# 3. Organic Acids

Organic acids are weakly acidic substances having carboxyl groups in their molecular structure, and are also referred to as carboxylic acids. When analyzing organic acids, ion exclusion, reversed-phase, ion exchange (ion chromatography), size exclusion, and other types of chromatography are used. Shodex columns for organic acid analysis include RSpak KC-811 (a combination of ion exclusion mode and reversed-phase mode), RSpak DE-413 (polymeric reversed-phase column), and IC SI-50 4E (anion chromatography column), as well as SUGAR SH1011 (column for analysis of saccharides and organic acids, described in Section 2). Table 3-1 shows a lineup of Shodex packing columns for organic acid analysis.

**Table 3-1. Shodex Columns for Organic Acid Analysis**

| Product Code | Product Name | Separation Mode*1 | Theoretical PlateNumber (TP/column) | Particle Size (μm) | ID x Length (mm) |
|---|---|---|---|---|---|
| F6378030 | RSpak KC-811 | IEX+RP | ≥17,000 | 6 | 8.0 x 300 |
| F6700030 | RSpak KC-G | IEX+RP | (Guard column) | 10 | 6.0 x 50 |
| F6700010 | RSpak KC-LG | IEX+RP | (Guard column) | 12 | 8.0 x 50 |
| F7008140 | RSpak NN-814 | IEX+RP | ≥9,000 | 10 | 8.0 x 250 |
| F6700510 | RSpak NN-G | IEX+RP | (Guard column) | 10 | 6.0 x 50 |
| F7001004 | RSpak DE-613 | RP | ≥7,000 | 6 | 6.0 x 150 |
| F7001005 | RSpak DE-413 | RP | ≥11,000 | 4 | 4.6 x 150 |
| F7009030 | RSpak DE-413L | RP | ≥17,000 | 4 | 4.6 x 250 |
| F7001006 | RSpak DE-413S | RP | ≥3,000 | 4 | 4.6 x 50 |
| F6700150 | RSpak DE-G | RP | (Guard column) | 10 | 4.6 x 10 |
| F7001007 | RSpak DE-213 | RP | ≥8,000 | 4 | 2.0 x 150 |
| F6700151 | RSpak DE-SG | RP | (Guard column) | 4 | 2.0 x 10 |
| F6378100 | SUGAR SH1011 | SEC + IEX | ≥17,000 | 6 | 8.0 x 300 |
| F6378101 | SUGAR SH1821 | SEC + IEX | ≥17,000 | 6 | 8.0 x 300 |
| F6700080 | SUGAR SH-G | - | (Guard column) | 10 | 6.0 x 50 |
| F6995244 | IC SI-90 4E | AEC | ≥5,000 | 9 | 4.0 x 250 |
| F6995245 | IC SI-50 4E | AEC | ≥10,000 | 5 | 4.0 x 250 |
| F6709620 | IC SI-90G | AEC | (Guard column) | 9 | 4.6 x 10 |

*1 : SEC (Size Exclusion), IEX (Ion Exclusion), RP (Reversed Phase) , AEC (Anion Exchange)

## 3-1. Separation Mechanism

Shodex can provide several columns for analysis of organic acids depending on other substances in the sample. (Table 3-2)

**Table 3-2. Column Selection for Samples**

| Column | Separation Mode | Sample |
|---|---|---|
| RSpak KC-811 | Ion Exclusion + Reversed Phase | General Organic Acids |
| RSpak NN-814 | Ion Exclusion + Reversed Phase | Aromatic Organic Acids |
| RSpak DE-413 | Reversed Phase | General Organic Acids |
| SUGAR SH1011<br>SUGAR SH1821 | Size Exclusion + Ion Exclusion | Organic Acids and Sugars |
| IC SI-90 4E<br>IC SI-50 4E | Anion Exchange | Organic Acids and Anions |

### 3-1-1. Ion Exclusion Mode

In organic acid analysis, a strong cation exchange resin with sulfo-groups bound to the packing surface is used. Weak acids, such as organic acids, exhibit only partial dissociation when dissolved in water. In a dissociated state (negatively charged), the positive charge of the cation is neutralized and excluded by the negative charge of the sulfo-groups on the resin surface. There is no concern over organic acid adsorption into the resin. In a non-dissociated state, on the other hand, ion exclusion does not occur; hydrophobic adsorption into the resin substrate occurs instead to allow organic acids to be retained by the resin (Figure 3-1).

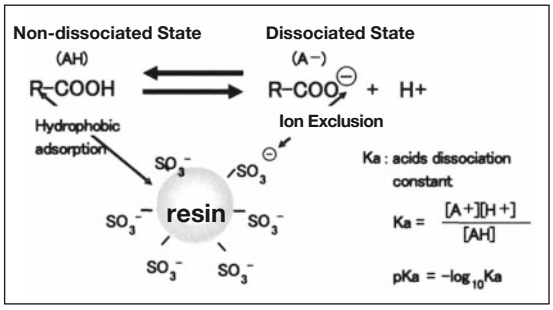



Fig. 3-1 Ion Exclusion Mode

Hence, organic acids are eluted in ascending order of pKa (descending order of acidity) and in descending order of polarity. The packing substrate of KC-811 is a styrene-divinylbenzene copolymer, therefore it is recommended to use NN-814, which has a higher polarity of packing substrate than KC-811, when analyzing aromatic organic acids of low polarity. The packing substrate of NN-814 is poly(hydroxymethacrylate).

### 3-1-2. Reversed Phase Mode

The reversed-phase mode is the most commonly used separation mode of HPLC. This mode is based on the hydrophobic interaction between the portion of low polarity of the packing and the portion of low polarity of the sample. Hence, elution occurs in descending order of polarity. A key to successful organic acid analysis in reversed-phase mode resides in thoroughly suppressing the dissociation of organic acids and reducing the polarity of the sample. Organic acids are generally reported to become non-dissociated when the eluent has a pH value lower than the pKa by 1.5.

### 3-1-3. Anion Exchange Mode

The resin surface is positively charged. The cation on the resin is scrambled by the anion of the component mobile phase and the organic acid anion; elution occurs in ascending order of ionic adsorptivity.

### 3-1-4. Size Exclusion Mode

Organic acids are eluted in ascending order of molecular size. Molecules larger than the pore size of resin are eluted $V_0$ position.

### 3-1-5. Detection of Organic Acids

The functional group shared by organic acids is the carboxyl group, which has a UV absorption band between 200 and 210nm, thus enabling the use of a UV detector. However, because many other organic substances have the same wavelength band for UV absorption, and also because the molar extinction coefficient is low at 50 to 70, detection of organic acids is likely to be influenced by impurities. The RI detector lacks selectivity and remains somewhat problematic with regard to sensitivity. Detection using the conductivity detector (CD) is prone to sensitivity variation depending on the kind of organic acid. As a method of selectively detecting organic acids, the post-column method using a pH indicator is available which employs a visible absorption spectrum (VIS) detector. (Fig. 3-2)

| | | |
|---|---|---|
| Column | : | Shodex RSpak KC-G (6.0mmID x 50mm) + KC-811 (8.0mmID x 300mm)x2 |
| Eluent | : | 3mM $HClO_4$ aq. |
| Flow Rate | : | 1.0mL/min |
| Column Temp. | : | 50℃ |

Sample:
1. 800ppm Citric Acid
2. 900ppm Tartaric Acid
3. 800ppm Malic Acid
4. 700ppm Succinic Acid
5. 1100ppm Lactic Acid
6. 600ppm Formic Acid
7. 700ppm Acetic Acid
8. 1500ppm Levulinic Acid
9. 1500ppm Pyroglutamic Acid

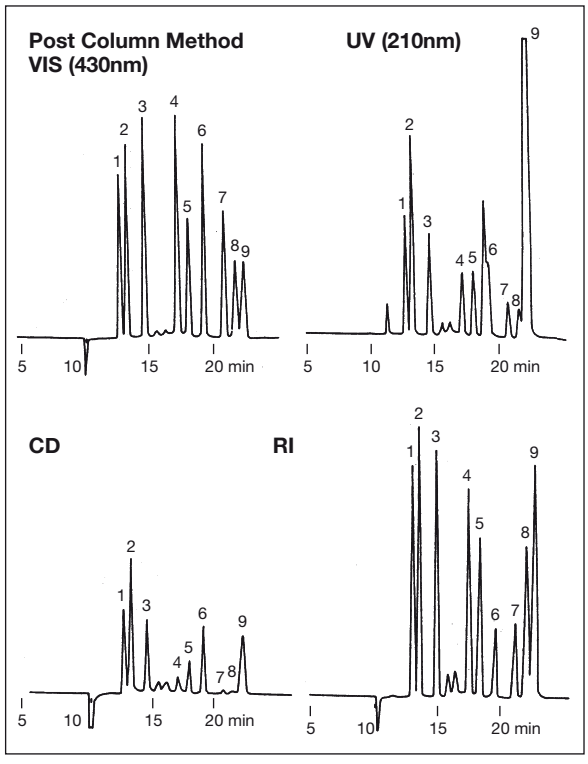
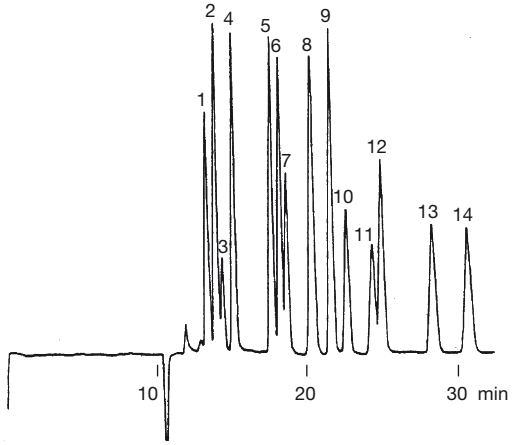



Fig. 3-2 Comparison of Chromatograms with Various Detectors

## 3-2. Organic Acids

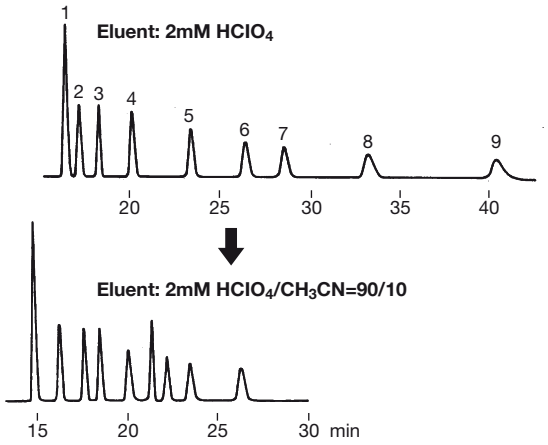



Sample:
1. Citric Acid
2. Tartaric Acid
3. Pyrubic Acid
4. Malic Acid
5. Succinic Acid
6. Glyconic Acid
7. Lactic Acid
8. Fumaric Acid
9. Acetic Acid
10. Levulinic Acid
11. Pyroglutamic Acid
12. Propionic Acid
13. Isobutyric Acid
14. n-Butyric Acid

General organic acids were analyzed with the post column method. After separation with the KC-811 column, a reaction reagent was added and detected with 430nm.

| | | |
|---|---|---|
| Column | : | Shodex RSpak KC-811 (8.0mmID x 300mm) x 2 |
| Eluent | : | 6mM $HClO_4$ aq. |
| Reagent | : | 10-fold diluted ST3-R (For Post Column Method) |
| Flow Rate | : | 1.0mL/min |
| Detector | : | VIS (430nm) |
| Column Temp. | : | 50℃ |

**Fig. 3-3. Analysis of Organic Acids with Post Column Method**

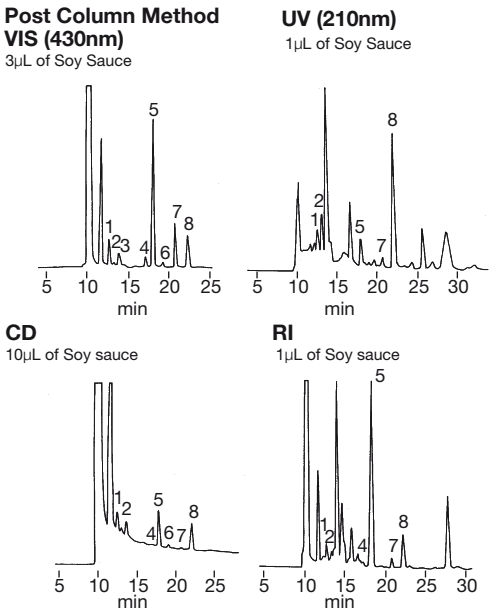



Sample:
1. Succinic Acid
2. Lactic Acid
3. Formic Acid
4. Acetic Acid
5. Propionic Acid
6. Isobutyric Acid
7. n-Butyric Acid
8. Isovaleric Acid
9. n-Valeric Acid

The elution time of organic acids with strong hydrophobicity can be shortened by adding 10% acetonitrile to the eluent.

| | | |
|---|---|---|
| Column | : | Shodex RSpak KC-LG (8.0mmID x 50mm) + KC-811 (8.0mmID x 300mm) x 2 |
| Reagent | : | 10-fold diluted ST3-R (For Post Column Method) |
| Flow Rate | : | (Eluent); 1.0mL/min, (Reagant); 0.5mL/min |
| Detector | : | VIS (430nm) |
| Column Temp. | : | 47℃ |

**Fig. 3-4 Effect of Elution of Organic Acids with Addition of Acetonitrile**

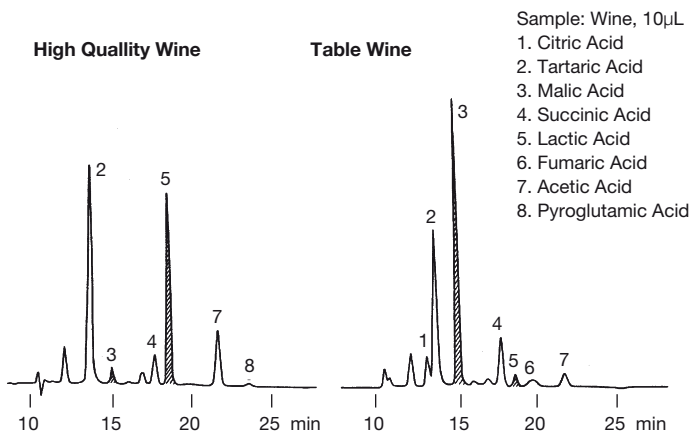



Sample: Soy Sauce
1. Citric Acid
2. Pyruvic Acid
3. Malic Acid
4. Succinic Acid
5. Lactic Acid
6. Formic Acid
7. Acetic Acid
8. Pyroglutamic Acid

For actual samples such as foods containing amino acids, sugars, NaCl, ions, organic substances, etc., the post column method is recommended because it enables highly selective analysis. UV detection (210 nm) analyses many organic substances other than organic acids, and conductivity detection produces a major peak of $Cl^-$ ions excluded at a position corresponding to nearly 10 minutes; therefore, the quantifiability of organic acids is affected with these methods. In the case of RI detection, organic acids are difficult to quantify because almost all components, including sugars, are detected.

| | | |
|---|---|---|
| Column | : | Shodex RSpak KC-G (6.0mmID x 50mm) + KC-811 (8.0mmID x 300mm) x 2 |
| Eluent | : | 3mM $HClO_4$ aq. |
| Flow Rate | : | 1.0mL/min |
| Column Temp. | : | 50℃ |

**Fig. 3-5 Comparison of Results of Soy Sauce with Various Detectors**

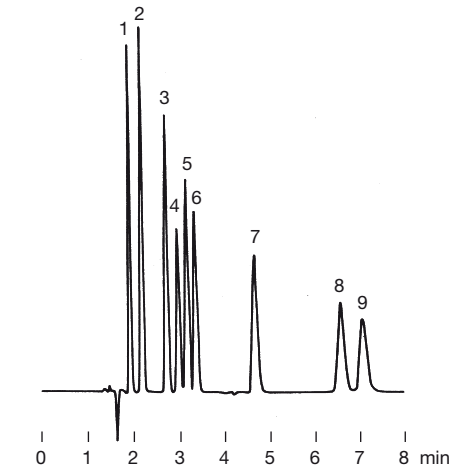



Sample: Wine, 10μL
1. Citric Acid
2. Tartaric Acid
3. Malic Acid
4. Succinic Acid
5. Lactic Acid
6. Fumaric Acid
7. Acetic Acid
8. Pyroglutamic Acid

Grapes are rich in malic acid. Wine reportedly becomes mellow as a result of malic acid conversion to lactic acid by the action of enzymes during malo-lactic fermentation. Vintage items prove to have almost all malic acid converted to lactic acid.

| | | |
|---|---|---|
| Column | : | Shodex RSpak KC-LG (8.0mmID x 50mm) + KC-811 (8.0mmID x 300mm) x 2 |
| Eluent | : | 3mM $HClO_4$ aq. |
| Reagent | : | 1/10 ST3-R (For Post Column Method) |
| Flow Rate | : | (Eluent); 1.0mL/min (Reagent); 0.7mL/min |
| Detector | : | VIS (430nm) |
| Column Temp. | : | 45℃ |

**Fig. 3-6 Organic Acids in White Wine**

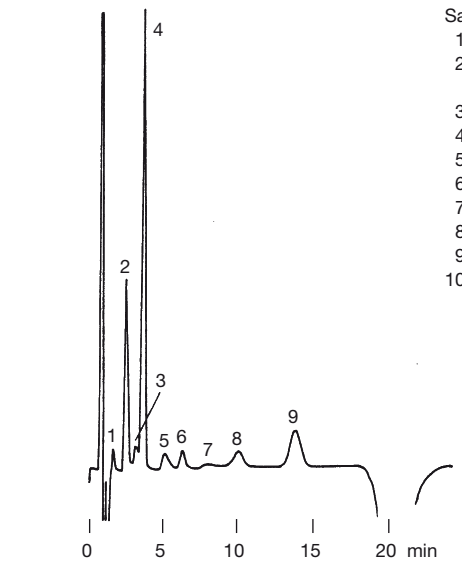



Sample:
1. Glyoxylic Acid, 1.78mg/mL
2. Tartaric Acid, 1.95mg/mL
3. Malic Acid, 2.06mg/mL
4. Lactic Acid, 2μL/mL
5. Malonic Acid, 1.95mg/mL
6. Acetic Acid, 2μL/mL
7. Succinic Acid, 2.05mg/mL
8. Levulinic Acid, 1.95mg/mL
9. Propionic Acid, 2μL/mL

When water or 100% buffer is used as the eluent, the column life of ODS columns is usually shortened. Since the packing material of RSpak DE-413 is chemically stable, long column life and stable analysis results can be obtained even when water or 100% buffer was used as the eluent.

| | | |
|---|---|---|
| Column | : | Shodex RSpak DE-413 (4.6mmID x 150mm) |
| Eluent | : | 10mM $H_3PO_4$ aq. |
| Flow Rate | : | 1.0mL/min |
| Detector | : | Shodex RI |
| Column Temp. | : | 50℃ |

**Fig. 3-7 Organic Acids (Reversed Phase Mode)**




Sample: 10-fold diluted Beer, 30μL
1. Acetic Acid
2. $H_2PO_4^-$, Succinic Acid, Pyroglutamic Acid
3. Lactic Acid, Pyruvic Acid
4. $Cl^-$
5. Malic Acid, $Br^-$
6. $NO_3^-$
7. Oxalic Acid
8. Citric Acid
9. $SO_4^{2-}$
10. Tartaric Acid

The eluent used here is suitable for the simultaneous analysis of inorganic anions and dibasic and tribasic organic acids using IC I-524A. Organic acids are contained in beer and they interfere with the quantification of anions. $Cl^-$, $NO_3^-$ and $SO_4^{2-}$ can be separated without such interference of organic acids. Organic acids, oxalic acid and citric acid also can be analyzed without interference.

| | | |
|---|---|---|
| Column | : | Shodex IC I-524A (4.6mmID x 100mm) |
| Eluent | : | 1.5mM Phthalic acid + 1.38mM Tris(hydroxymethyl) aminomethane(pH4.0) + 300mM Boric Acid |
| Flow Rate | : | 1.2mL/min |
| Detector | : | Shodex CD |
| Column Temp. | : | 40℃ |

**Fig. 3-8 Organic Acids and Anions in Beer (Anion Exchange Mode)**

Tables of elution volume of organic acids with each column are shown in appendix-B, C and D.