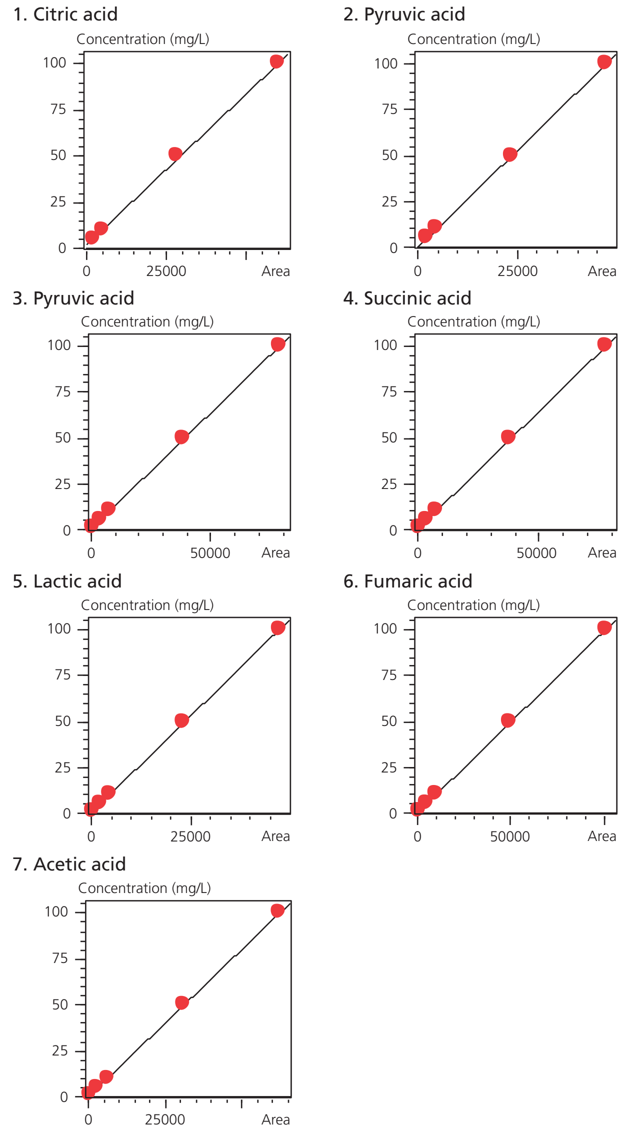


Fig. 11 Calibration Curves for Target Components

6. Conclusion

- Shim-pack Fast-OA high-speed analytical columns for organic acid analysis can shorten analysis cycle times.
- When the post-column pH-buffered electrical conductivity detection method is used in combination with a high-speed analytical column for organic acid analysis, organic acids can be selectively detected, with minimal impact from the contaminants in samples.

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