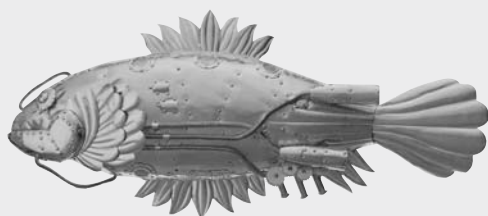




SHOWA
DENKO

Food Analysis with Shodex Columns

Saccharides, Organic Acids, Vitamins, Fatty Acids and Amino Acids



Shodex[®]

TECHNICAL NOTEBOOK

No.3

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1. Introduction

In recent years, a broad range of analyses, including agrochemical residues and food additives, have been performed on foods with increasingly greater concern about their safety. This article describes analyses of general food ingredients, especially those based on high performance liquid chromatography (HPLC).

2. Saccharides

HPLC-based analyses of saccharides and sugar alcohols are performed in various modes, such as normal phase, ligand exchange, size exclusion, and ion exchange. There are two series of Shodex columns for saccharide analysis: those operated mainly in ligand exchange mode (SUGAR series), and those in normal phase mode (Asahipak NH2P-50 polymeric amino columns). In the SUGAR series columns, a hard styrene-divinylbenzene copolymer designed for saccharide analysis is used as the base material, and a strong cation exchange resin incorporating functional sulfo-groups coupled with metal counter ions is used as the packing. Columns of the SUGAR series are available in 14 types according to counter ion, pore size, and pore structure, showing differential selectivity for different saccharides. On the other hand, the Asahipak NH2P-50 columns comprise a hydrophilic polymer gel [poly(vinyl)alcohol] and a polyamine that is chemically and stably bound to the gel. As such, these columns have excellent durability without the problem of time-related deterioration in silica-based amino columns. Both these series are suitable for saccharide separation and analysis of foods, areas of biochemistry, and natural substances. Other items include the Asahipak GS-220 HQ polymeric packed columns for size exclusion mode to analyze water-soluble, low molecular weight dietary fibers, and the OHPak SB-800 HQ polymeric packed columns for size exclusion mode to analyze polysaccharides, as well as the SUGAR KS-800 columns. Table 1 shows the lineup of Shodex packed columns for saccharide analysis.

Table 2-1. Columns for Saccharide Analysis (1)

| Product Code | Product Name | Counter Ion | Separation Mode*1 | ExclusionLimit (Pullulan) | Theoretical Plate Number (TP/column) | Particle Size (µm) | ID x Length (mm) |
|--------------|----------------------|------------------|-------------------|---------------------------|--------------------------------------|--------------------|------------------|
| F6378100 | SUGAR SH1011 | H ⁺ | SEC + IEX | 1,000 | ≥17,000 | 6 | 8.0 x 300 |
| F6378101 | SUGAR SH1821 | H ⁺ | SEC + IEX | 10,000 | ≥17,000 | 6 | 8.0 x 300 |
| F6700080 | SUGAR SH-G | H ⁺ | - | Guard column | - | 10 | 6.0 x 50 |
| F6378102 | SUGAR SC1011 | Ca ²⁺ | SEC + LEX | 1,000 | ≥13,000 | 6 | 8.0 x 300 |
| F6378103 | SUGAR SC1821 | Ca ²⁺ | SEC + LEX | 10,000 | ≥13,000 | 6 | 8.0 x 300 |
| F6700090 | SUGAR SC-LG | Ca ²⁺ | - | Guard column | - | 10 | 6.0 x 50 |
| F6378105 | SUGAR SP0810 | Pb ²⁺ | SEC + LEX | 1,000 | ≥11,000 | 7 | 8.0 x 300 |
| F6700081 | SUGAR SP-G | Pb ²⁺ | - | Guard column | - | 10 | 6.0 x 50 |
| F7001400 | SUGAR SC1211 | Ca ²⁺ | NP + LEX | - | ≥ 5,500 | 6 | 6.0 x 250 |
| F6700120 | SUGAR SC-G | Ca ²⁺ | - | Guard column | - | 10 | 4.6 x 10 |
| F7001300 | SUGAR SZ5532 | Zn ²⁺ | NP + LEX | - | ≥ 5,500 | 6 | 6.0 x 150 |
| F6700110 | SUGAR SZ-G | Zn ²⁺ | - | Guard column | - | 6 | 4.6 x 10 |
| F6378010 | SUGAR KS-801 | Na ⁺ | SEC + LEX | 1,000 | ≥17,000 | 6 | 8.0 x 300 |
| F6378020 | SUGAR KS-802 | Na ⁺ | SEC + LEX | 10,000 | ≥17,000 | 6 | 8.0 x 300 |
| F6378025 | SUGAR KS-803 | Na ⁺ | SEC | 50,000 | ≥17,000 | 6 | 8.0 x 300 |
| F6378035 | SUGAR KS-804 | Na ⁺ | SEC | 400,000 | ≥17,000 | 7 | 8.0 x 300 |
| F6378050 | SUGAR KS-805 | Na ⁺ | SEC | 5,000,000 | ≥ 9,000 | 17 | 8.0 x 300 |
| F6378060 | SUGAR KS-806 | Na ⁺ | SEC | (50,000,000)*2 | ≥ 9,000 | 17 | 8.0 x 300 |
| F6700020 | SUGAR KS-G | Na ⁺ | - | Guard column | - | 10 | 6.0 x 50 |
| F6378070 | SUGAR KS-807 | Na ⁺ | SEC | (200,000,000)*2 | ≥ 4,000 | 30 | 8.0 x 300 |
| F6700021 | SUGAR KS-807G | Na ⁺ | - | Guard column | - | 30 | 8.0 x 50 |
| F7600005 | Asahipak GS-220 HQ | - | SEC | 3,000 | ≥19,000 | 6 | 7.5 x 300 |
| F6710019 | Asahipak GS-2G 7B | - | - | Guard column | - | 9 | 7.5 x 50 |
| F7630002 | Asahipak NH2P-50 4D | - | NP | - | ≥ 5,000 | 5 | 4.6 x 150 |
| F7630001 | Asahipak NH2P-50 4E | - | NP | - | ≥ 7,500 | 5 | 4.6 x 250 |
| F6710016 | Asahipak NH2P-50G 4A | - | - | Guard column | - | 5 | 4.6 x 10 |
| F7630006 | Asahipak NH2P-50 2D | - | NP | - | ≥ 3,500 | 5 | 2.0 x 150 |
| F6713000 | Asahipak NH2P-50G 2A | - | - | Guard column | - | 5 | 2.0 x 10 |
| F6710100 | Asahipak NH2P-LF | - | - | Line filter | - | - | 8.0 x 75 |
| F6379230 | USPpak MN-431 | Ca ²⁺ | SEC + LEX | - | ≥ 4,000 | - | 4.0 x 250 |

*1 : SEC (Size exclusion), IEX (Ion exclusion), LEX (Ligand exchange), NP (Normal phase)

*2 : Figures in () are estimated values

Table 2-2. Columns for Saccharide Analysis (2)

| Product Code | Product Name | Exclusion Limit (Pullulan) | Usable Organic Solvents (Max.%) | | | Theoretical PlateNumber (TP/column) | Particle Size (μm) | ID x Length (mm) |
|--------------|-------------------|-----------------------------|---------------------------------|--------------|-----|-------------------------------------|--------------------|------------------|
| | | | Methanol | Acetonitrile | DMF | | | |
| F6429100 | OHpak SB-802 HQ | 4,000 | 0 | 0 | 0 | ≥12,000 | 8 | 8.0 x 300 |
| F6429101 | OHpak SB-802.5 HQ | 10,000 | 100 | 75 | 100 | ≥16,000 | 6 | 8.0 x 300 |
| F6429102 | OHpak SB-803 HQ | 100,000 | 100 | 75 | 100 | ≥16,000 | 6 | 8.0 x 300 |
| F6429103 | OHpak SB-804 HQ | 1,000,000 | 75 | 75 | 100 | ≥16,000 | 10 | 8.0 x 300 |
| F6429104 | OHpak SB-805 HQ | 4,000,000 | 75 | 75 | 100 | ≥12,000 | 13 | 8.0 x 300 |
| F6429105 | OHpak SB-806 HQ | (20,000,000)* ¹ | 75 | 75 | 100 | ≥12,000 | 13 | 8.0 x 300 |
| F6429106 | OHpak SB-806M HQ | (20,000,000)* ¹ | 75 | 75 | 100 | ≥12,000 | 13 | 8.0 x 300 |
| F6709430 | OHpak SB-G | Guard column | 75 | 75 | 100 | - | 10 | 6.0 x 50 |
| F6429108 | OHpak SB-807 HQ | (500,000,000)* ¹ | 75 | 75 | 100 | ≥15,000 | 35 | 8.0 x 300 |
| F6709431 | OHpak SB-807 G | Guard column | 75 | 75 | 100 | - | 35 | 6.0 x 50 |

*1 : Figures in () are estimated values

2-1. Separation Mechanism

2-1-1. Anomer Separation of Saccharides

Reducing saccharides can adopt various cyclic structures, as well as a linear structure. In such cases, two tautomers, α type and β type, are produced because carbonyl carbon atoms become asymmetric. The relation between α type and β type is called anomer. (Fig. 2-1)

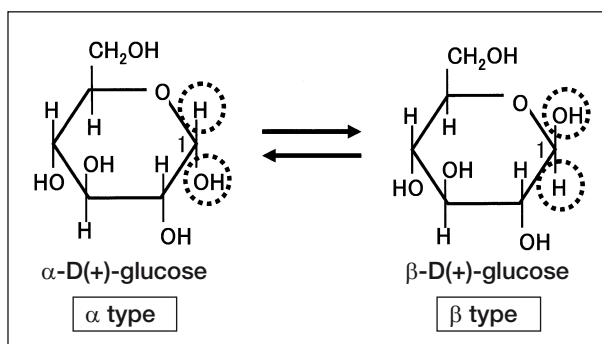


Fig. 2-1 Anomers

Under conditions in which the conversion rate between the tautomers is low, α and β anomers are separated by the column causing the peak tops to split or widen. (Fig. 2-2)

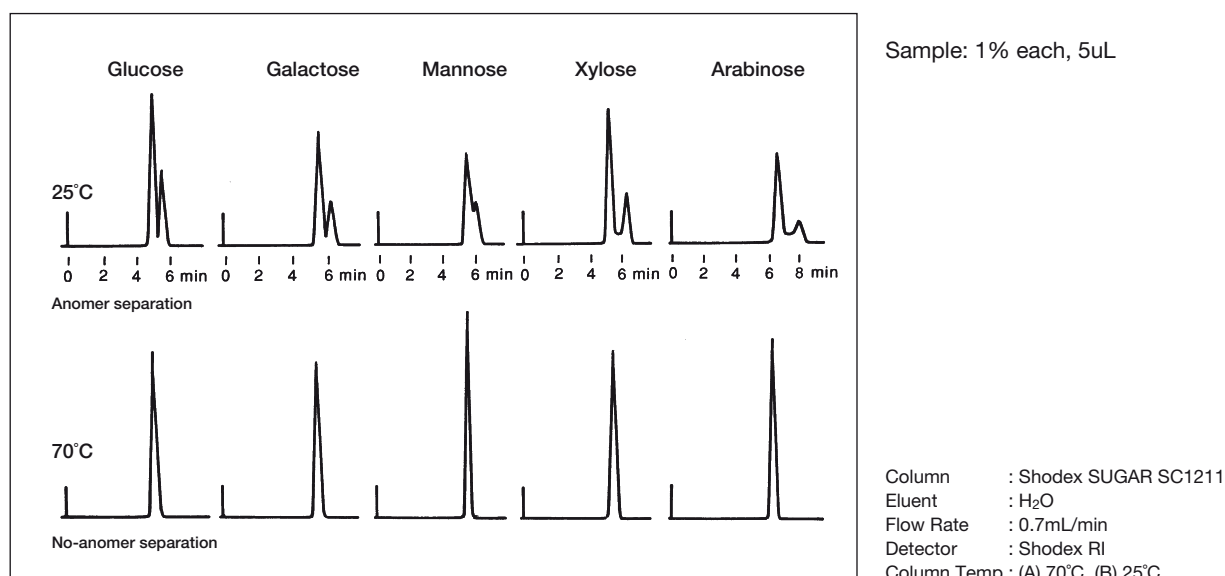


Fig. 2-2 Influence of Anomer Separation in Saccharide Analysis

Two methods are available to prevent anomer separation during analysis:

- * Analysis at high temperature
- * Analysis under strong alkaline conditions

2-1-2. Shodex SUGAR Series

A strong feature of the Shodex SUGAR series columns resides in the mechanism of separation in the ligand exchange mode. Ligand exchange refers to a mode of separation based on the interaction (ligand exchange potential) between hydroxyl groups and metal ions to form a complex. Saccharides have a 5-membered (furanose) or 6-membered (pyranose) ring structure, containing a large number of hydroxyl groups. These hydroxyl groups bind together either equatorially or axially with respect to the carbon plane. This conformation of the hydroxyl groups differs depending on the kind of saccharide.

Figure 2-3 shows the relationship between hydroxyl group conformation and counter ion interaction.

In Figure 2-3(a), three hydroxyl groups form a complex with the metal ion. In Figure 2-3(b), only two hydroxyl groups form a complex with the metal ion due to the hydroxyl group conformation. Hence, ligand exchange potential is higher for (a) than for (b).

The complex formation potential also differs depending on the kind of metal ion.

* When using a column of the SUGAR series, analysis should be performed at increased column temperatures to prevent anomer separation. Under strongly alkaline conditions, saccharides are likely to isomerize with the fear of decomposition of polysaccharides.

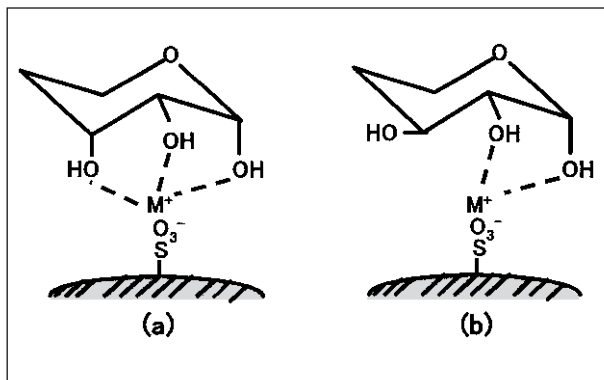


Fig. 2-3 Difference of counter ion interaction

2-1-3. Shodex Asahipak NH2P-50 Series

Columns of the Shodex Asahipak NH2P-50 series are greatly improved amino columns which not only maintain the high separation performance of the conventional silica-based amino columns, but also solve the problem of declines in retention over time. This is due to stable chemical bonding of polyamine with hydrophilic polymer gel. Other advantages are:

- * Analysis under moderate conditions (around pH 7 and room temperature) is possible.
- * Sharp, near-symmetric peaks can be obtained for a wide variety of saccharides.
- * Accurate quantitative determination can be made.
- * A wide range of eluents, such as various buffer solutions, alkaline solutions, or acidic solutions can be used.
- * Alkaline washing of columns is possible.

With amino columns, saccharides elute in order of increasing polarity due to the function of normal phase chromatography. Usually, a mixed solvent of acetonitrile and water is used as the eluent. When the mixing ratio of acetonitrile is increased, the polarity of the eluent becomes lower. This results in a stronger interaction between saccharides and the column and a larger elution volume.

As NH2P-50 columns have weak alkaline amino groups, the condition inside the column is alkaline. This enables saccharides to be analyzed without causing separation of anomers even at room temperature.

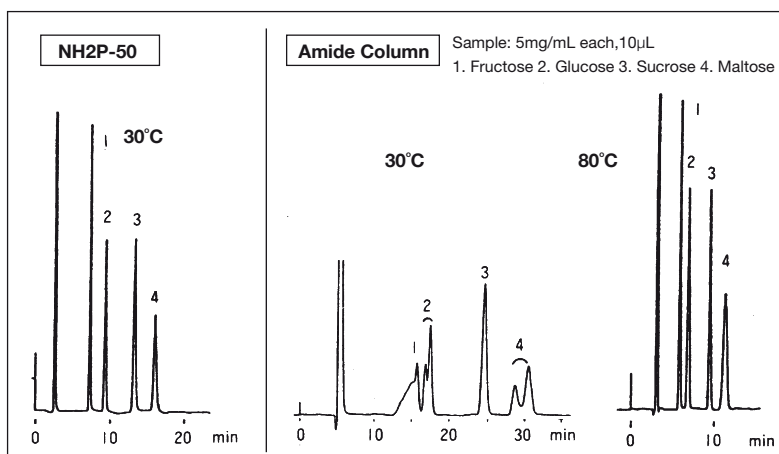


Fig. 2-4 Effects of Column Temperature on Elution Patterns (Comparison with Amide Column)

There are columns, called amide columns, which are used for analysis of saccharides under the same elution conditions as those for amino columns. Although amide columns have acrylamide groups introduced, analysis has to be made at high temperatures because the acrylamide group is not alkaline. (Fig. 2-4)

Column : Shodex Asahipak NH2P-50 4E
(4.6x250mm)
Amide Column from Company-A
(4.6x250mm)
Eluent : CH₃CN/H₂O=75/25
Flow Rate : 1.0mL/min
Detector : Shodex RI
Column Temp.: 30°C, 80°C

NH2P-50 columns show good reproducibility for a long time, because the chemical structure of the packing material is stable. With silica-based amino columns, retention of saccharide weakens over time, resulting in the significant widening of every peak. This clearly indicates deterioration of the column. (Fig. 2-5)

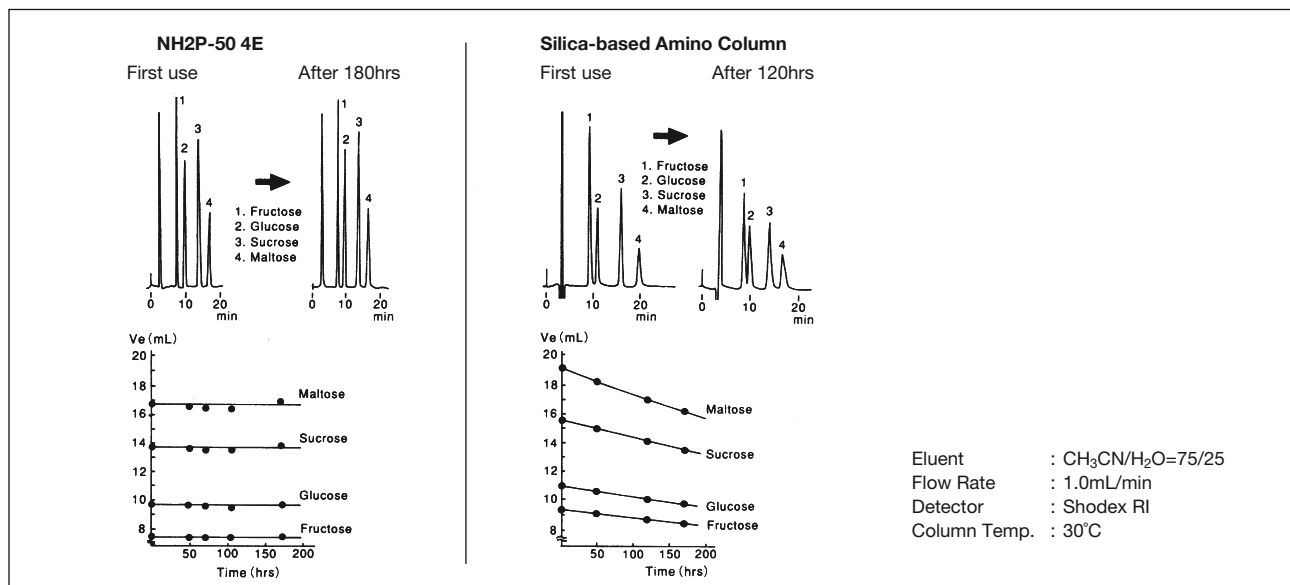


Fig. 2-5 Reproducibility of Chromatograms with NH2P-50 4E Column and Silica-based Amino Column

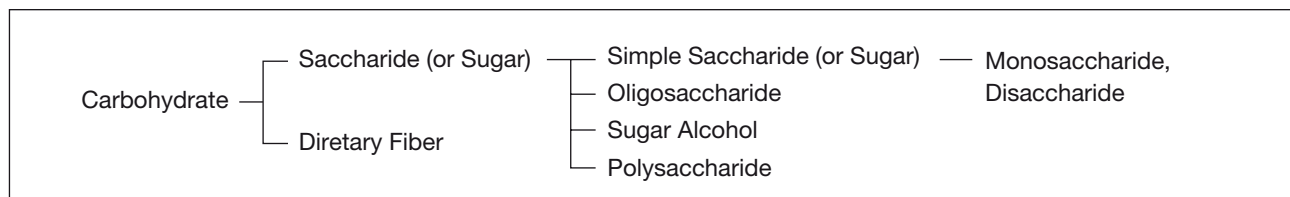
2-1-4. Detection of Saccharides

The most commonly used approach for detecting saccharides is the use of the differential refractive index (RI) detector, which is based on refractive index differences between sample components and a mobile phase. Although the RI detector is highly versatile, it exhibits low selectivity and is not suitable for gradient elution. Another drawback is the tendency for lower sensitivity than other types of detectors. In cases where high selectivity and high sensitivity are required, the ultraviolet absorption (UV) detector and the fluorescence (FL) detector are effective. It should be noted, however, that saccharides must be derivatized because they lack a structure for absorbing UV or emitting fluorescence. Other available methods of detection include the mass spectrum (MS) analyzer, the electrochemical (EC) detector which electrochemically detects ionized saccharides under alkaline conditions, and the evaporation light scattering (ELS) detector which detects saccharides by evaporating an eluent and an irradiating laser.

2-2. Monosaccharides and Disaccharides

The term "Saccharide" generically refers to simple saccharides, oligosaccharides, polysaccharides, sugar alcohols and the like. Simple saccharides, i.e., monosaccharides and disaccharides, exclude sugar alcohols. Emphasized labeling such as, "Very Low Sugar" or "Low Sugar," and "Sugarless" is not permitted unless the content of the relevant type of sugar is not more than 5 grams per 100 grams (2.5 g/100 mL for refreshing beverages), and less than 0.5 grams per 100 grams (0.5 g/100 mL for refreshing beverages), respectively. Japan's nutritional facts labeling standards specify the use of an amino column (4.6 mm inner diameter x 250 mm length) to analyze simple saccharides and oligosaccharides (Figure 2-6).

Shodex can provide many kinds of columns for saccharide analysis.



| Monosaccharide, Disaccharide | Oligosaccharide |
|--|--|
| <p>Column : A column packed with a silica or polymer gel bounded amino (propyl) group (4.6mmID x 250mm) → Shodex Asahipak NH2P-50 4E</p> <p>Eluent : CH₃CN/H₂O = 75/25</p> <p>Flow Rate : 1.0 mL/min</p> <p>Detector : RI</p> <p>Column Temp.: Room Temp.</p> <p>Injection Vol. : 20μL</p> | <p>Column : A column packed with a silica or polymer gel bounded amino (propyl) group (4.6mmID x 250mm) → Shodex Asahipak NH2P-50 4E</p> <p>Eluent : CH₃CN/H₂O = 75/25</p> <p>Flow Rate : 1.0 mL/min</p> <p>Detector : RI</p> <p>Column Temp.: Room Temp.</p> <p>Injection Vol. : 20μL</p> |
| Japan's Nutritional Facts Labeling Standards Ver. 3 (Japan Health Food Authorization) | |

Fig. 2-6 Analysis Condition of Saccharides and Oligosaccharides

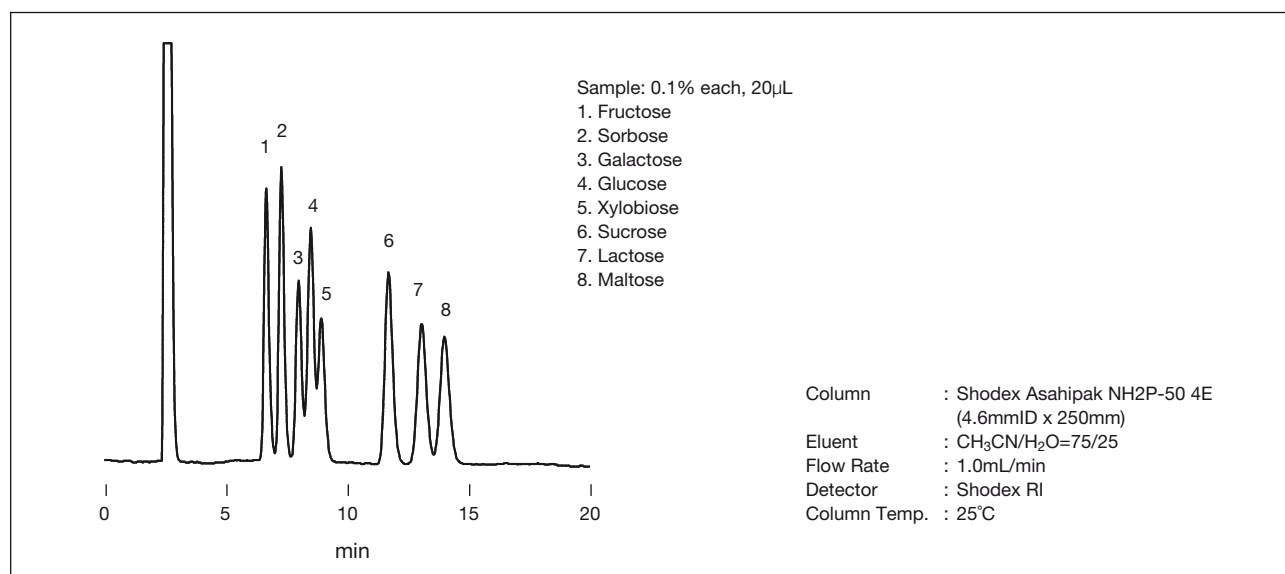


Fig. 2-7 Standard Saccharides with NH2P-50 4E

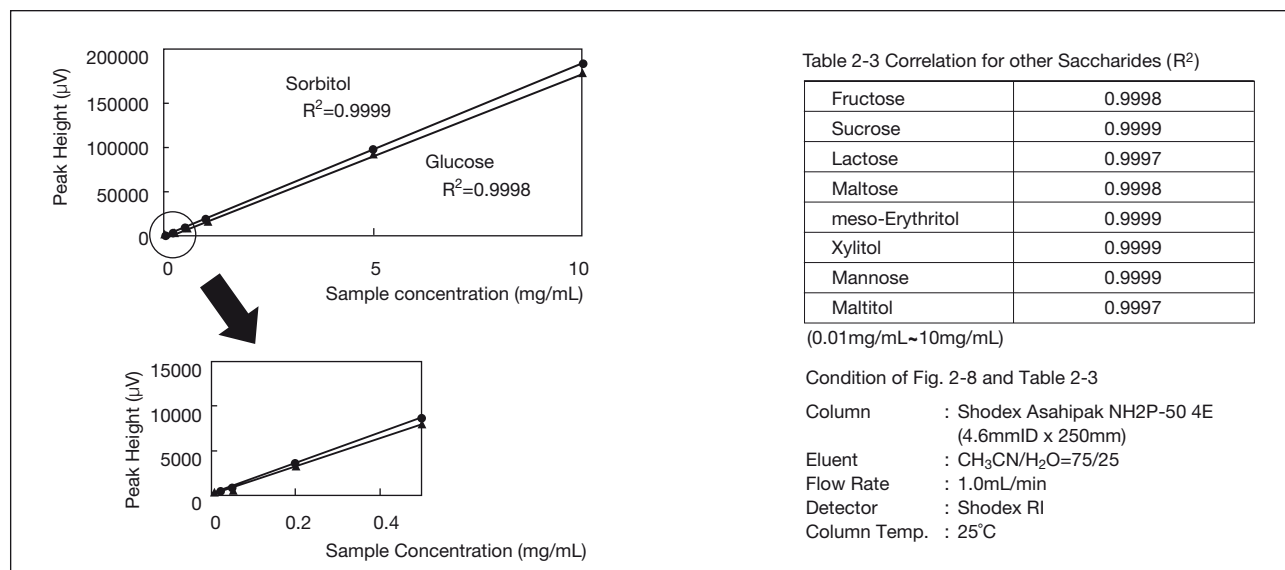


Fig. 2-8 Calibration Curves with NH2P-50

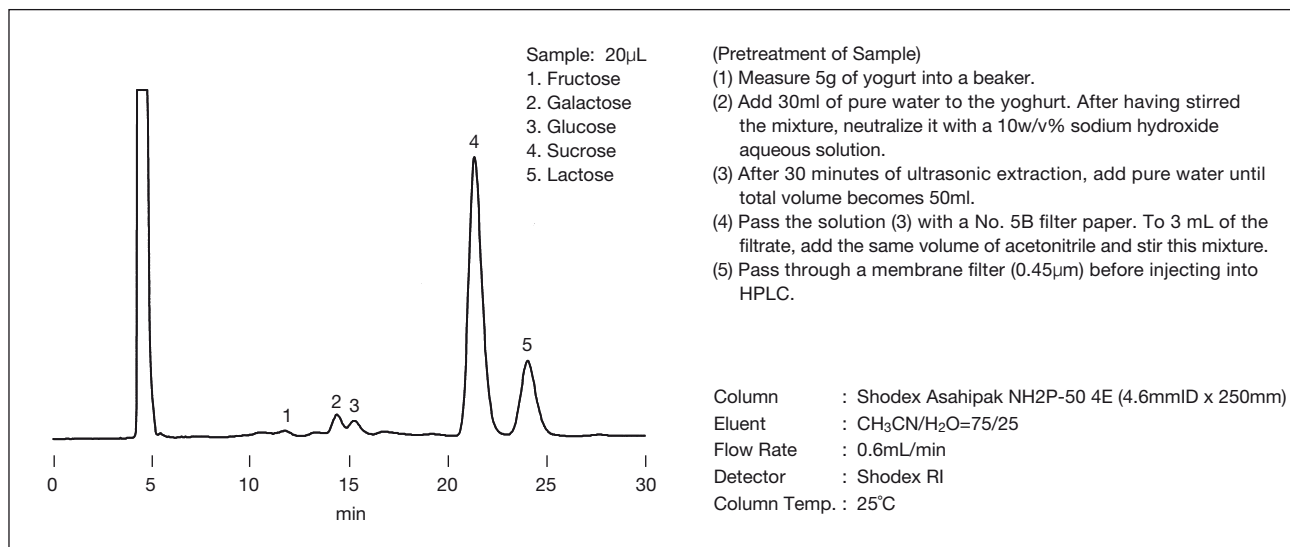


Fig. 2-9 Saccharides in Sugar-added Yoghurt

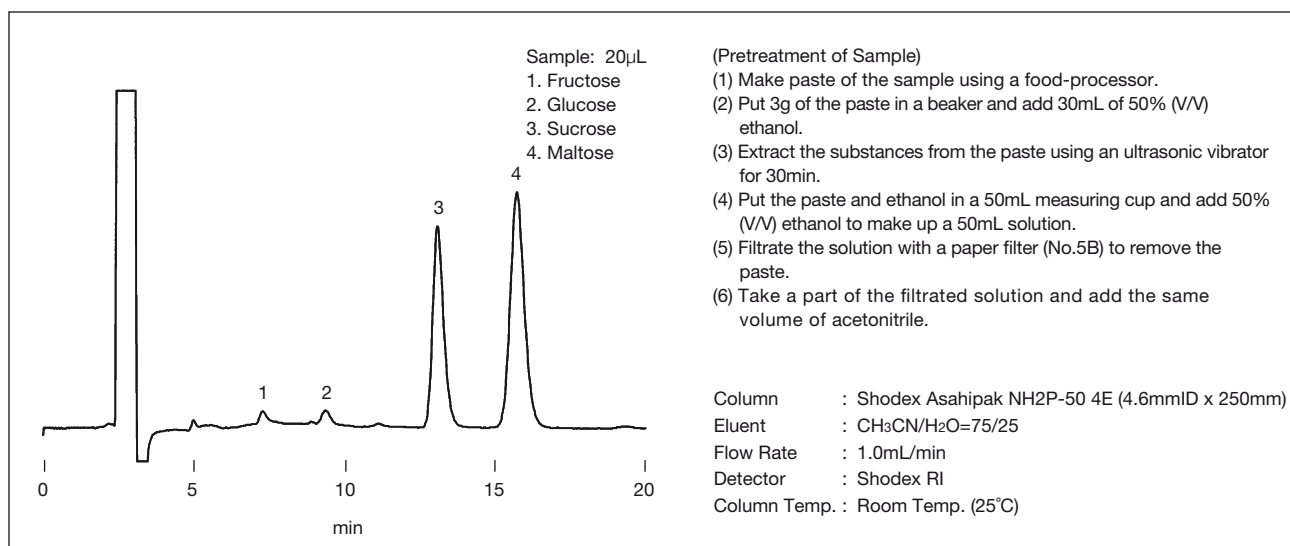


Fig. 2-10 Saccharides in Sweet Potato, Roasted

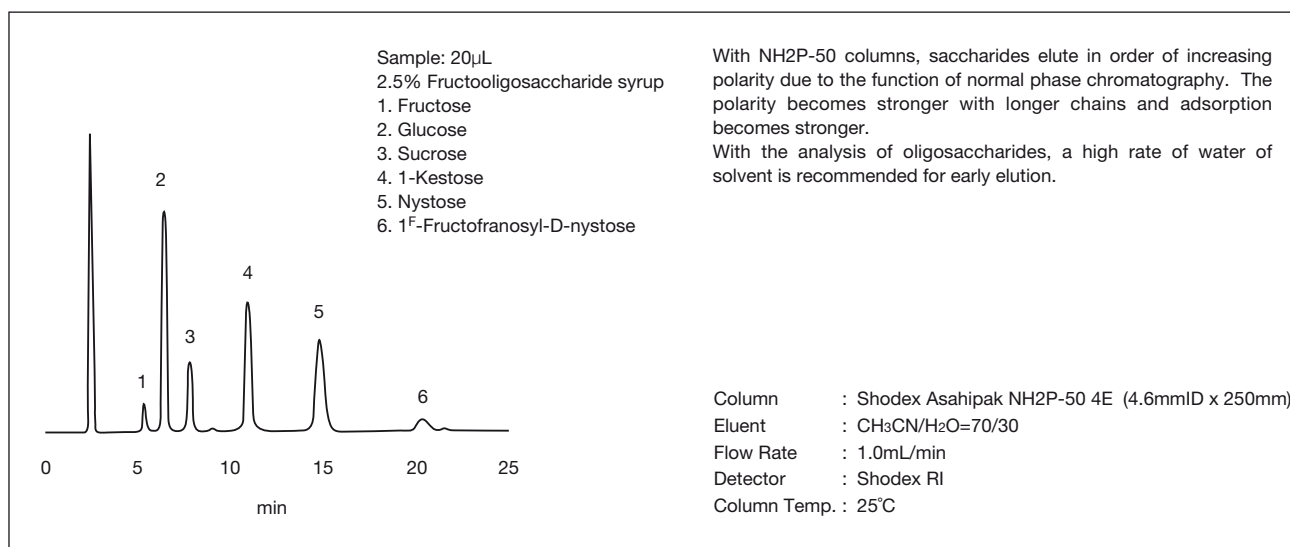


Fig. 2-11 Fructo-oligosaccharide Syrup

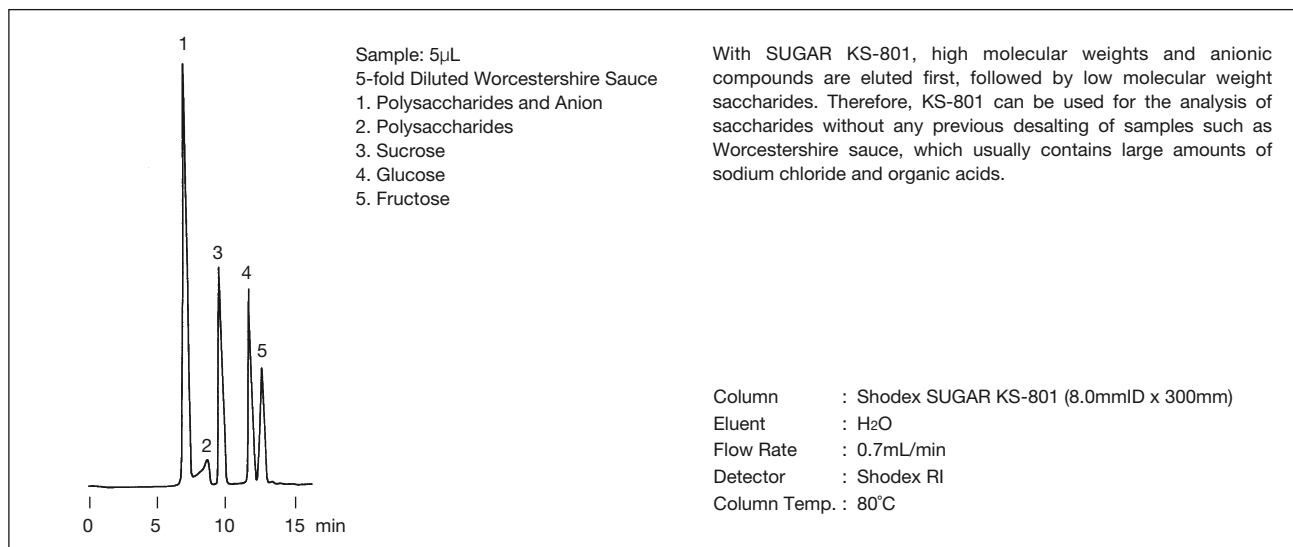


Fig. 2-12 Saccharides in Worcestershire Sauce

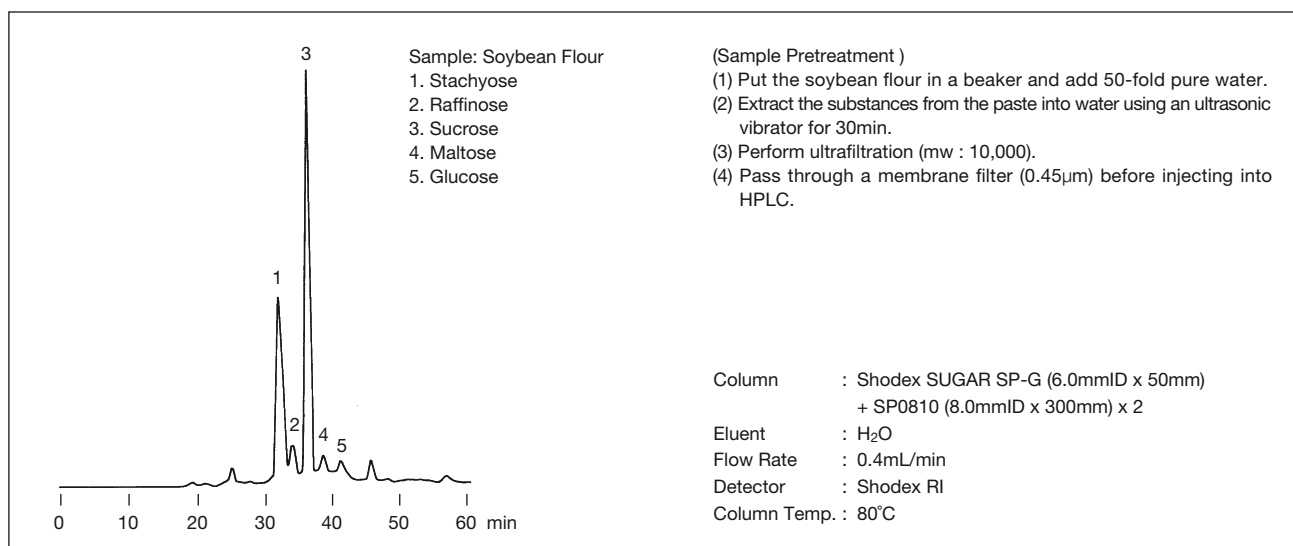


Fig. 2-13 Saccharides in Soybean Flour

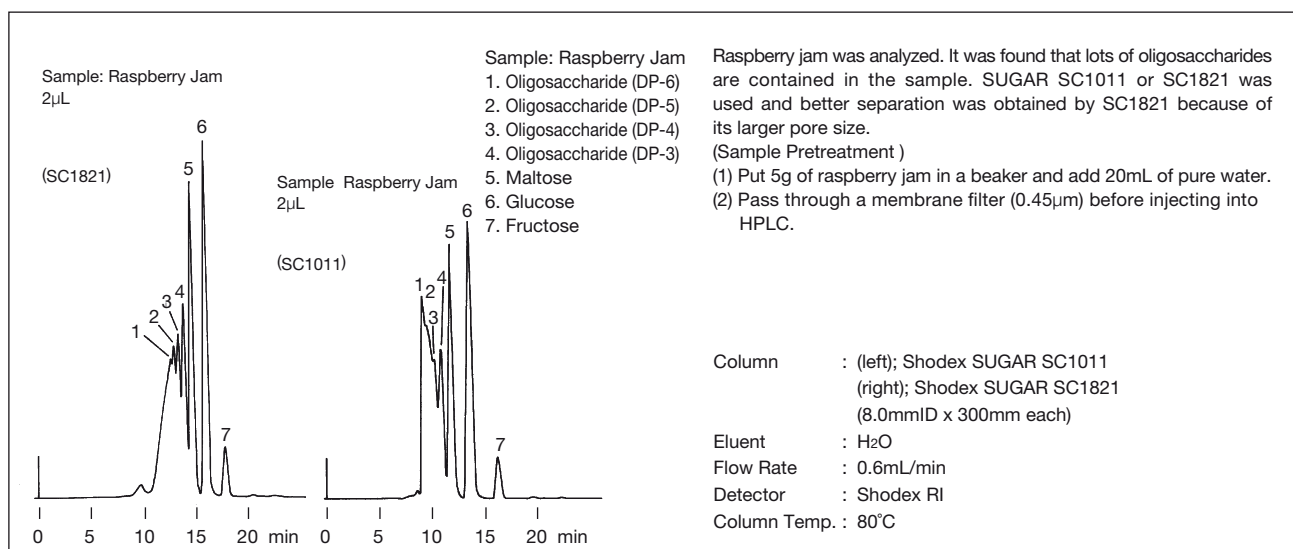


Fig. 2-14 Saccharides in Raspberry Jam

2-3. Saccharides and Sugar Alcohols

Amino columns (4.6mmID x 250mm) and ligand exchange mode columns are one of the official methods of analysis for sugar alcohols. (Fig. 2-15)

| Sugar Alcohol (1) | Sugar Alcohol (2) |
|---|---|
| <p>Column : A column packed with a silica or polymer gel bounded amino (propyl) group (4.6mmID x 250mm) → Shodex Asahipak NH2P-50 4E</p> <p>Eluent : CH₃CN/H₂O = 75/25</p> <p>Flow Rate : 1.0mL/min</p> <p>Detector : RI</p> <p>Column Temp.: Room Temp.</p> <p>Injection Vol. : 20μL</p> | <p>Column : A column packed with sulfonated poly styrene gel (Pb or Ca ligand) (7.8-8.0mmID x 300mm) → Shodex SUGAR SP0810, SC1011</p> <p>Eluent : H₂O</p> <p>Flow Rate : 0.6mL/min</p> <p>Detector : RI</p> <p>Column Temp.: 85°C</p> <p>Injection Vol. : 5μL</p> |
| <p>Japan's Nutritional Facts Labeling Standards Ver. 3 (Japan Health Food Authorization)</p> | |

Fig. 2-15 Analysis Condition of Sugar Alcohols

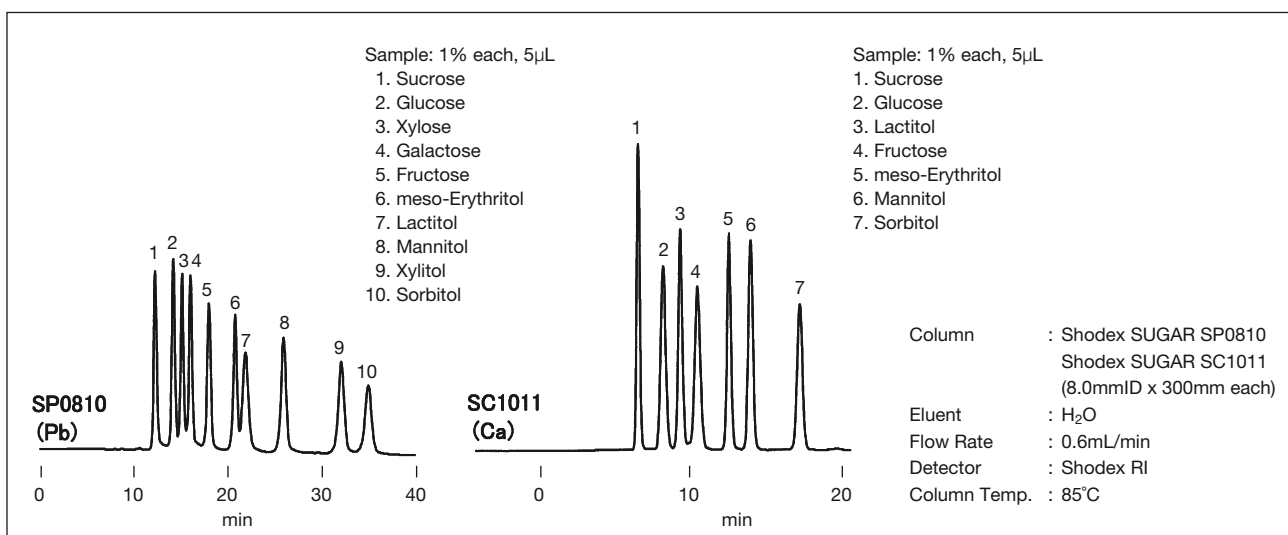


Fig. 2-16 Standard Saccharides and Sugar Alcohol with SP0810, SC1011

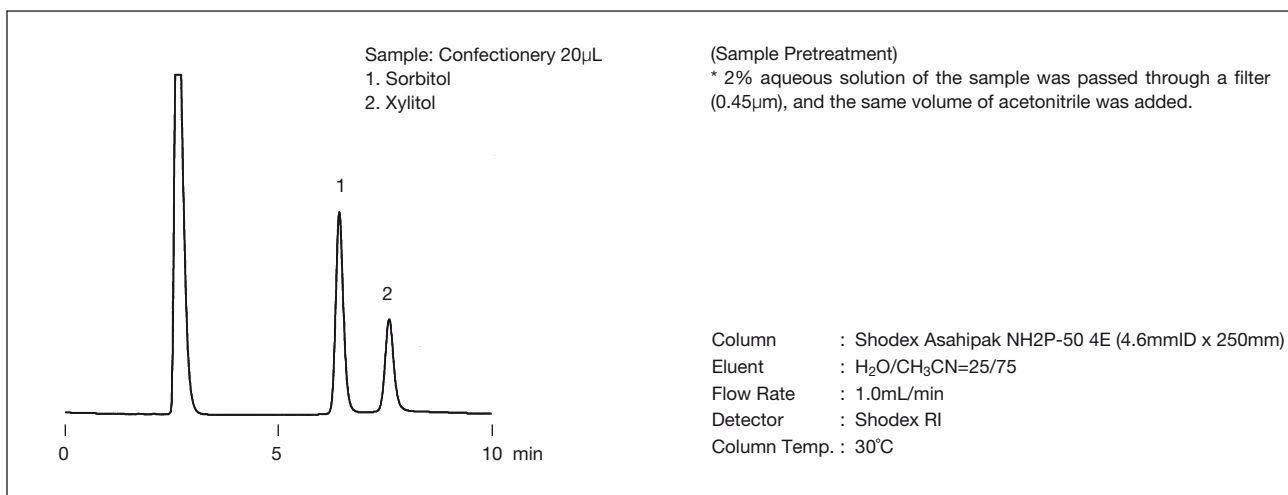


Fig. 2-17 Xylitol in Confectionery

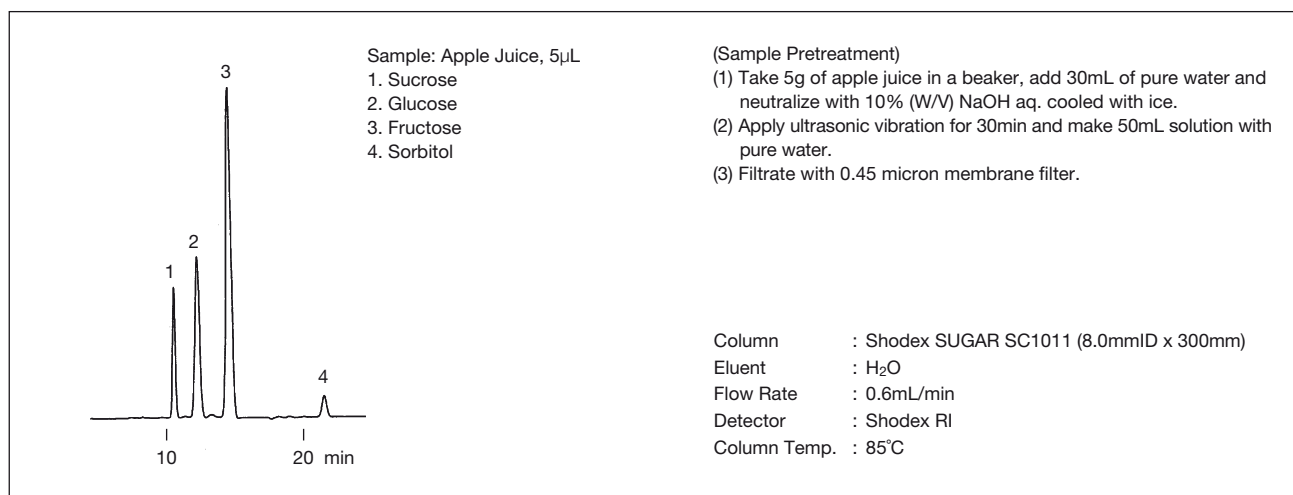


Fig. 2-18 Apple Juice

2-4. Water-soluble Low Molecular Weight Dietary Fibers

Dietary fibers are usually quantified by the Prosky method (enzyme-gravimetric method). However, for water-soluble low molecular weight dietary fibers unquantifiable by the Prosky method, the enzyme-HPLC method is used. In this analysis, the sample is divided into two fractions: dietary fibers (tri- and higher saccharides), and mono- and disaccharides. Subsequently, the peak area ratio of the dietary fiber fraction and glucose (or internal standard) is determined.

Water-soluble Low Molecular Weight Dietary Fibers

Column : Size exclusion chromatography columns
 (Exclusion limit MW : ca. 5,000)
 or Ligand exchange columns
 (Na or Ca type)

Eluent : H₂O
 Flow Rate : 0.5mL/min
 Detector : RI
 Column Temp. : 80°C
 Injection Vol. : 20 μ L

Japan's Nutritional Facts Labeling Standards Ver. 3
 (Japanese Health and Food Nutrition Society)

Fig. 2-19 Analysis Condition of Water Soluble Dietary Fiber

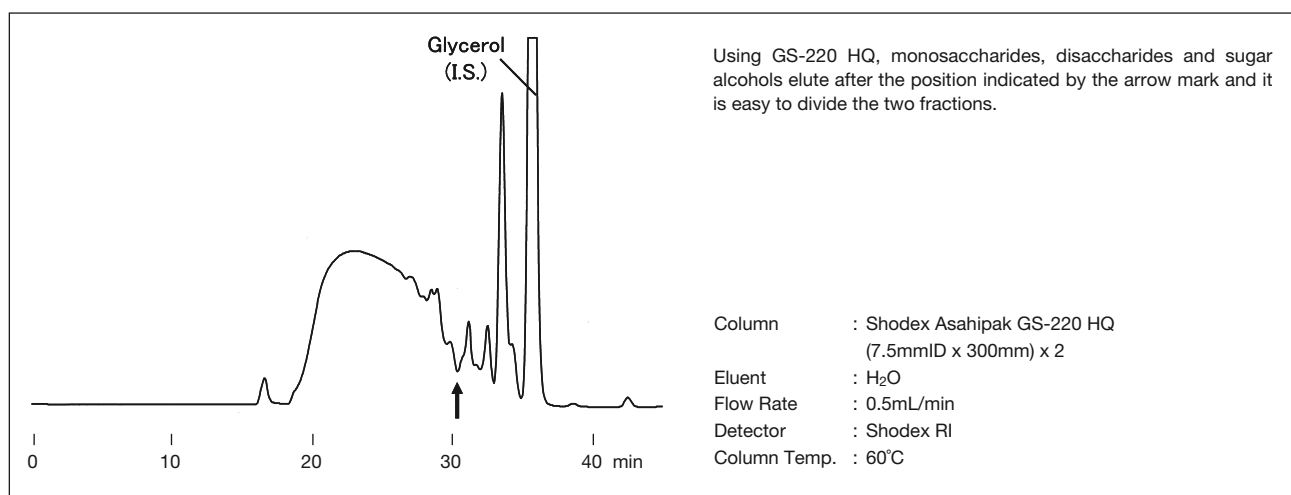


Fig. 2-20 Water-soluble Dietary Fiber

2-5. Saccharides with Amino Acids or Organic Acids

Amino acids and organic acids are often contained with saccharides in foods.

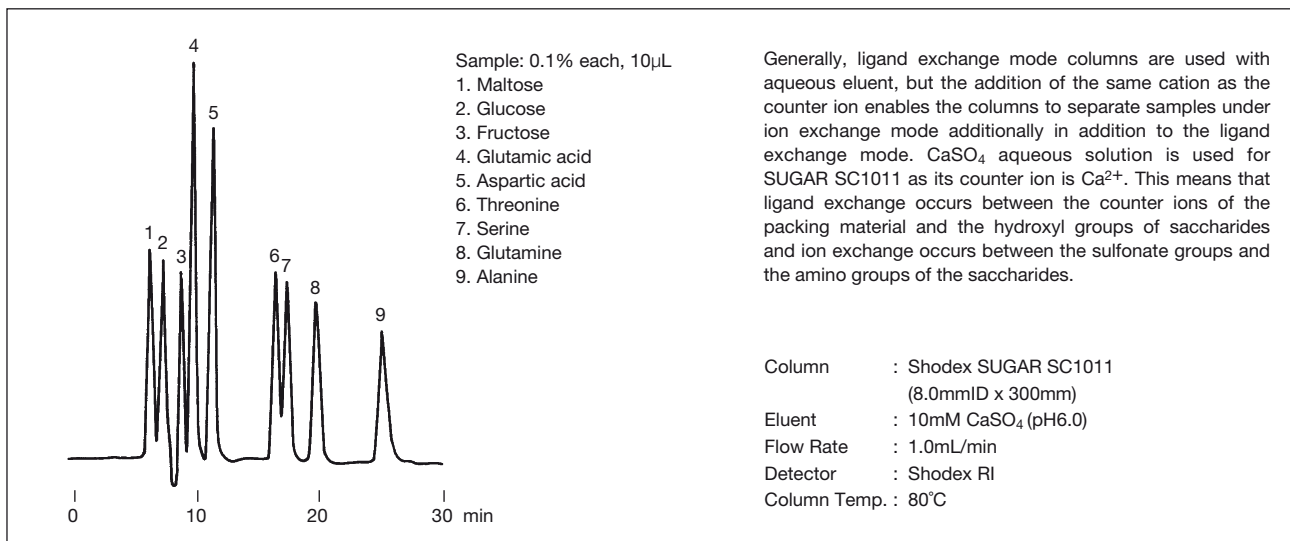


Fig. 2-21 Saccharides and Amino Acids (1)

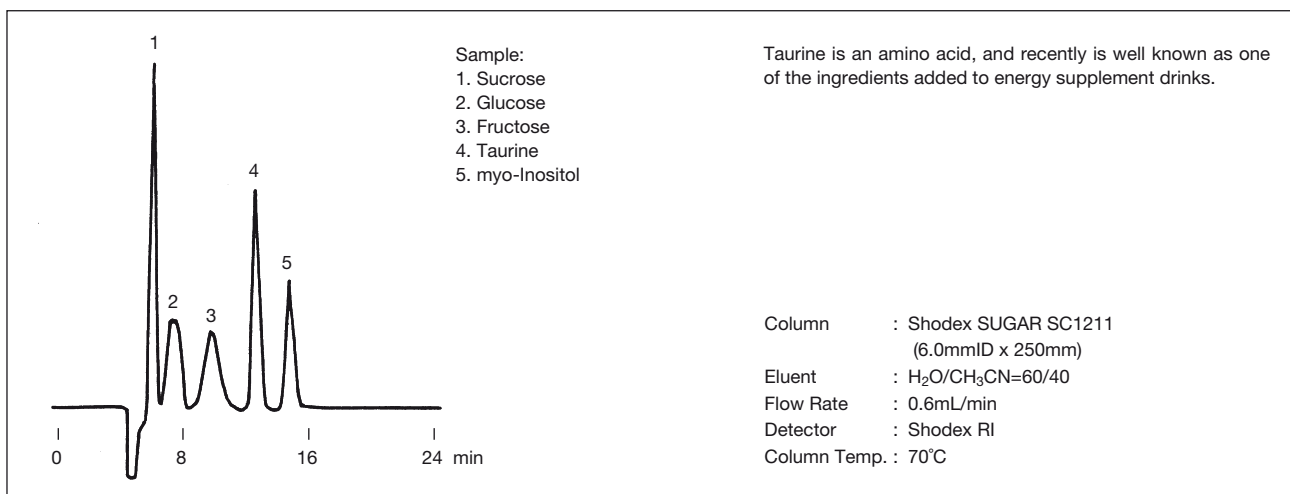


Fig. 2-22 Saccharides and Amino Acids (2)

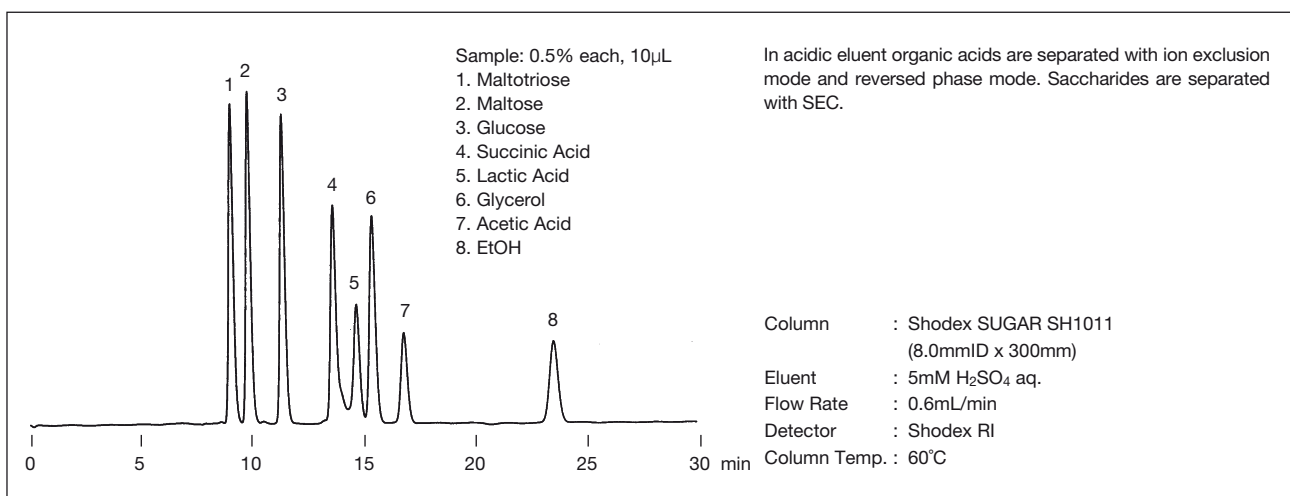


Fig. 2-23 Saccharides and Organic Acids

2-6. Polysaccharides

Polysaccharides are used as thickeners/stabilizers to thicken and/or solidify foods and for other purposes. Polysaccharides have a broad range of molecular weights, therefore their analyses are performed not only for quantification but also for molecular weight determination.

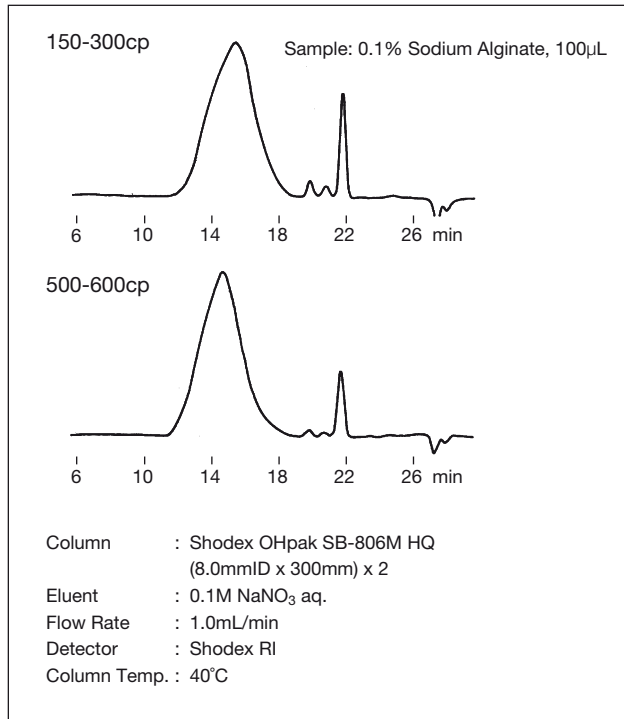


Fig. 2-24 Sodium Alginate

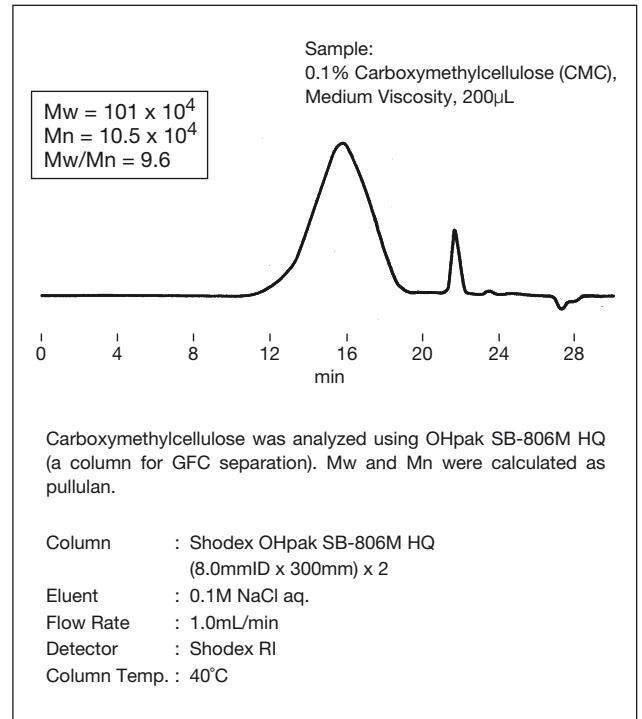


Fig. 2-25 Carboxymethylcellulose

2-7. Sweeteners

Generally food additives are analyzed with reversed phase mode columns: however, saccharides are difficult to analyze because of their high polarity. So columns for saccharide analysis are recommended to analyze saccharides and sweeteners at the same time.

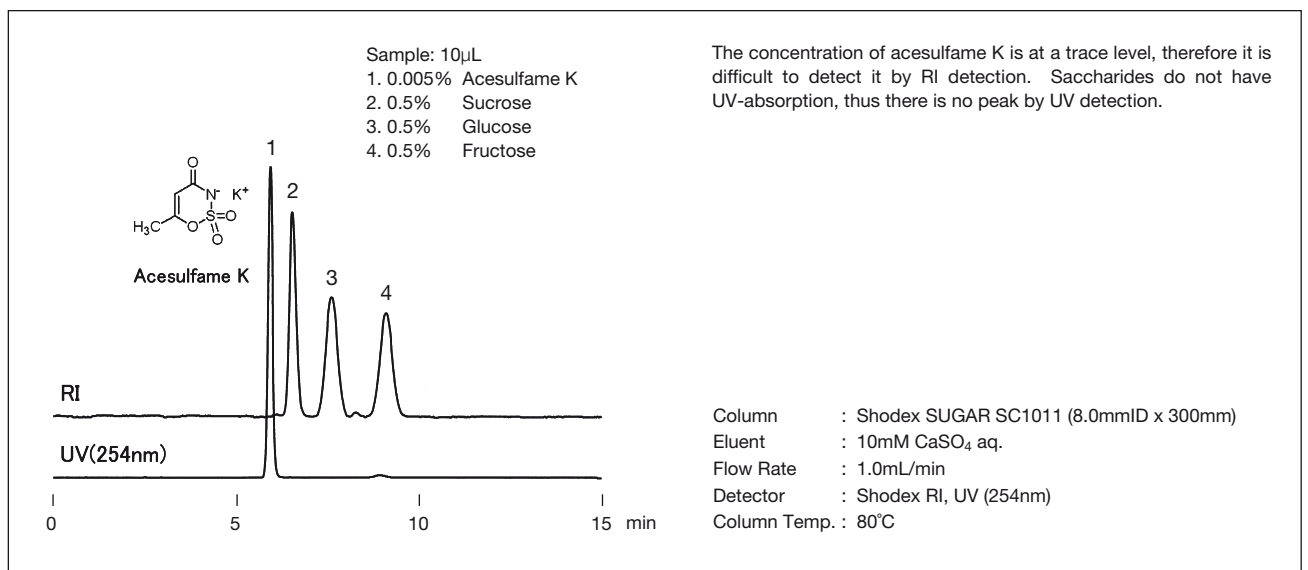


Fig. 2-26 Acesulfame K and Saccharides

Tables of elution volume of saccharides with each column are shown in Appendix-A.

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 SUGAR SH1821 | Size Exclusion + Ion Exclusion | Organic Acids and Sugars |
| IC SI-90 4E 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).

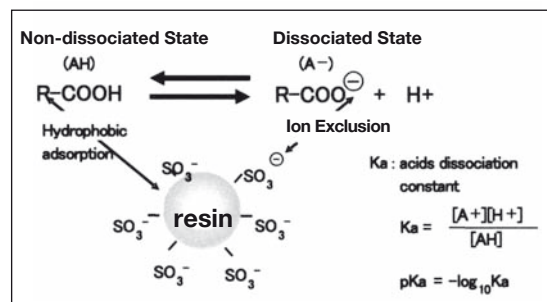


Fig. 3-1 Ion Exclusion Mode

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 V₀ 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 | Sample: |
| Eluent | : 3mM HClO ₄ aq. | 1. 800ppm Citric Acid |
| Flow Rate | : 1.0mL/min | 2. 900ppm Tartaric Acid |
| Column Temp. | : 50°C | 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 |

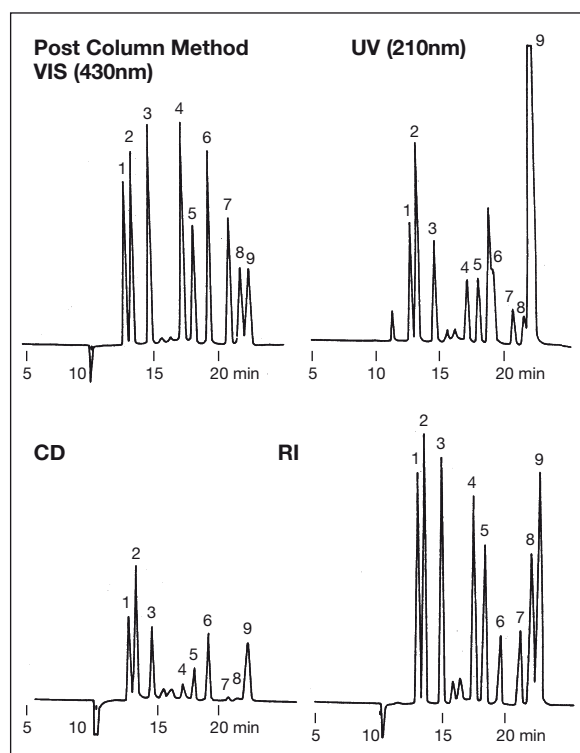


Fig. 3-2 Comparison of Chromatograms with Various Detectors

3-2. Organic Acids

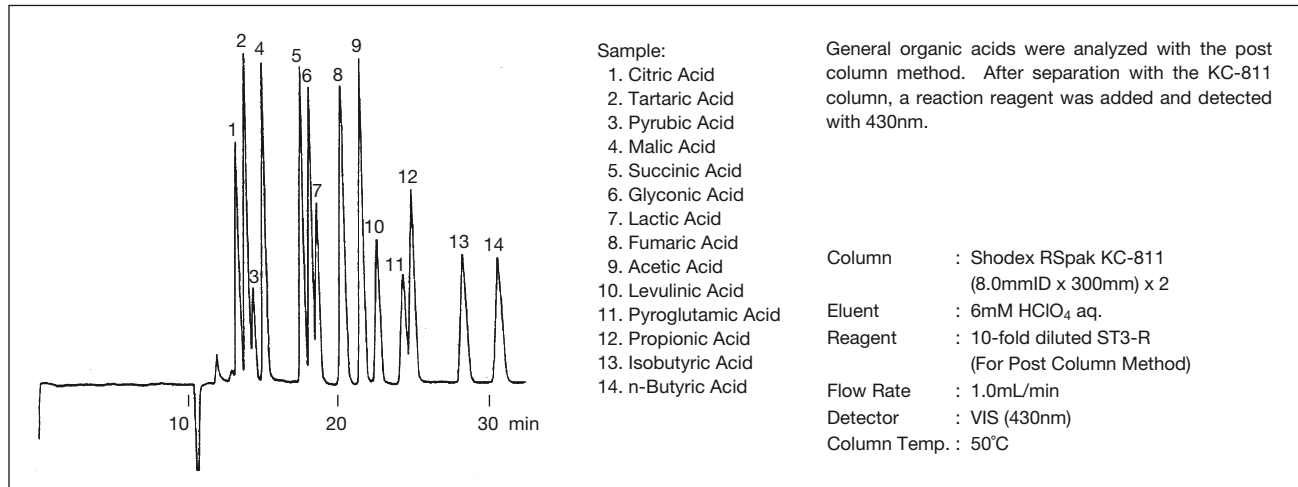


Fig. 3-3. Analysis of Organic Acids with Post Column Method

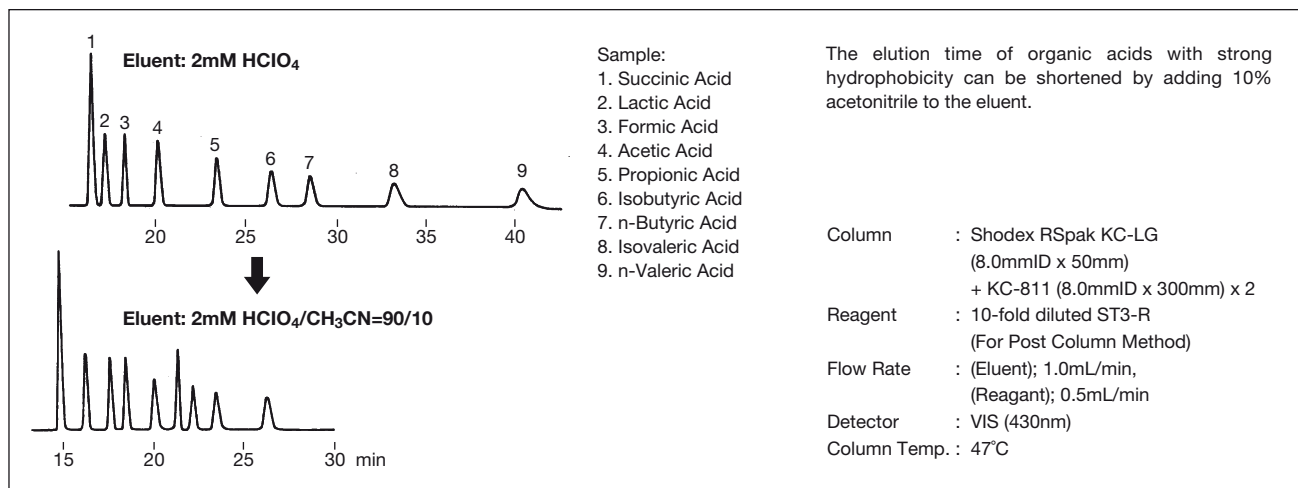


Fig. 3-4 Effect of Elution of Organic Acids with Addition of Acetonitrile

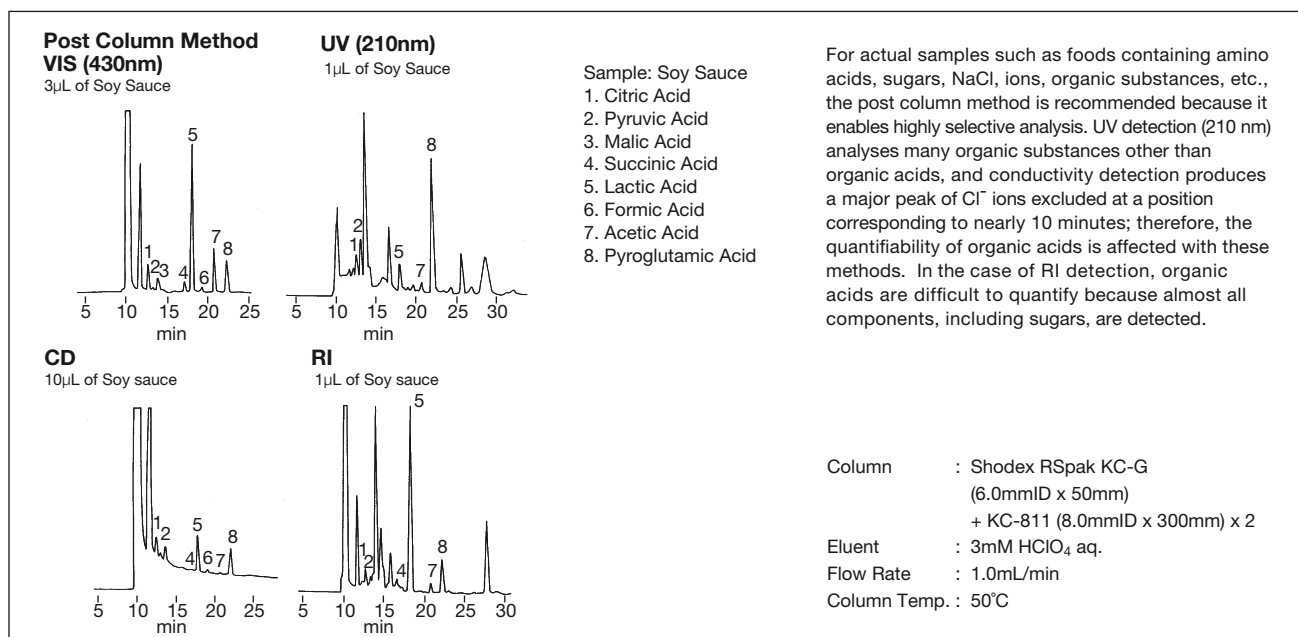


Fig. 3-5 Comparison of Results of Soy Sauce with Various Detectors

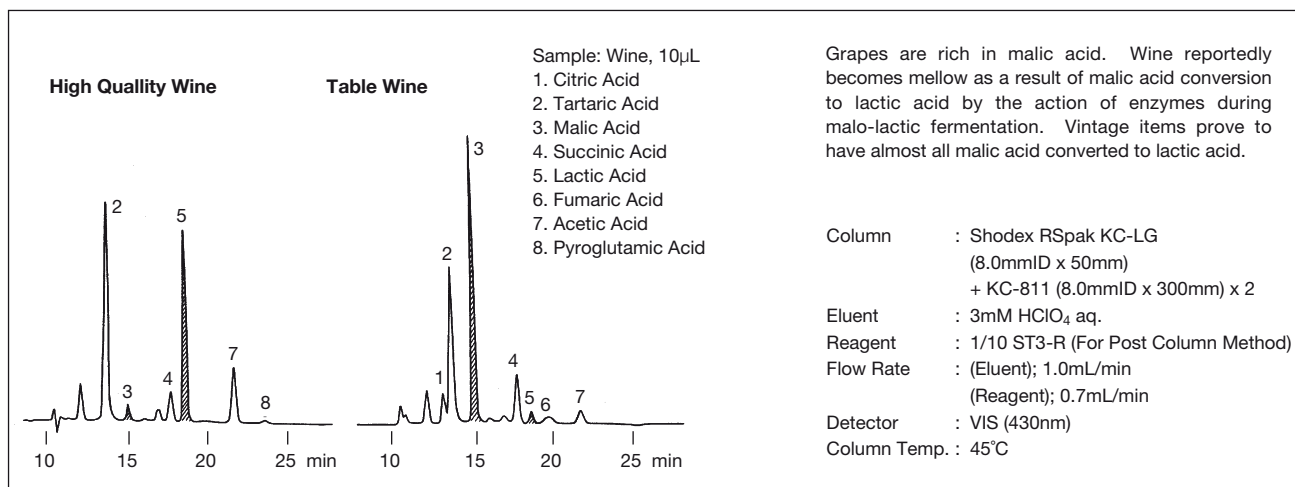


Fig. 3-6 Organic Acids in White Wine

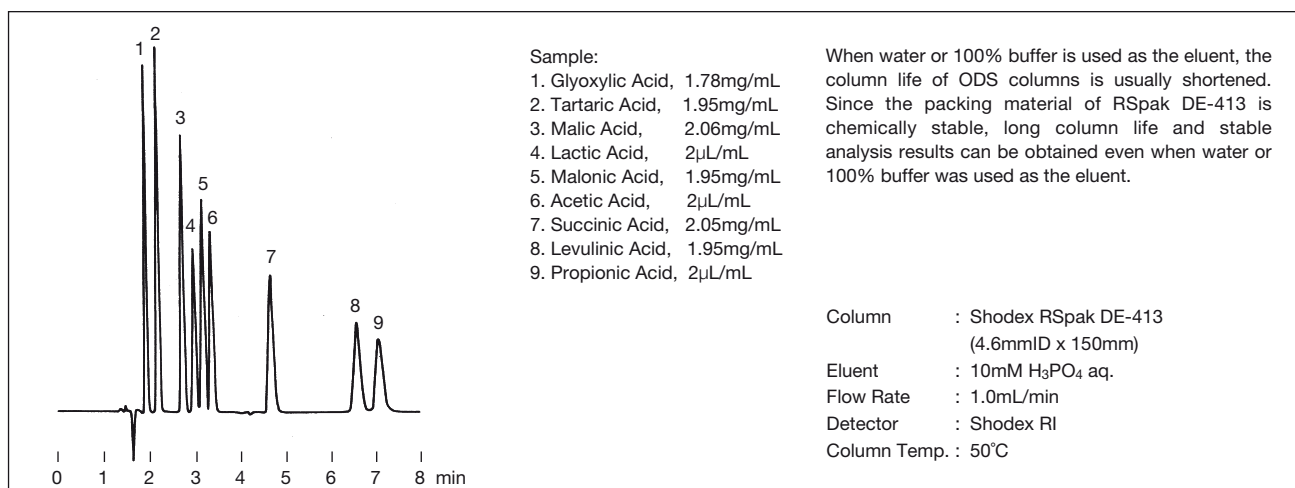


Fig. 3-7 Organic Acids (Reversed Phase Mode)

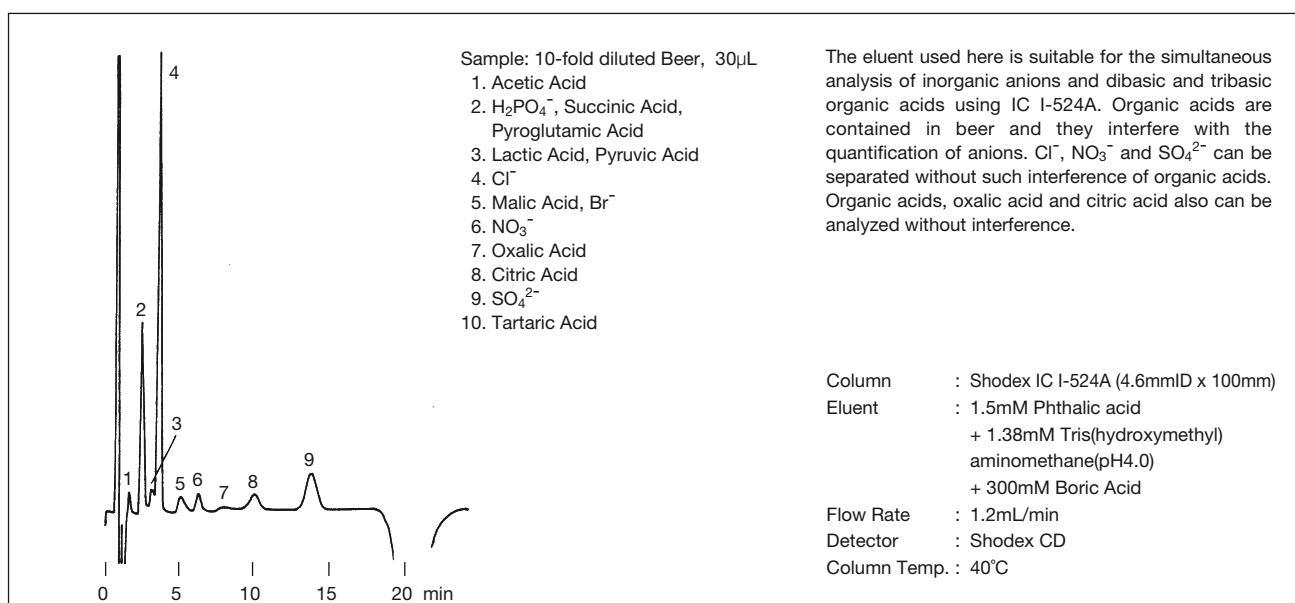


Fig. 3-8 Organic Acids and Anions in Beer (Anion Exchange Mode)

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

4. Vitamins

There are two types of vitamins: fat-soluble vitamins and water-soluble vitamins. (Fig. 4-1)

It is possible to analyze vitamins simultaneously, though several official methods show the analysis methods respectively. The major analysis methods by HPLC are reversed phase or normal phase, additionally size exclusion chromatography can be applied. Table 4-1 shows the lineup of Shodex packed columns for vitamin analysis with separation modes.

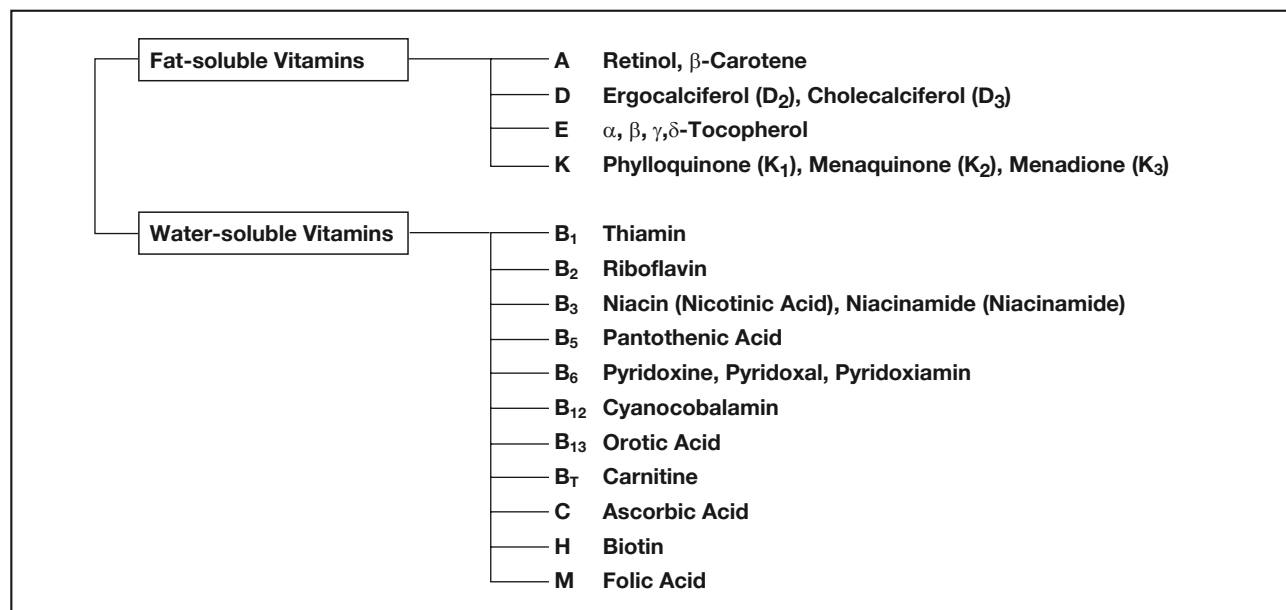


Fig. 4-1 Categories of Vitamins

Table 4-1 Shodex Columns for Vitamin Analysis
(Absorption and Partition)

| Product Code | Product Name | Theoretical Plate Number (TP/column) | Particle Size (μm) | ID x Length (mm) |
|--------------|----------------------|--------------------------------------|--------------------|------------------|
| F7621001 | Asahipak ODP-40 4D | ≥11,000 | 4 | 4.6 x 150 |
| F7621002 | Asahipak ODP-40 4E | ≥17,000 | 4 | 4.6 x 250 |
| F7620002 | Asahipak ODP-50 6D | ≥ 9,000 | 5 | 6.0 x 150 |
| F7620001 | Asahipak ODP-50 6E | ≥14,000 | 5 | 6.0 x 250 |
| F6710001 | Asahipak ODP-50G 6A | (Guard column) | 5 | 6.0 x 10 |
| F6710023 | Asahipak ODP-50 4B | ≥2,500 | 5 | 4.6 x 50 |
| F7620004 | Asahipak ODP-50 4D | ≥9,000 | 5 | 4.6 x 150 |
| F7620003 | Asahipak ODP-50 4E | ≥14,000 | 5 | 4.6 x 250 |
| F6710022 | Asahipak ODP-50G 4A | (Guard column) | 5 | 4.6 x 10 |
| F7620009 | Asahipak ODP-50 2D | ≥5,000 | 5 | 2.0 x 150 |
| F6713001 | Asahipak ODP-50G 2A | (Guard column) | 5 | 2.0 x 10 |
| F7001004 | RSpak DE-613 | ≥7,000 | 6 | 6.0 x 150 |
| F7001005 | RSpak DE-413 | ≥11,000 | 4 | 4.6 x 150 |
| F7009030 | RSpak DE-413L | ≥17,000 | 4 | 4.6 x 250 |
| F7001006 | RSpak DE-413S | ≥3,000 | 4 | 4.6 x 50 |
| F6700150 | RSpak DE-G | (Guard column) | 10 | 4.6 x 10 |
| F7001007 | RSpak DE-213 | ≥8,000 | 4 | 2.0 x 150 |
| F6700151 | RSpak DE-SG | (Guard column) | 4 | 2.0 x 10 |
| F7630002 | Asahipak NH2P-50 4D | ≥5,500 | 5 | 4.6 x 150 |
| F7630001 | Asahipak NH2P-50 4E | ≥7,500 | 5 | 4.6 x 250 |
| F6710016 | Asahipak NH2P-50G 4A | (Guard column) | 5 | 4.6 x 10 |
| F7630006 | Asahipak NH2P-50 2D | ≥3,500 | 5 | 2.0 x 150 |
| F6713000 | Asahipak NH2P-50G 2A | (Guard column) | 5 | 2.0 x 10 |
| F6650050 | Silica 5SIL 4D | ≥9,000 | 5 | 4.6 x 150 |
| F6650051 | Silica 5SIL 4E | ≥15,000 | 5 | 4.6 x 250 |

(Size Exclusion)

| Product Code | Product Name | Theoretical Plate Number (TP/column) | Particle Size (μm) | Exclusion Limit (Polystyrene) | ID x Length (mm) |
|--------------|--------------|--------------------------------------|--------------------|-------------------------------|------------------|
| F6028010 | GPC KF-801 | ≥18,000 | 6 | 1,500 | 8.0 x 300 |
| F6700300 | GPC KF-G | (Guard column) | 8 | | 4.6 x 10 |

(Multimode)

| Product Code | Product Name | Theoretical Plate Number (TP/column) | Particle Size (μm) | ID x Length (mm) |
|--------------|--------------|--------------------------------------|--------------------|------------------|
| F7008140 | RSpak NN-814 | ≥9,000 | 10 | 8.0 x 250 |
| F7008150 | RSpak NN-614 | ≥4,000 | 10 | 6.0 x 150 |
| F6700510 | RSpak NN-G | (Guard column) | 10 | 6.0 x 50 |
| F7008160 | RSpak NN-414 | ≥6,000 | 10 | 4.6 x 150 |

4-1. Fat-Soluble Vitamins

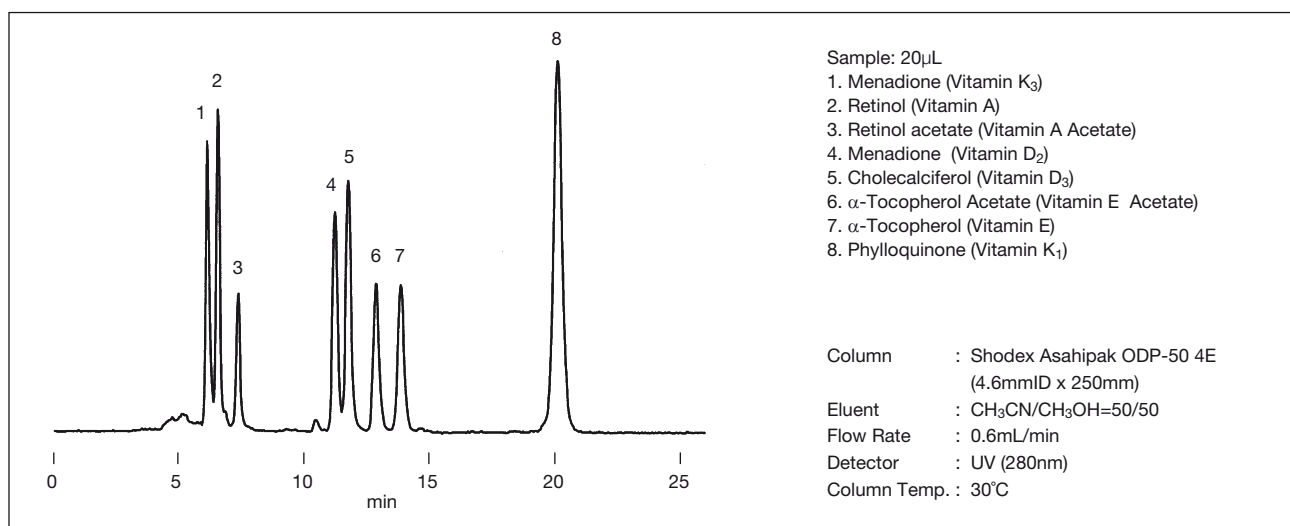


Fig. 4-2 Simultaneous Analysis of Fat-soluble Vitamins (Reversed Phase Mode)

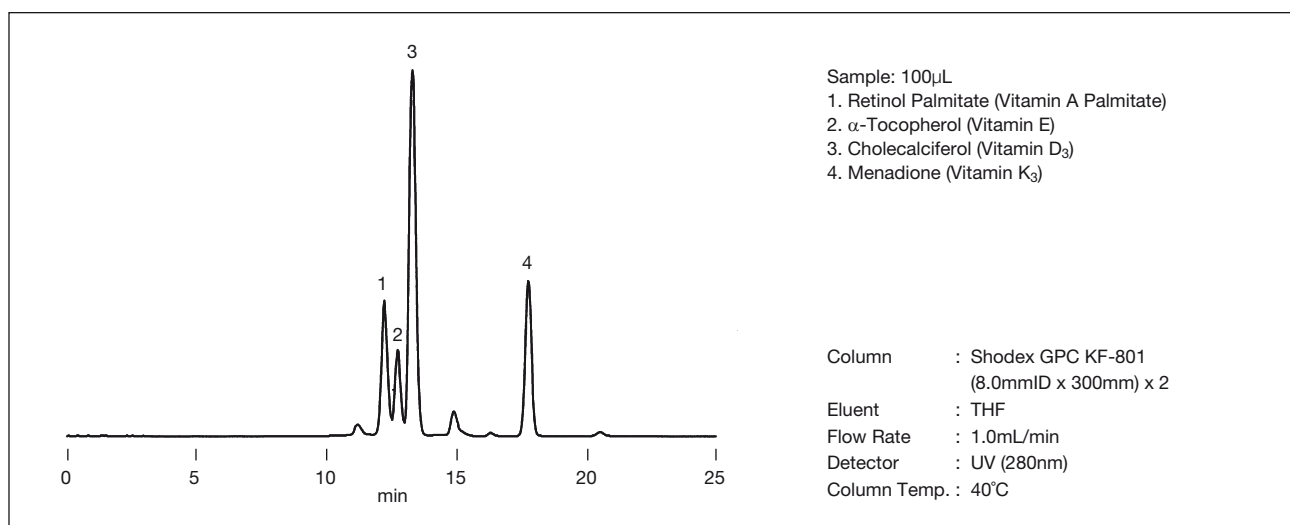


Fig. 4-3 Simultaneous Analysis of Fat-soluble Vitamins (Size Exclusion Mode)

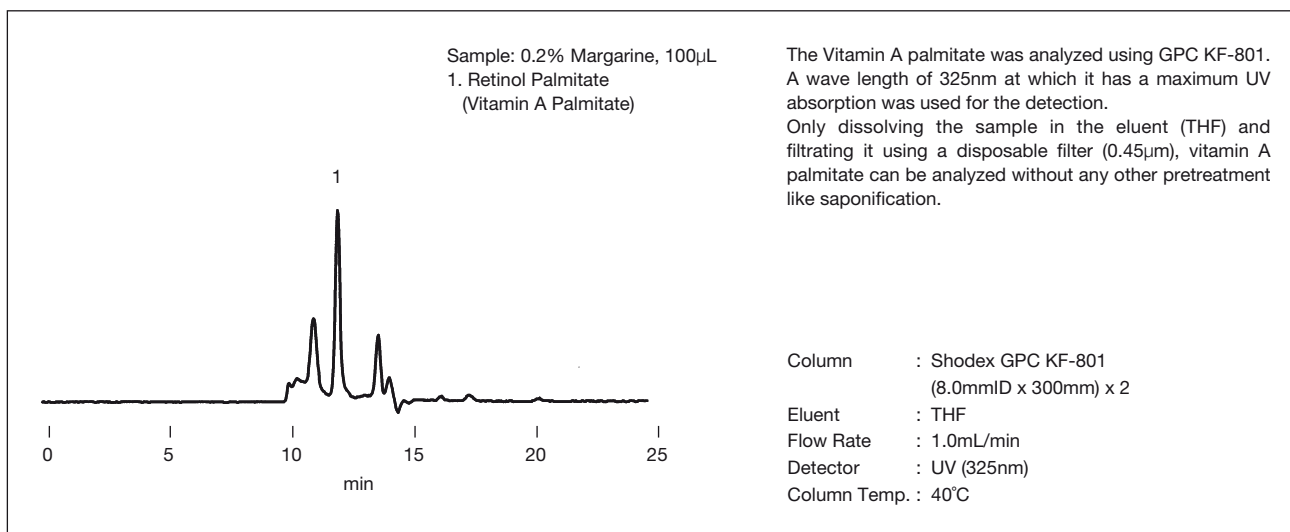


Fig. 4-4 Vitamin A Palmitate in Margarine (Size Exclusion Mode)

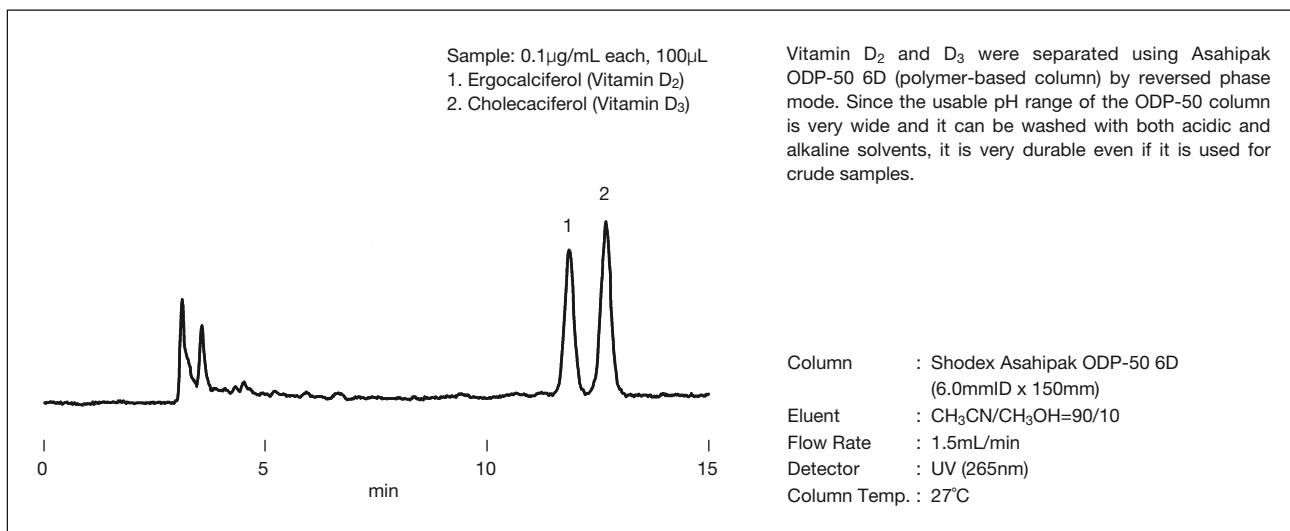


Fig. 4-5 Vitamin D₂ and D₃ (Reversed Phase Mode)

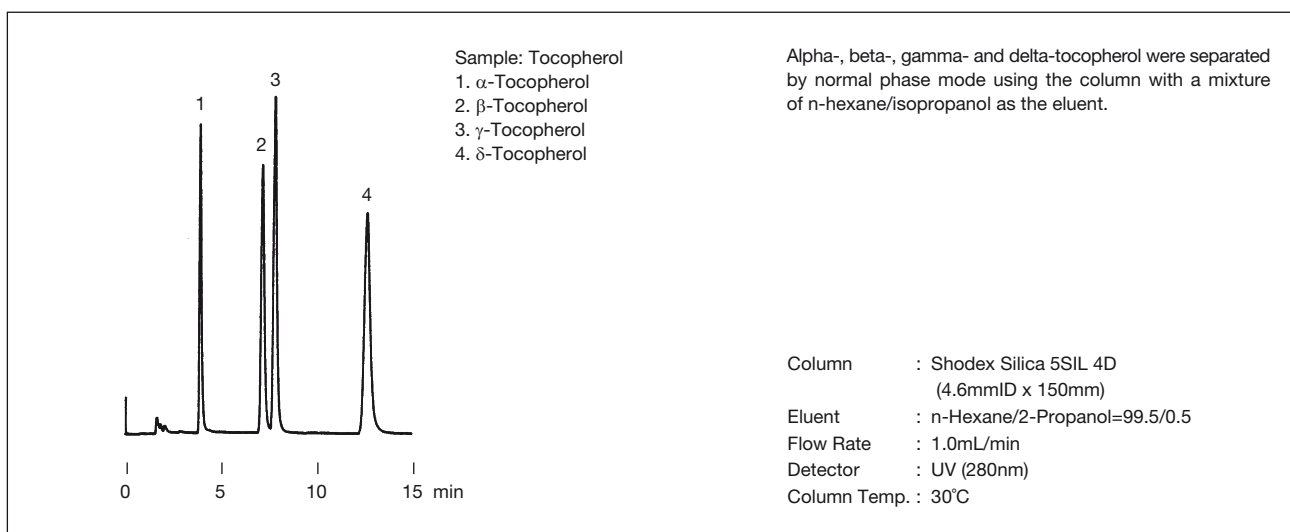


Fig. 4-6 Vitamin E (Normal Phase Mode)

4-2. Water-soluble Vitamins

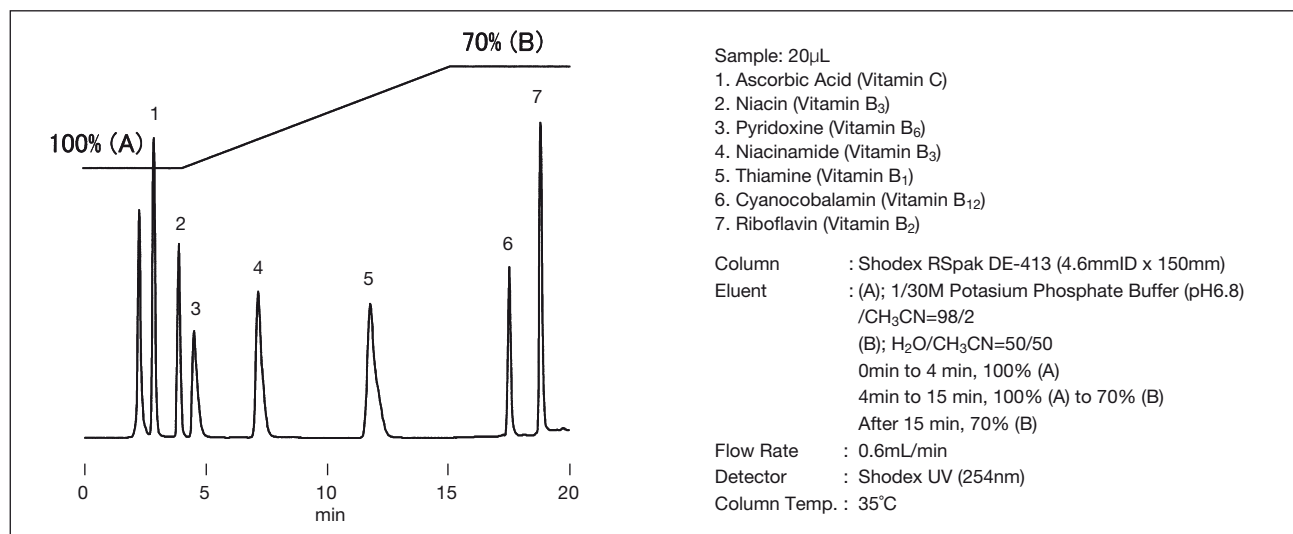


Fig. 4-7 Simultaneous Analysis of Water-soluble Vitamins (Reversed Phase Mode)

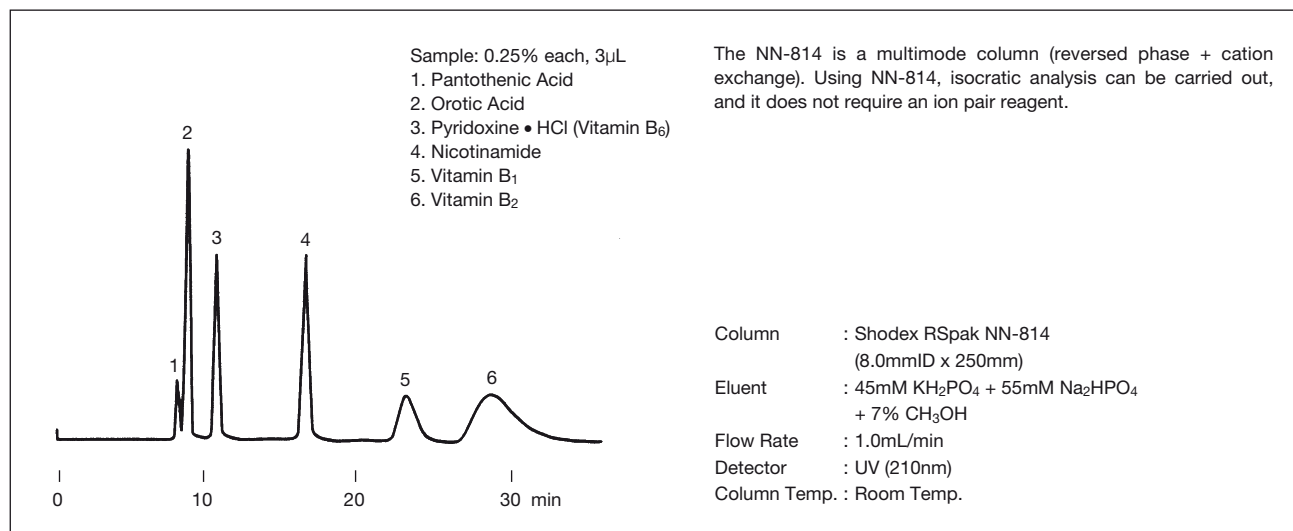


Fig. 4-8 Simultaneous Analysis of Water-soluble Vitamins (Multimode)

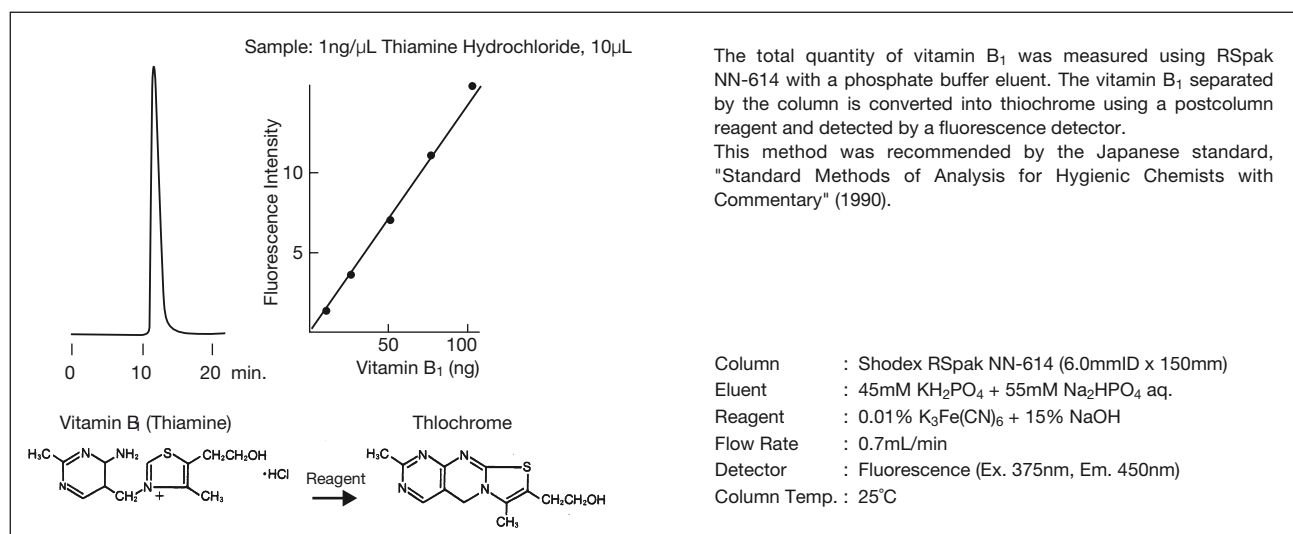


Fig. 4-9 Vitamin B1 (Multimode)

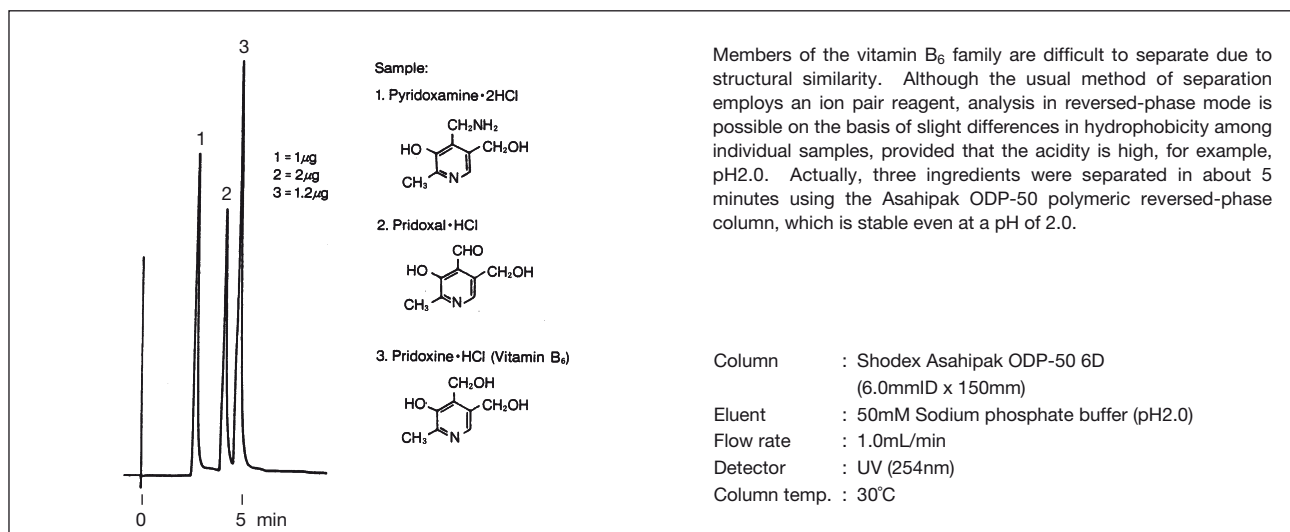


Fig. 4-10 Vitamin B₆ (reversed phase mode)

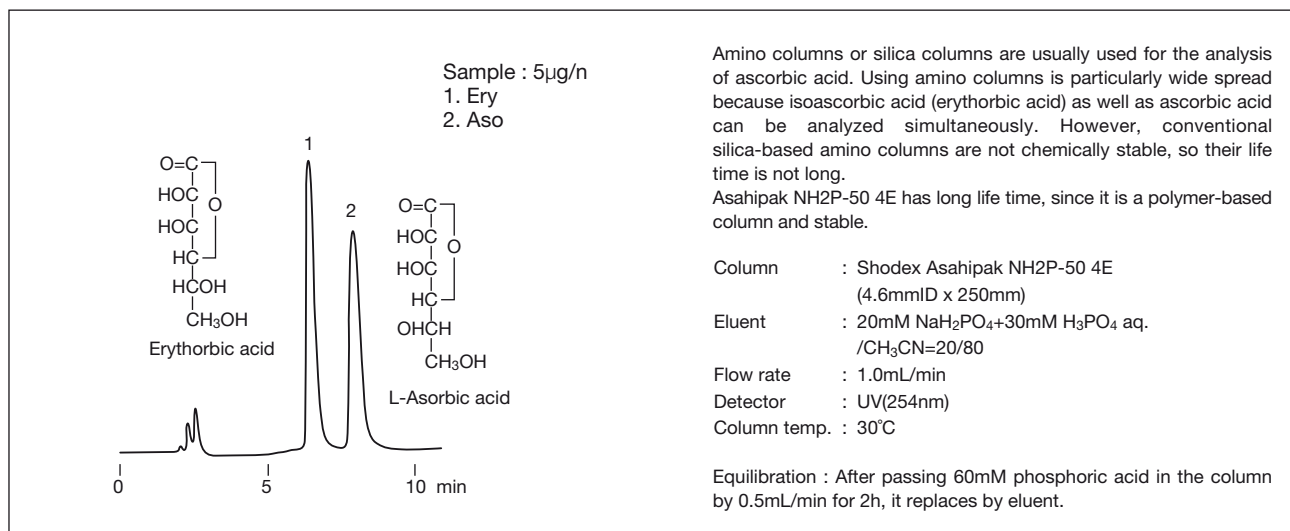


Fig. 4-11 Vitamin C (normal phase mode)

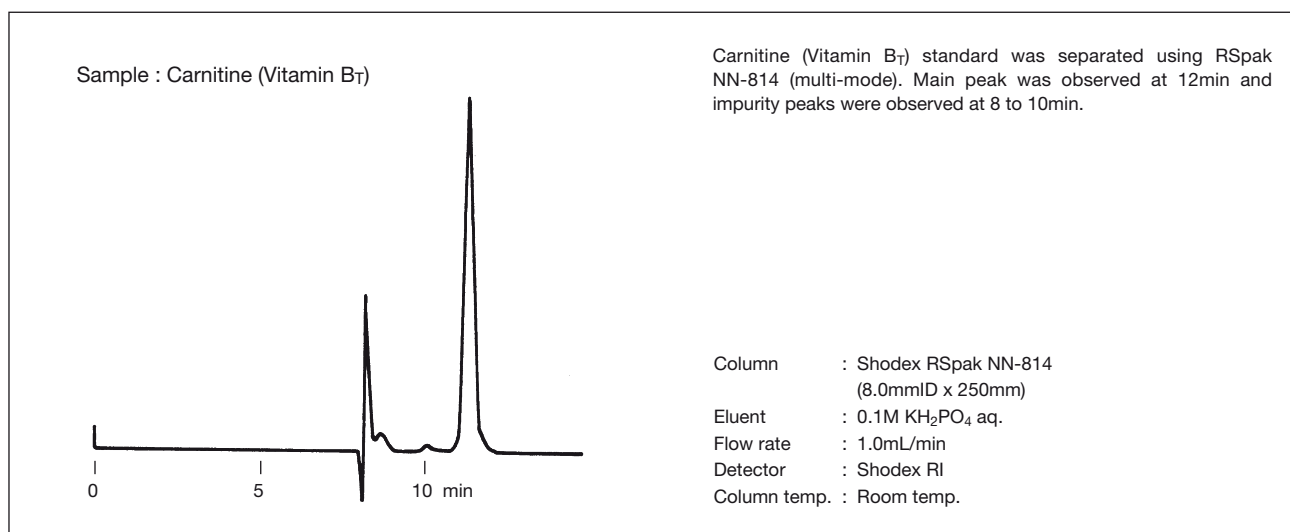


Fig. 4-12 Vitamin B₇ (multi- mode)

5. Other Ingredients in Foods

5-1. Higher Fatty Acids

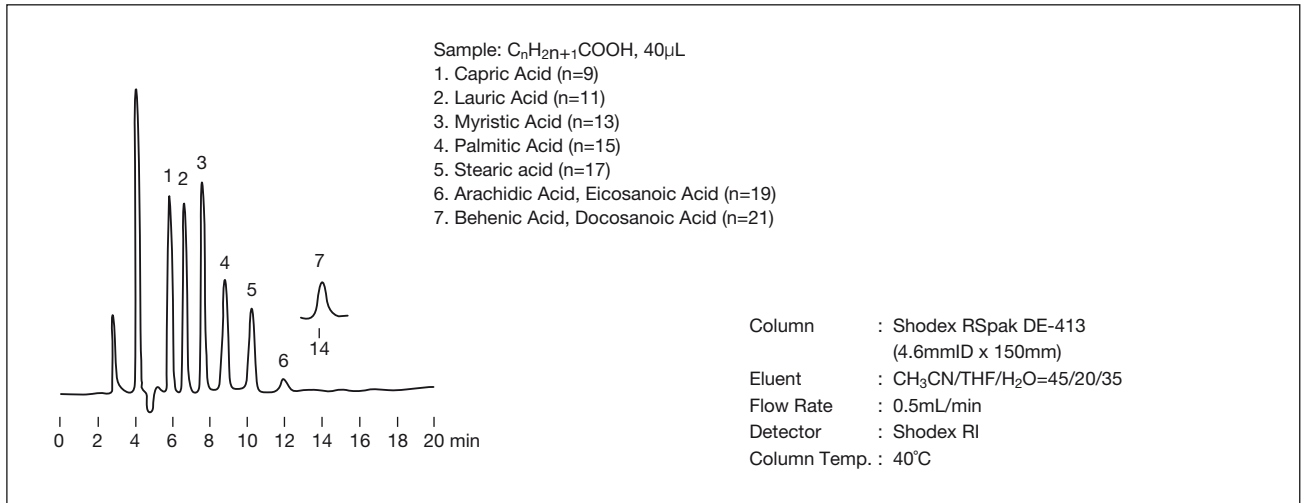


Fig. 5-1 Higher Fatty Acids (1)

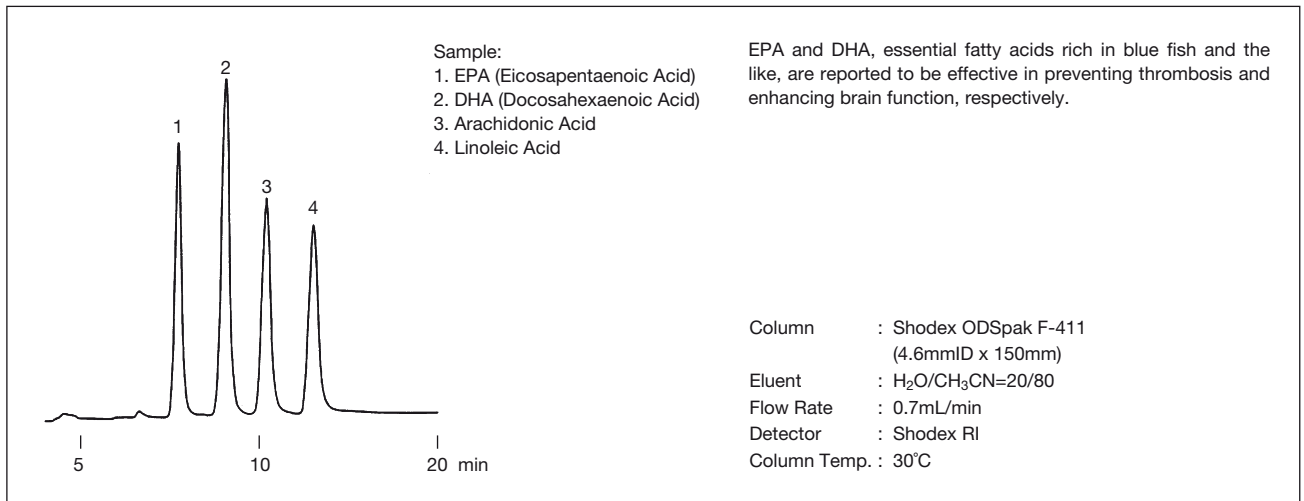


Fig. 5-2 Higher Fatty Acids (2)

Table 5-1 Shodex Columns for Higher Fatty Acid Analysis
(Absorption and Partition)

| Product Code | Product Name | Theoretical Plate Number (TP/column) | Particle Size (μ m) | Carbon Content (%) | ID x Length (mm) |
|--------------|----------------|--------------------------------------|--------------------------|--------------------|------------------|
| F7001004 | RSpak DE-613 | $\geq 7,000$ | 6 | — | 6.0 x 150 |
| F7001005 | RSpak DE-413 | $\geq 11,000$ | 4 | — | 4.6 x 150 |
| F7009030 | RSpak DE-413L | $\geq 17,000$ | 4 | — | 4.6 x 250 |
| F7001006 | RSpak DE-413S | $\geq 3,000$ | 4 | — | 4.6 x 50 |
| F6700150 | RSpak DE-G | (Guard column) | 10 | — | 4.6 x 10 |
| F7001007 | RSpak DE-213 | $\geq 8,000$ | 4 | — | 2.0 x 150 |
| F6700151 | RSpak DE-SG | (Guard column) | 4 | — | 2.0 x 10 |
| F6604112 | ODSpak F-411 | $\geq 8,000$ | 5 | 14 | 4.6 x 150 |
| F6605110 | ODSpak F-511 | $\geq 14,000$ | 5 | 14 | 4.6 x 250 |
| F6604113 | ODSpak F-411/S | $\geq 10,000$ | 3 | 14 | 4.6 x 100 |

5-2. Nucleic Acids as Taste Components

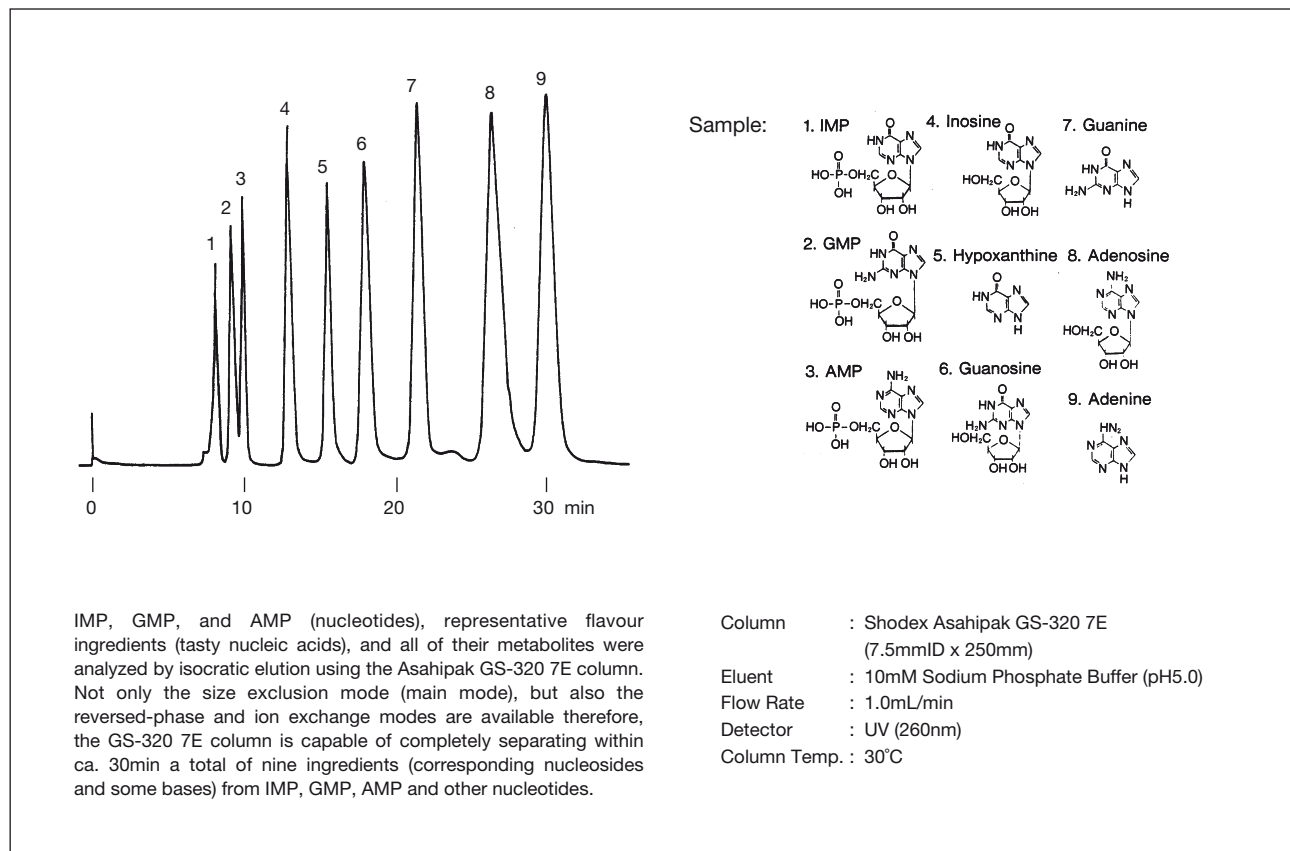


Fig. 5-3 Nucleic Acids as Taste Components

Table 5-2 Shodex Columns for Flavour Compounds Analysis (Multimode)

| Product Code | Product Name | ID x Length (mm) |
|--------------|--------------------|------------------|
| F7610005 | Asahipak GS-320 7E | 7.5 x 300 |
| F6710019 | Asahipak GS-2G 7B | 7.5 x 50 |

5-3. Amino Acids

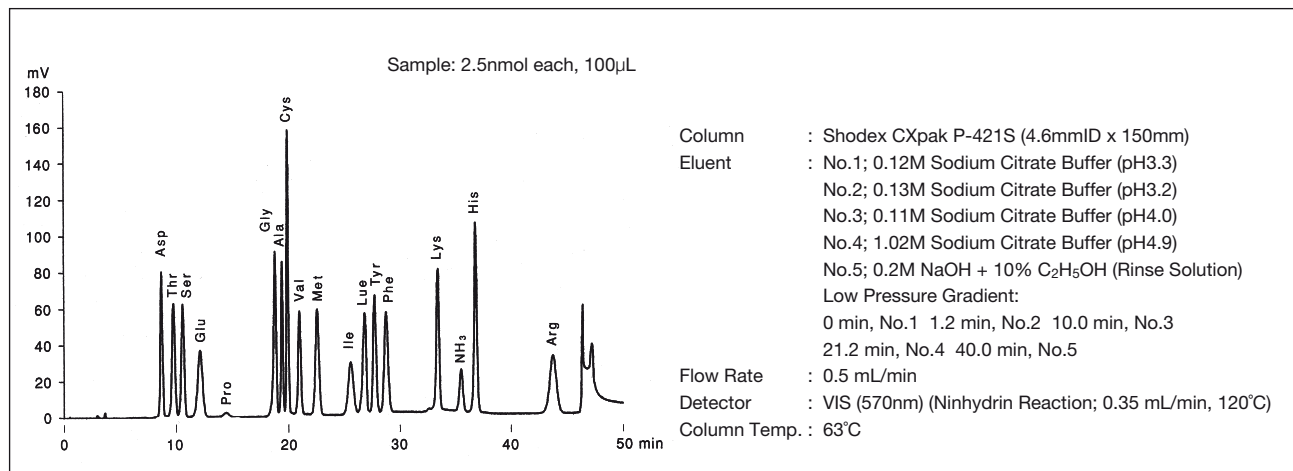


Fig. 5-4 Amino Acids (Strong Cation Exchange Mode)

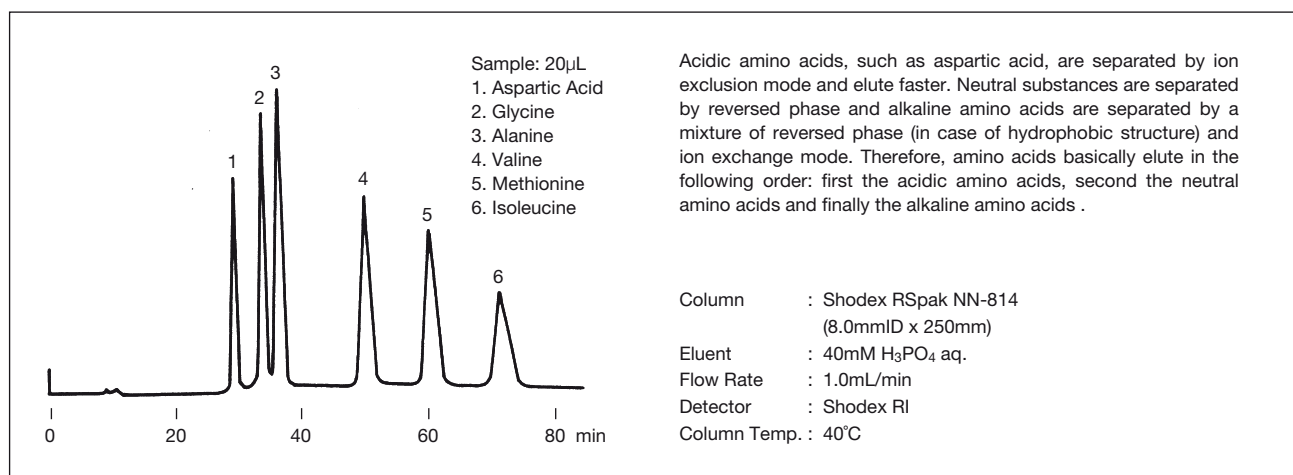


Fig. 5-5 Amino Acids (Multimode)

Table 5-3 Shodex Columns for Amino Acids Analysis (Strong Cation Exchange)

| Product Code | Product Name | SeparationMode | Particle Size (µm) | ID x Length (mm) |
|--------------|--------------|------------------------|--------------------|------------------|
| F6354211 | CXpak P-421S | Strong Cation Exchange | 6 | 4.6 x 150 |
| F6700210 | CXpak P-G | | 10 | 4.6 x 10 |

Table 5-3 Shodex Columns for Amino Acids Analysis (Multimode)

| Product Code | Product Name | SeparationMode | Particle Size (µm) | ID x Length (mm) |
|--------------|--------------|------------------|--------------------|------------------|
| F7008140 | RSpak NN-814 | Ion Exclusion | 10 | 8.0 x 250 |
| F7008150 | RSpak NN-614 | + Ion Exchange | 10 | 6.0 x 150 |
| F6700510 | RSpak NN-G | + Reversed Phase | 10 | 6.0 x 50 |
| F7008160 | RSpak NN-414 | | 10 | 4.6 x 150 |

Appendix

A. List of Elution volume of Saccharides

| Substances | Elution Volume (mL) | | | | | | |
|-------------------------------------|----------------------------|----------------------------|---------------------------|----------------------------|----------------------------|------------|-----------------|
| | SUGAR Series | | | | | Asahipak | Asahipak |
| | SP0810 Pb ²⁺ | SC1011 Ca ²⁺ | KS-801 Na ⁺ | SZ5532 Zn ²⁺ | SC1211 Ca ²⁺ | NH2P-50 4E | GS-220 HQ ×2 |
| N-Acetyl- α -D-glucosamine | 8.9 | 7.8 | 6.7 | - | 4.1 | 6.7 | |
| D(+)-Arabinose | 10.4 | 8.9 | 8.2 | 5.1 | 5.6 | 6.2 | |
| D-Arabitol | 15.9 | 11.3 | 7.6 | 7.3 | 8.2 | 6.3 | |
| Aspartame | - | - | - | - | 34.0 | - | |
| 2-Deoxy-D-glucose | 8.8 | 7.6 | 7.2 | 4.3 | 4.0 | 6.0 | |
| Difructose Anhydride (II) | 7.1 | 6.3 | 5.8 | 4.3 | * | 7.8 | |
| Dulcitol | 20.2 | 12.8 | 7.4 | 9.5 | 11.3 | 7.5 | |
| meso-Erythritol | 12.7 | 10.1 | 7.9 | 5.7 | 6.3 | 5.4 | 17.4 |
| Ethanol | 11.1 | 11.3 | 10.1 | - | 4.3 | - | |
| 1-Fructofuranosyl-D-nystose | 6.1 | 5.3 | 4.8 | 31.4 | * | - | 14.2 |
| D(-)-Fructose | 11.1 | 8.9 | 7.7 | 5.4 | 5.9 | 6.8 | 17.1 |
| D(+)-Fucose | 10.5 | 8.8 | 8.1 | 4.5 | 5.0 | 5.4 | |
| D(+)-Galactose | 9.7 | 8.0 | 7.6 | 6.5 | 5.0 | 8.1 | 16.6 |
| 4'-Galactosyllactose | 7.4 | 6.0 | 5.4 | 19.0 | * | 21.7 | |
| α -D-Galacturonic Acid | - | 6.3 | 4.4 | - | 5.6 | - | |
| Gentiobiose | 7.2 | 6.1 | 5.8 | 10.5 | * | 16.4 | |
| Glucose | 8.6 | 7.3 | 7.2 | 5.9 | 4.8 | 8.6 | 16.8 |
| Glycerol | | | | | | | 17.8 |
| Glycyrrhizin | - | - | - | - | 2.7 | - | |
| myo-Inositol | 12.8 | 8.9 | 8.0 | 12.6 | 7.9 | 10.0 | |
| Isomaltose | 7.7 | 6.3 | 6.0 | 10.6 | * | 15.2 | 15.6 |
| Isomaltotriose | 7.1 | 5.8 | 5.3 | 21.2 | * | 27.6 | 14.7 |
| 1-Kestose | 6.8 | 5.8 | 5.3 | 13.1 | * | 20.1 | 15.3 |
| Kojibiose | 7.6 | 6.2 | 5.9 | 9.7 | * | 14.8 | |
| Lactitol | 13.3 | 8.1 | 6.1 | 16.4 | 6.7 | 11.8 | 15.5 |
| Lactose | 8.1 | 6.5 | 6.0 | 10.1 | 4.1 | 13.3 | 15.7 |
| Lactosylfructoside | 7.1 | 5.9 | 5.3 | 14.7 | * | 19.0 | |
| Lactulose | 9.1 | 7.0 | 6.2 | 9.2 | 4.7 | 10.7 | |
| Maltitol | 12.2 | 8.3 | 6.0 | 13.0 | 6.8 | 11.8 | 15.8 |
| Maltoheptaose | | | | | | | 14.8 |
| Maltohexaose | | | | | | | 14.6 |
| Maltopentaose | | | | | | | 14.3 |
| Maltose | 7.9 | 6.3 | 5.9 | 8.7 | * | 14.2 | 16.1 |
| Maltotriose | 7.5 | 5.9 | 5.4 | 13.8 | * | 25.0 | 15.6 |
| Mannitol | 15.8 | 11.1 | 7.2 | 8.8 | 9.0 | 7.4 | |
| D-Mannose | 10.7 | 8.2 | 7.6 | 5.8 | 5.0 | 7.8 | |
| D(+)-Melezitose | 6.9 | 5.8 | 5.2 | 13.6 | * | 19.3 | |
| Melibiose | 8.2 | 6.5 | 6.0 | 11.7 | 4.2 | 14.7 | |
| Methyl- α -D-mannopyranoside | 11.1 | 8.9 | 7.8 | 4.0 | 4.4 | 4.7 | |
| Nystose | 6.4 | 5.5 | 4.9 | 20.1 | * | 31.9 | 14.7 |
| Palatinit | 2peaks | 2peaks | 5.9 | 2peaks | 2peaks | 12.7 | |

(-) → cannot be detected (*) → cannot be separated from solvent peak

| Substances | Elution Volume (mL) | | | | | | |
|--------------------|----------------------------|----------------------------|---------------------------|----------------------------|----------------------------|------------|-----------------|
| | SUGAR Series | | | | | Asahipak | Asahipak |
| | SP0810 Pb ²⁺ | SC1011 Ca ²⁺ | KS-801 Na ⁺ | SZ5532 Zn ²⁺ | SC1211 Ca ²⁺ | NH2P-50 4E | GS-220 HQ x2 |
| Palatinose | 7.8 | 6.5 | 5.9 | 8.1 | 4.0 | 12.1 | |
| Panose | 7.1 | 5.8 | 5.3 | 16.9 | * | 25.6 | |
| L-Phenylalanine | - | - | - | - | 31.6 | - | |
| D(+)-Raffinose | 7.1 | 5.8 | 5.3 | 16.4 | * | 20.3 | 15.1 |
| D(+)-Rhamnose | 9.8 | 8.2 | 7.4 | 3.9 | 4.4 | 5.5 | |
| D(-)-Ribose | 19.4 | 13.7 | 9.0 | 4.8 | 8.6 | 5.5 | |
| Rutinose | 7.8 | 6.5 | 5.8 | 6.7 | * | 10.9 | |
| Saccharin Sodium | 7.0 | 5.4 | 4.3 | 6.8 | * | 5.7 | |
| D(-)-Sorbitol | 21.6 | 13.3 | 7.4 | 9.8 | 11.9 | 7.1 | 16.6 |
| D(+)-Sorbose | 9.7 | 8.0 | 7.4 | 5.1 | 4.9 | 7.4 | |
| Stachyose | 6.8 | 5.6 | 5.0 | - | * | 36.2 | 14.3 |
| Stevioside | - | - | - | 4.1 | * | 6.1 | |
| Sucrose | 7.5 | 6.3 | 5.9 | 7.9 | * | 11.9 | 16.3 |
| α -D-Talose | 21.3 | 12.6 | 8.8 | 5.7 | 8.5 | 6.5 | |
| Theanderose | 2peaks | 2peaks | 2peaks | 2peaks | * | 2peaks | |
| Trehalose | 7.6 | 6.3 | 5.8 | 10.9 | * | 13.3 | |
| Trehalulose | 8.9 | 7.0 | 6.1 | 9.5 | 4.8 | 11.7 | |
| Xylitol | 19.9 | 13.1 | 7.9 | 7.8 | 10.2 | 6.1 | 16.9 |
| Xylobiose | 8.2 | 6.7 | 6.4 | 5.7 | * | 9.1 | |
| D(+)-Xylose | 9.2 | 7.1 | 7.7 | 4.6 | 4.5 | 6.6 | 17.5 |
| D-Xylulose | 10.6 | 9.0 | 8.0 | 4.1 | 5.1 | 5.4 | |

(-) → cannot be detected (*) → cannot be separated from solvent peak

Column : Shodex SUGAR SP0810, SC1011, KS-801
(8.0mmID x 300mm each)
Eluent : H₂O
Flow Rate : 1.0mL/min
Detector : Shodex RI
Column Temp. : 80°C

Column : Shodex Asahipak NH2P-50 4E
(4.6mmID x 250mm)
Eluent : H₂O/CH₃CN=25/75
Flow Rate : 1.0mL/min
Detector : Shodex RI
Column Temp. : 30°C

Column : Shodex SUGAR SZ5532
(6.0mmID x 150mm)
Eluent : H₂O/CH₃CN=25/75
Flow Rate : 1.0mL/min
Detector : Shodex RI
Column Temp. : 60°C

Column : Shodex Asahipak GS-220 HQ
(7.5mmID x 300mm) x 2
Eluent : H₂O
Flow Rate : 0.5mL/min
Detector : Shodex RI
Column Temp. : 60°C

Column : Shodex SUGAR SC1211
(6.0mmID x 250mm)
Eluent : H₂O/CH₃CN=65/35
Flow Rate : 1.0mL/min
Detector : Shodex RI
Column Temp. : 70°C

B. List of Elution Volume of Saccharides and Organic Acids (SH1011)

| Sample | Eluent | |
|-----------------------------|--------|------|
| | 5mM | 20mM |
| Oxalic Acid | 5.1 | 5.6 |
| Stachyose | 5.2 | 5.2 |
| Isomaltotriose | 5.3 | 5.3 |
| Panose | 5.4 | 5.4 |
| Maltotriose | 5.4 | 5.5 |
| Gentiobiose | 5.7 | 5.7 |
| Isomaltose | 5.8 | 5.8 |
| Melibiose | 5.9 | 5.9 |
| Kojibiose | 5.9 | 5.9 |
| Maltose | 5.9 | 5.9 |
| Trehalose | 5.9 | 5.9 |
| Nigerose | 5.9 | 5.9 |
| Palatinit | 6.0 | 6.0 |
| Palatinose | 6.0 | 5.9 |
| Trehalulose | 6.0 | 6.0 |
| Lactose | 6.0 | 6.0 |
| Xylobiose | 6.1 | 6.1 |
| Maltitol | 6.1 | 6.2 |
| D-Glucuronic Acid | 6.1 | 6.2 |
| Citric acid | 6.2 | 6.3 |
| α -Ketoglutaric Acid | 6.2 | 6.9 |
| Maleic Acid | 6.2 | 7.1 |
| Lactitol | 6.3 | 6.3 |
| N-Acetylneuramic Acid | 6.3 | 6.6 |
| Tartaric Acid | 6.4 | 6.6 |
| Glucose | 6.8 | 6.8 |
| Pyruvic Acid | 6.9 | 7.7 |
| Sorbose | 6.9 | 6.9 |

| Sample | Eluent | |
|-------------------------------------|--------|------|
| | 5mM | 20mM |
| myo-Inositol | 7.0 | 7.1 |
| Malic Acid | 7.0 | 7.2 |
| D-Mannose | 7.1 | 7.1 |
| D(+)-Galactose | 7.1 | 7.2 |
| D(+)-Xylose | 7.2 | 7.2 |
| Malonic Acid | 7.2 | 7.5 |
| D(-)-Fructose | 7.2 | 7.2 |
| trans-Aconitic Acid | 7.2 | 7.5 |
| Mannitol | 7.4 | 7.4 |
| D(-)-Sorbitol | 7.5 | 7.5 |
| D(+)-Rhamnose | 7.5 | 7.5 |
| D(+)-Arabinose | 7.6 | 7.7 |
| D(-)-Ribose | 7.8 | 7.8 |
| D-Arabitol | 7.8 | 7.9 |
| Xylitol | 8.0 | 8.0 |
| D(+)-Fucose | 8.0 | 8.0 |
| Succinic Acid | 8.3 | 8.3 |
| meso-Erythritol | 8.3 | 8.3 |
| N-Acetyl- α -D-glucosamine | 8.4 | 8.4 |
| DL-Lactic Acid | 8.8 | 8.9 |
| N-Acetyl- α -D-galactosamine | 9.3 | 9.3 |
| Formic Acid | 9.4 | 9.5 |
| Acetic Acid | 10.1 | 10.2 |
| Adipic Acid | 10.4 | 10.5 |
| Mesaconic Acid | 11.0 | 11.4 |
| L-Pyroglyutamic Acid | 11.4 | 11.7 |
| Propionic Acid | 11.7 | 11.7 |
| Ethanol | 14.1 | 14.1 |

Column : Shodex SUGAR SH1011 (8.0mmID x 300mm)
 Eluent : 5mM or 20mM H₂SO₄ aq.
 Flow Rate : 0.6mL/min
 Detector : Shodex RI
 Column Temp. : 60°C

C. List of Elution Volume of Organic Acids (KC-811)

| Substance | Elution Volume (mL) | | | |
|--|-----------------------|-----------------------|-----------------------|-----------------------|
| | 1mM HClO ₄ | 2mM HClO ₄ | 3mM HClO ₄ | 5mM HClO ₄ |
| Oxalic Acid | 9.3 | 9.4 | 9.6 | 9.7 |
| Maleic Acid | 9.7 | 10.2 | 10.5 | 11.1 |
| alpha-Ketoglutaric Acid | 10.0 | 10.4 | 10.9 | 11.4 |
| Oxalacetic Acid | 10.5 | 11.2 | 11.8 | 12.5 |
| Pyruvic Acid | 10.5 | 11.2 | 11.8 | 12.5 |
| 2-Ketogluconic Acid | 10.6 | 11.1 | 11.5 | 11.7 |
| Phosphoric Acid | 10.6 | 11.0 | 11.3 | 11.6 |
| Citric Acid | 10.8 | 11.3 | 11.7 | 11.8 |
| Tartaric Acid | 11.1 | 11.7 | 12.1 | 12.3 |
| Isocitric Acid | 11.1 | 11.5 | 11.9 | 11.9 |
| Glucuronic Acid | 11.2 | 11.4 | 11.7 | 11.8 |
| trans-Aconitic Acid | 11.5 | 12.4 | 13.1 | 13.5 |
| Malonic Acid | 11.5 | 12.4 | 13.0 | 13.4 |
| Galacturonic Acid | 11.8 | 12.1 | 12.4 | 12.4 |
| Gluconic Acid | 12.3 | 12.5 | 12.8 | 12.5 |
| Glyoxylic Acid | 12.4 | 12.9 | 13.3 | 13.9 |
| Malic Acid | 12.5 | 13.0 | 13.4 | 13.4 |
| alpha-Hydroxyglutaric Acid | 13.5 | 13.9 | 14.0 | 14.2 |
| Ascorbic Acid | 13.5 | 13.7 | 13.9 | 13.8 |
| Fumaric Acid | 14.5 | 16.1 | 17.2 | 17.8 |
| Succinic Acid | 15.3 | 15.5 | 15.8 | 15.7 |
| Glycolic Acid | 15.6 | 15.9 | 16.3 | 16.2 |
| Itaconic Acid | 15.9 | 16.4 | 16.8 | 16.8 |
| Lactic Acid | 16.0 | 16.4 | 16.7 | 16.7 |
| 2-Hydroxyisobutyric Acid | 16.5 | 16.8 | 17.1 | 17.1 |
| Adipic Acid | 16.7 | 19.8 | 20.2 | 20.0 |
| beta-Hydroxypropionic Acid | 16.9 | 17.0 | 17.2 | 17.1 |
| Formic Acid | 17.2 | 17.6 | 17.9 | 17.9 |
| Mesaconic Acid | 17.2 | 19.2 | 20.5 | 21.1 |
| Uric Acid | 17.9 | 17.9 | 18.3 | 18.0 |
| Pyroglutamic Acid | 18.6 | 20.3 | 21.0 | 21.7 |
| Acetic Acid | 19.2 | 19.3 | 19.4 | 19.4 |
| Levulinic Acid | 20.0 | 20.2 | 20.3 | 20.2 |
| Propionic Acid | 22.3 | 22.4 | 22.5 | 22.5 |
| CO ₃ ²⁻ , HCO ₃ ³⁻ | 24.7 | 24.7 | 24.5 | 24.7 |
| Isobutyric Acid | 25.4 | 25.5 | 25.6 | 25.6 |
| n-Butyric Acid | 27.3 | 27.5 | 27.6 | 27.6 |
| Isovaleric Acid | 31.9 | 32.1 | 32.3 | 32.2 |
| n-Valeric Acid | 38.4 | 38.6 | 38.9 | 38.8 |

Column : Shodex RSpak KC-811 (8.0mmID x 300mm) x 2
 Eluent : HClO₄ aq.
 Flow Rate : 1.0mL/min
 Column Temp. : 50°C

D. List of Elution Volume of Organic Acids (KC-811)

| Substance | Elution Volume (mL) | | | | | |
|---|---------------------|------|------|------|------|------|
| | 30°C | 40°C | 50°C | 60°C | 70°C | 80°C |
| Oxalic Acid | 9.7 | 9.6 | 9.6 | 9.6 | 9.5 | 9.5 |
| Maleic Acid | 10.7 | 10.7 | 10.5 | 10.5 | 10.5 | 10.4 |
| Phosphoric Acid | 11.0 | 11.1 | 11.3 | 11.4 | 11.5 | 11.7 |
| α -Ketoglutaric Acid | 11.1 | 11.0 | 10.9 | 10.8 | 10.7 | 10.6 |
| 2-Ketogluconic Acid | 11.6 | 11.5 | 11.5 | 11.5 | 11.4 | 11.4 |
| Glucuronic Acid | 11.7 | 11.7 | 11.7 | 11.7 | 11.7 | * |
| Pyruvic Acid | 11.9 | 11.8 | 11.8 | 11.7 | 11.7 | 11.7 |
| Citric Acid | 11.9 | 11.8 | 11.7 | 11.6 | 11.5 | 11.4 |
| Oxalacetic Acid | 12.0 | 11.9 | 11.8 | 11.8 | 11.7 | 11.7 |
| Isocitric Acid | 12.1 | 12.0 | 11.9 | 11.7 | 11.6 | 11.5 |
| Tartaric Acid | 12.4 | 12.2 | 12.1 | 12.0 | 11.9 | 11.8 |
| Galacturonic Acid | 12.5 | 12.4 | 12.4 | 12.3 | 12.3 | 12.2 |
| Gluconic Acid | 12.8 | 12.8 | 12.8 | 12.8 | 12.9 | 12.9 |
| Malonic Acid | 13.3 | 13.1 | 13.0 | 12.8 | 12.7 | 12.6 |
| trans-Aconitic Acid | 14.0 | 13.5 | 13.1 | 12.7 | 12.5 | 12.2 |
| Glyoxylic Acid | 13.3 | 13.3 | 13.3 | 13.2 | 13.1 | 13.1 |
| Malic Acid | 13.7 | 13.5 | 13.4 | 13.2 | 13.0 | 12.9 |
| Ascorbic Acid | 14.1 | 14.0 | 13.9 | 13.7 | 13.6 | * |
| α -Hydroxyglutaric Acid | 14.6 | 14.3 | 14.1 | 13.8 | 14.0 | 13.9 |
| Succinic Acid | 16.5 | 16.1 | 15.8 | 15.4 | 15.1 | 14.8 |
| Glycolic Acid | 16.6 | 16.4 | 16.3 | 16.1 | 15.9 | 15.7 |
| Lactic Acid | 16.8 | 16.8 | 16.7 | 16.5 | 16.4 | 16.2 |
| 2-Hydroxyisobutyric Acid | 17.0 | 17.1 | 17.1 | 16.9 | 16.8 | 16.7 |
| β -Hydroxypropionic Acid | 17.5 | 17.3 | 17.2 | 17.0 | 16.8 | 16.6 |
| Itaconic Acid | 17.8 | 17.3 | 16.8 | 16.3 | 15.9 | 15.5 |
| Formic Acid | 18.2 | 18.0 | 17.9 | 17.6 | 17.5 | 17.3 |
| Fumaric Acid | 19.2 | 18.0 | 17.2 | 16.4 | 15.8 | 15.3 |
| Acetic Acid | 19.9 | 19.7 | 19.4 | 19.1 | 18.8 | 18.6 |
| Uric Acid | 20.7 | 19.3 | 18.3 | 17.4 | 16.8 | 16.2 |
| Levulinic Acid | 22.1 | 21.1 | 20.3 | 19.5 | 18.9 | 18.3 |
| Pyroglutamic Acid | 22.9 | 21.8 | 21.0 | 20.3 | 19.8 | 19.3 |
| Adipic Acid | 23.1 | 21.4 | 20.2 | 19.0 | 18.1 | 17.3 |
| Propionic Acid | 23.5 | 23.0 | 22.5 | 22.0 | 21.5 | 20.7 |
| Mesaconic Acid | 24.0 | 21.9 | 20.5 | 19.2 | 18.1 | 17.3 |
| CO ₃ ²⁻ , HCO ₃ ⁻ | 24.3 | 24.5 | 24.5 | 24.5 | 24.4 | 24.2 |
| Isobutyric Acid | 27.1 | 26.4 | 25.6 | 24.8 | 24.0 | 23.1 |
| n-Butyric Acid | 29.8 | 28.7 | 27.6 | 26.5 | 25.5 | 24.5 |
| Isovaleric Acid | 35.7 | 33.9 | 32.3 | 30.6 | 29.0 | 27.5 |
| n-Valeric Acid | 45.1 | 41.8 | 38.9 | 36.1 | 33.6 | 31.3 |

Column : Shodex RSpak KC-811 (8.0mmID x 300mm) x 2
 Eluent : 3mM HClO₄ aq.
 Flow Rate : 1.0mL/min

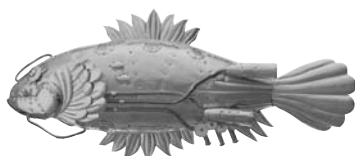
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