Shodex™

HPLC Columns

Food analysis with Shodex™ columns

Saccharides, Organic acids, Vitamins, Fatty acids and Amino acids

Technical notebook
No. 3

SHOWA DENKO EUROPE

Contents

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 exchage), 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)

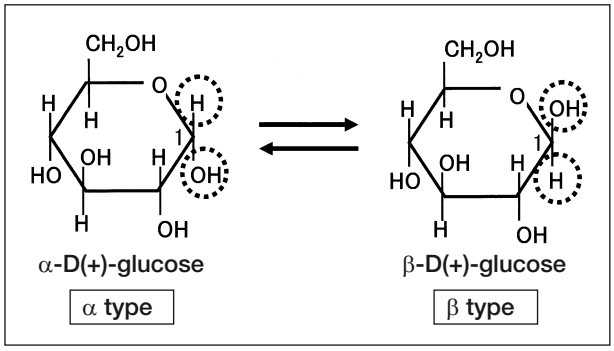
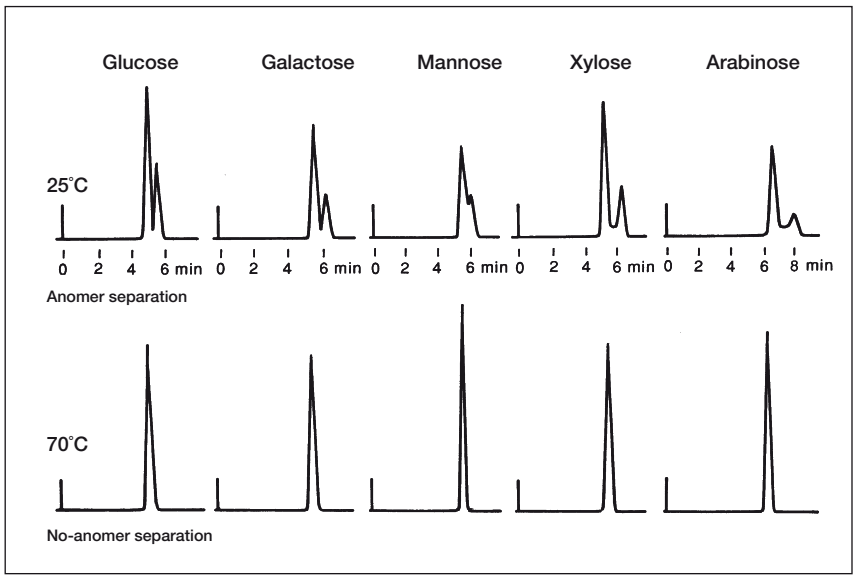


Fig. 2-1 Anomers

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)



Sample: 1% each, 5uL

Column : Shodex SUGAR SC1211
Eluent : H_2O
Flow Rate : 0.7mL/min
Detector : Shodex RI
Column Temp.: (A) 70°C, (B) 25°C

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.

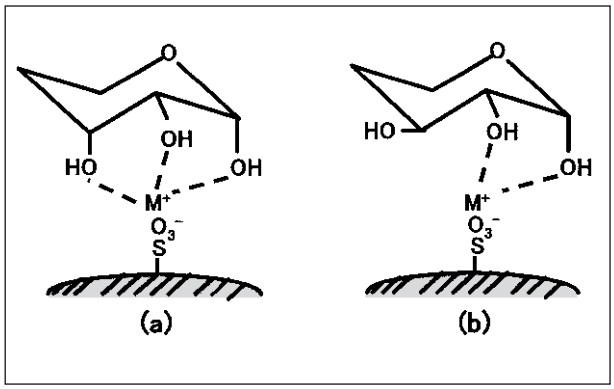


Fig. 2-3 Difference of counter ion interaction

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.

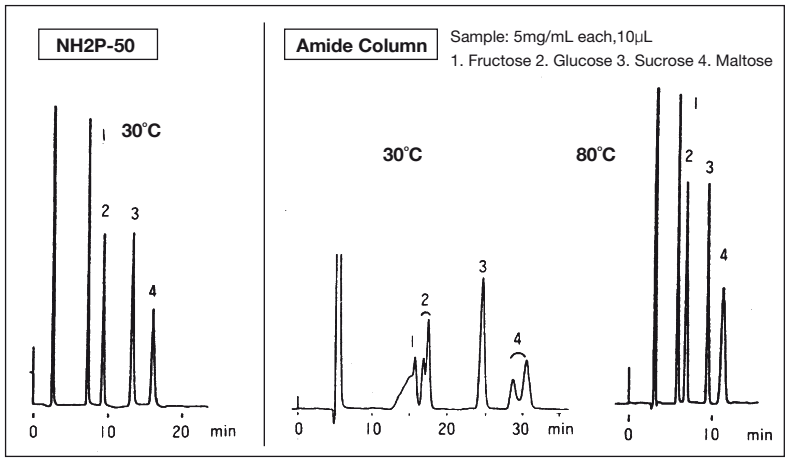


Fig. 2-4 Effects of Column Temperature on Elution Patterns (Comparison with Amide Column)

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	: CH_3CN/H_2O=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)

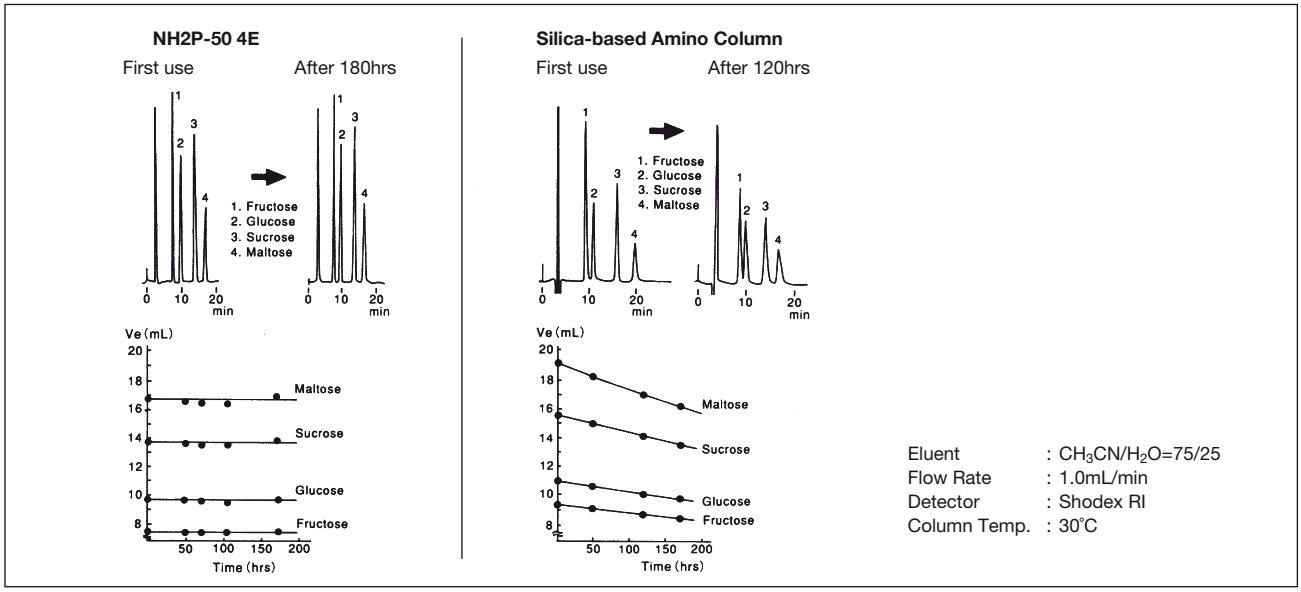


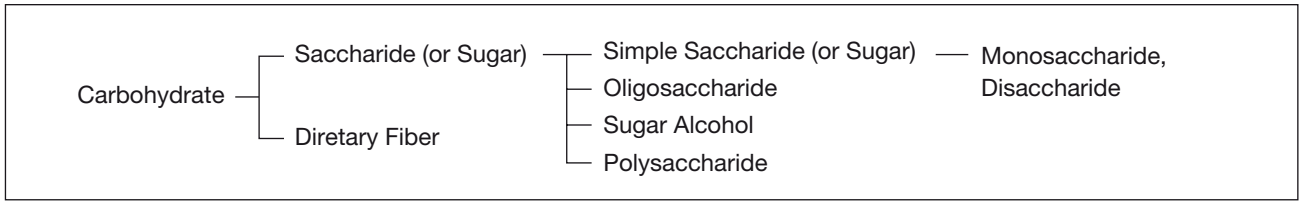
Fig. 2-5 Reproducibility of Chromatograms with NH2P-50 4E Column and Silica-based Amino Column

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

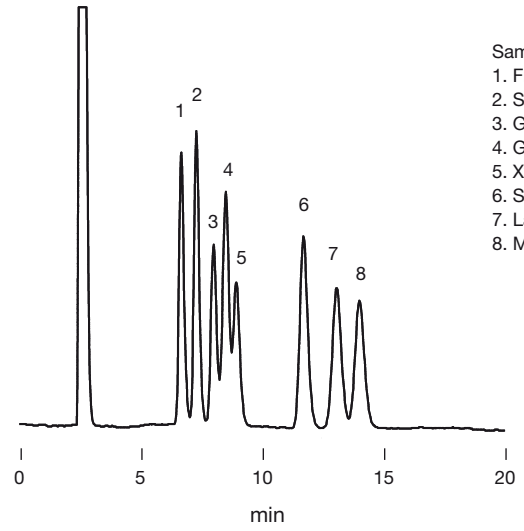
Column	: A column packed with a silica or polymer gel bounded amino (propyl) group (4.6mmID x 250mm) → Shodex Asahipak NH2P-50 4E
Eluent	: CH_3CN/H_2O = 75/25
Flow Rate	: 1.0 mL/min
Detector	: RI
Column Temp.	: Room Temp.
Injection Vol.	: 20μL

Oligosaccharide

Column	: A column packed with a silica or polymer gel bounded amino (propyl) group (4.6mmID x 250mm) → Shodex Asahipak NH2P-50 4E
Eluent	: CH_3CN/H_2O = 75/25
Flow Rate	: 1.0 mL/min
Detector	: RI
Column Temp.	: Room Temp.
Injection Vol.	: 20μL

Japan's Nutritional Facts Labeling Standards Ver. 3 (Japan Health Food Authorization)

Fig. 2-6 Analysis Condition of Saccharides and Oligosaccharides



Sample: 0.1% each, 20μL
1. Fructose
2. Sorbose
3. Galactose
4. Glucose
5. Xylobiose
6. Sucrose
7. Lactose
8. Maltose

Column	: Shodex Asahipak NH2P-50 4E (4.6mmID x 250mm)
Eluent	: CH_3CN/H_2O=75/25
Flow Rate	: 1.0mL/min
Detector	: Shodex RI
Column Temp.	: 25℃

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

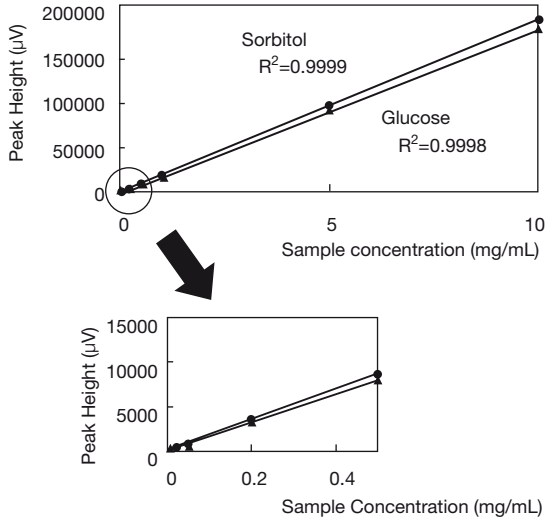


Table 2-3 Correlation for other Saccharides (R^2)

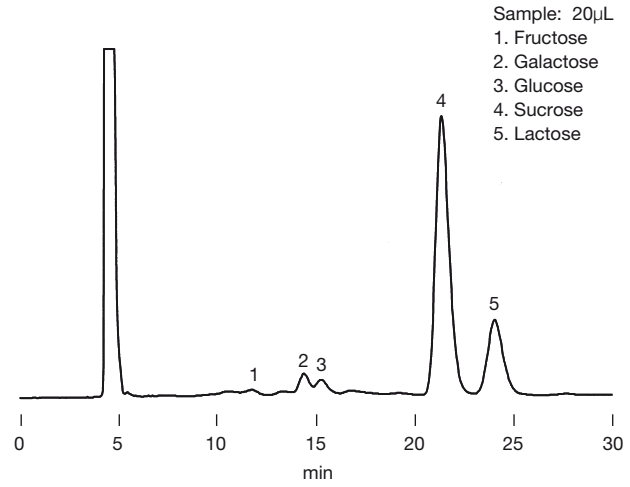
Fructose	0.9998
Sucrose	0.9999
Lactose	0.9997
Maltose	0.9998
meso-Erythritol	0.9999
Xylitol	0.9999
Mannose	0.9999
Maltitol	0.9997

(0.01mg/mL~10mg/mL)

Condition of Fig. 2-8 and Table 2-3

Column	: Shodex Asahipak NH2P-50 4E (4.6mmID x 250mm)
Eluent	: CH_3CN/H_2O=75/25
Flow Rate	: 1.0mL/min
Detector	: Shodex RI
Column Temp.	: 25℃

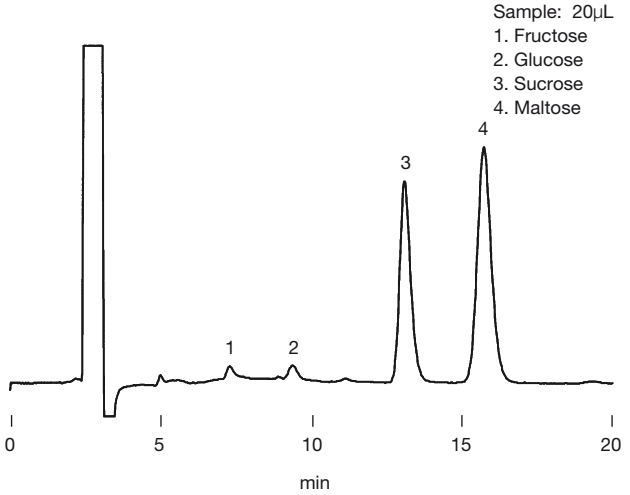
Fig. 2-8 Calibration Curves with NH2P-50



(Pretreatment of Sample)
(1) Measure 5g of yogurt into a beaker.
(2) Add 30ml of pure water to the yoghurt. After having stirred the mixture, neutralize it with a 10w/v% sodium hydroxide aqueous solution.
(3) After 30 minutes of ultrasonic extraction, add pure water until total volume becomes 50ml.
(4) Pass the solution (3) with a No. 5B filter paper. To 3 mL of the filtrate, add the same volume of acetonitrile and stir this mixture.
(5) Pass through a membrane filter (0.45μm) before injecting into HPLC.

Column	: Shodex Asahipak NH2P-50 4E (4.6mmID x 250mm)
Eluent	: CH_3CN/H_2O=75/25
Flow Rate	: 0.6mL/min
Detector	: Shodex RI
Column Temp.	: 25℃

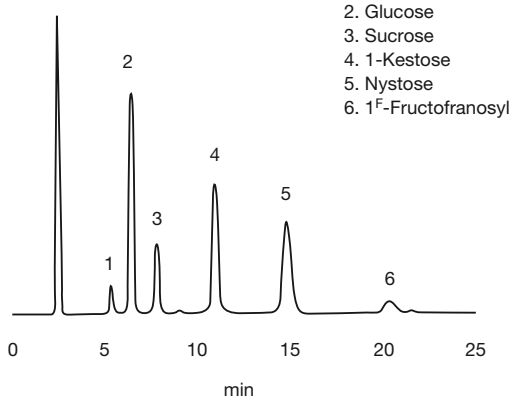
Fig. 2-9 Saccharides in Sugar-added Yoghurt



(Pretreatment of Sample)
(1) Make paste of the sample using a food-processor.
(2) Put 3g of the paste in a beaker and add 30mL of 50% (V/V) ethanol.
(3) Extract the substances from the paste using an ultrasonic vibrator for 30min.
(4) Put the paste and ethanol in a 50mL measuring cup and add 50% (V/V) ethanol to make up a 50mL solution.
(5) Filtrate the solution with a paper filter (No.5B) to remove the paste.
(6) Take a part of the filtrated solution and add the same volume of acetonitrile.

Column	: Shodex Asahipak NH2P-50 4E (4.6mmID x 250mm)
Eluent	: CH_3CN/H_2O=75/25
Flow Rate	: 1.0mL/min
Detector	: Shodex RI
Column Temp.	: Room Temp. (25℃)

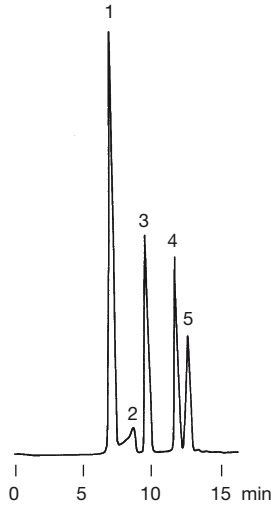
Fig. 2-10 Saccharides in Sweet Potato, Roasted



With NH2P-50 columns, saccharides elute in order of increasing polarity due to the function of normal phase chromatography. The polarity becomes stronger with longer chains and adsorption becomes stronger.
With the analysis of oligosaccharides, a high rate of water of solvent is recommended for early elution.

Column	: Shodex Asahipak NH2P-50 4E (4.6mmID x 250mm)
Eluent	: CH_3CN/H_2O=70/30
Flow Rate	: 1.0mL/min
Detector	: Shodex RI
Column Temp.	: 25℃

Fig. 2-11 Fructo-oligosaccharide Syrup

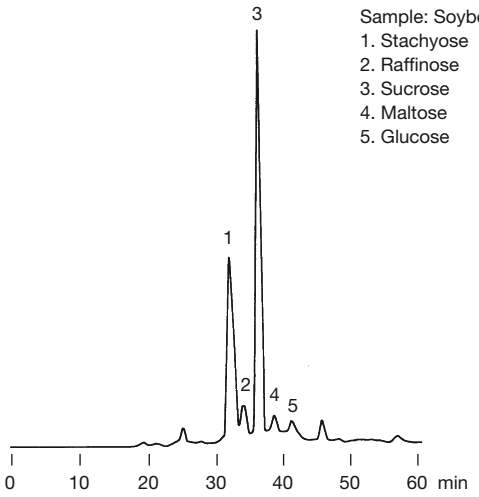


Sample: 5μL
5-fold Diluted Worcestershire Sauce
1. Polysaccharides and Anion
2. Polysaccharides
3. Sucrose
4. Glucose
5. Fructose

With SUGAR KS-801, high molecular weights and anionic compounds are eluted first, followed by low molecular weight saccharides. Therefore, KS-801 can be used for the analysis of saccharides without any previous desalting of samples such as Worcestershire sauce, which usually contains large amounts of sodium chloride and organic acids.

Column : Shodex SUGAR KS-801 (8.0mmID x 300mm)
Eluent : H_2O
Flow Rate : 0.7mL/min
Detector : Shodex RI
Column Temp. : 80°C

Fig. 2-12 Saccharides in Worcestershire Sauce

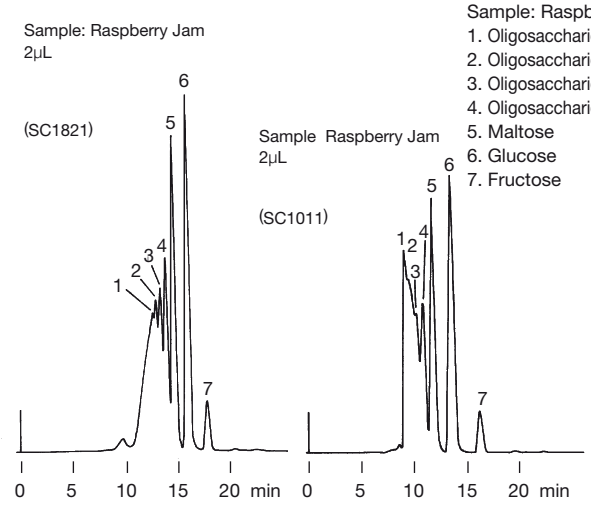


Sample: Soybean Flour
1. Stachyose
2. Raffinose
3. Sucrose
4. Maltose
5. Glucose

(Sample Pretreatment)
(1) Put the soybean flour in a beaker and add 50-fold pure water.
(2) Extract the substances from the paste into water using an ultrasonic vibrator for 30min.
(3) Perform ultrafiltration (mw : 10,000).
(4) Pass through a membrane filter (0.45μm) before injecting into HPLC.

Column : Shodex SUGAR SP-G (6.0mmID x 50mm)
+ SP0810 (8.0mmID x 300mm) x 2
Eluent : H_2O
Flow Rate : 0.4mL/min
Detector : Shodex RI
Column Temp. : 80°C

Fig. 2-13 Saccharides in Soybean Flour



Sample: Raspberry Jam
1. Oligosaccharide (DP-6)
2. Oligosaccharide (DP-5)
3. Oligosaccharide (DP-4)
4. Oligosaccharide (DP-3)
5. Maltose
6. Glucose
7. Fructose

Raspberry jam was analyzed. It was found that lots of oligosaccharides are contained in the sample. SUGAR SC1011 or SC1821 was used and better separation was obtained by SC1821 because of its larger pore size.
(Sample Pretreatment)
(1) Put 5g of raspberry jam in a beaker and add 20mL of pure water.
(2) Pass through a membrane filter (0.45μm) before injecting into HPLC.

Column : (left); Shodex SUGAR SC1011
(right); Shodex SUGAR SC1821
(8.0mmID x 300mm each)
Eluent : H_2O
Flow Rate : 0.6mL/min
Detector : Shodex RI
Column Temp. : 80°C

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)

Column	: A column packed with a silica or polymer gel bounded amino (propyl) group (4.6mmID x 250mm) → Shodex Asahipak NH2P-50 4E
Eluent	: CH_3CN/H_2O = 75/25
Flow Rate	: 1.0mL/min
Detector	: RI
Column Temp.	: Room Temp.
Injection Vol.	: 20μL

Sugar Alcohol (2)

Column	: A column packed with sulfonated poly styrene gel (Pb or Ca ligand) (7.8-8.0mmID x 300mm) → Shodex SUGAR SP0810, SC1011
Eluent	: H_2O
Flow Rate	: 0.6mL/min
Detector	: RI
Column Temp.	: 85℃
Injection Vol.	: 5μL

Japan's Nutritional Facts Labeling Standards Ver. 3 (Japan Health Food Authorization)

Fig. 2-15 Analysis Condition of Sugar Alcohols

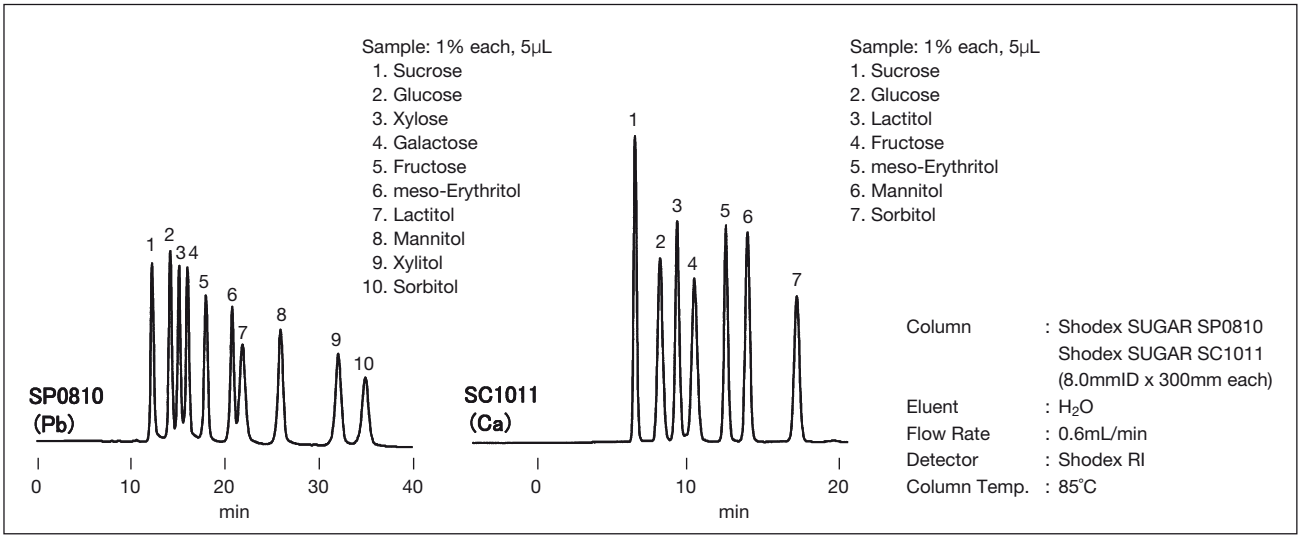


Fig. 2-16 Standard Saccharides and Sugar Alcohol with SP0810, SC1011

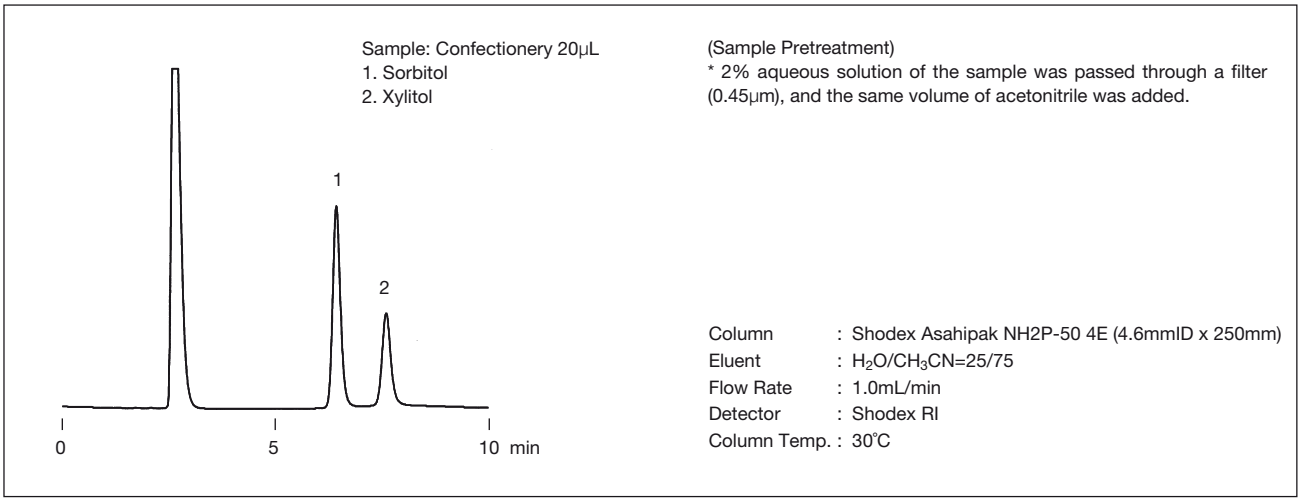
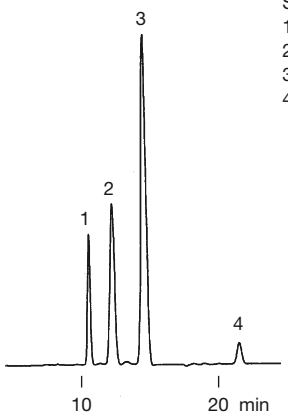


Fig. 2-17 Xylitol in Confectionary



Sample: Apple Juice, 5μL
1. Sucrose
2. Glucose
3. Fructose
4. Sorbitol

(Sample Pretreatment)
(1) Take 5g of apple juice in a beaker, add 30mL of pure water and neutralize with 10% (W/V) NaOH aq. cooled with ice.
(2) Apply ultrasonic vibration for 30min and make 50mL solution with pure water.
(3) Filtrate with 0.45 micron membrane filter.

Column	: Shodex SUGAR SC1011 (8.0mmID x 300mm)
Eluent	: H_2O
Flow Rate	: 0.6mL/min
Detector	: Shodex RI
Column Temp.	: 85°C

Fig. 2-18 Apple Juice

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

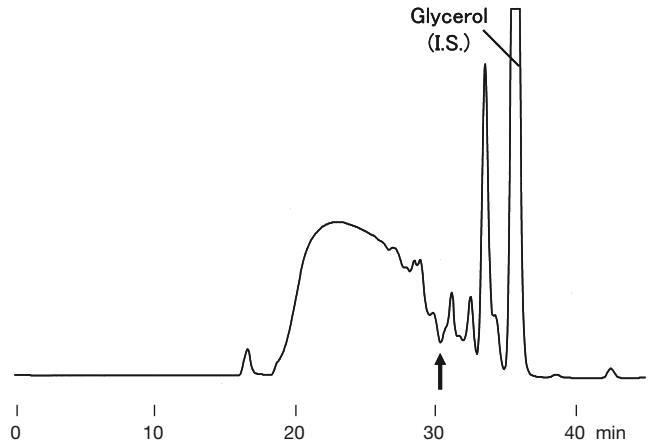
Dietary fibers are usually quantified by the Prosky method (enzyme-gravimetric method). However, for water-soluble low molecular weight dietary fibers unquantifiable by the Prosky method, the enzyme-HPLC method is used. In this analysis, the sample is divided into two fractions: dietary fibers (tri- 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	: H_2O
Flow Rate	: 0.5mL/min
Detector	: RI
Column Temp.	: 80°C
Injection Vol.	: 20μL

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

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



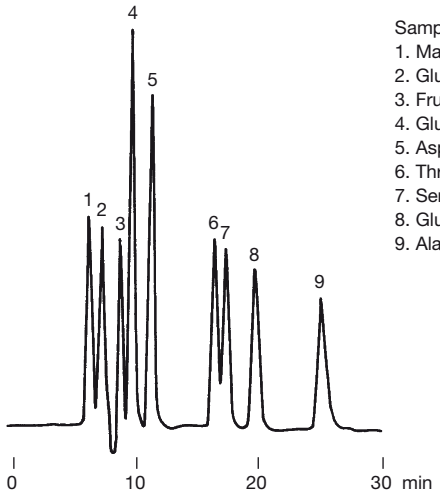
Using GS-220 HQ, monosaccharides, disaccharides and sugar alcohols elute after the position indicated by the arrow mark and it is easy to divide the two fractions.

Column	: Shodex Asahipak GS-220 HQ (7.5mmID x 300mm) x 2
Eluent	: H_2O
Flow Rate	: 0.5mL/min
Detector	: Shodex RI
Column Temp.	: 60°C

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.

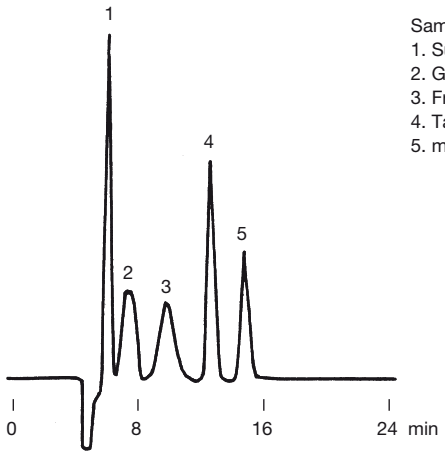


Sample: 0.1% each, 10μL
1. Maltose
2. Glucose
3. Fructose
4. Glutamic acid
5. Aspartic acid
6. Threonine
7. Serine
8. Glutamine
9. Alanine

Generally, ligand exchange mode columns are used with aqueous eluent, but the addition of the same cation as the counter ion enables the columns to separate samples under ion exchange mode additionally in addition to the ligand exchange mode. $CaSO_4$ aqueous solution is used for SUGAR SC1011 as its counter ion is Ca^{2+}. This means that ligand exchange occurs between the counter ions of the packing material and the hydroxyl groups of saccharides and ion exchange occurs between the sulfonate groups and the amino groups of the saccharides.

Column : Shodex SUGAR SC1011 (8.0mmID x 300mm)
Eluent : 10mM $CaSO_4$ (pH6.0)
Flow Rate : 1.0mL/min
Detector : Shodex RI
Column Temp. : 80℃

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

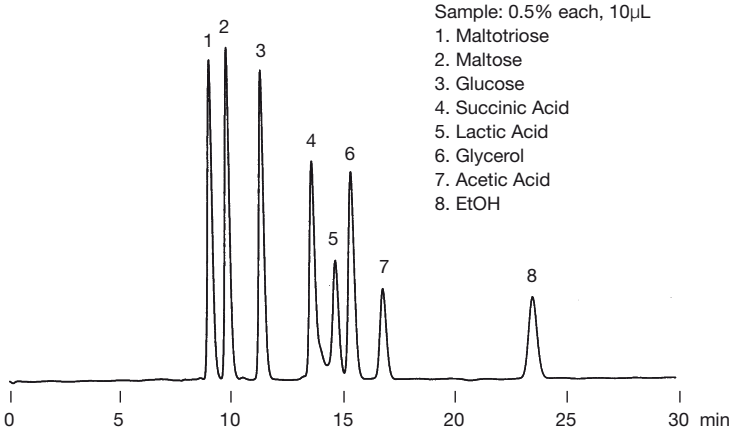


Sample:
1. Sucrose
2. Glucose
3. Fructose
4. Taurine
5. myo-Inositol

Taurine is an amino amino acid, and recently is well known as one of the ingredients added to energy supplement drinks.

Column : Shodex SUGAR SC1211 (6.0mmID x 250mm)
Eluent : H_2O/CH_3CN=60/40
Flow Rate : 0.6mL/min
Detector : Shodex RI
Column Temp. : 70℃

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



Sample: 0.5% each, 10μL
1. Maltotriose
2. Maltose
3. Glucose
4. Succinic Acid
5. Lactic Acid
6. Glycerol
7. Acetic Acid
8. EtOH

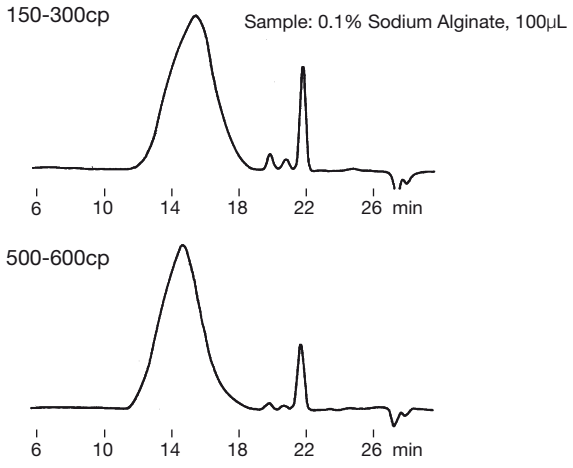
In acidic eluent organic acids are separated with ion exclusion mode and reversed phase mode. Saccharides are separated with SEC.

Column : Shodex SUGAR SH1011 (8.0mmID x 300mm)
Eluent : 5mM H_2SO_4 aq.
Flow Rate : 0.6mL/min
Detector : Shodex RI
Column Temp. : 60℃

Fig. 2-23 Saccharides and Organic Acids

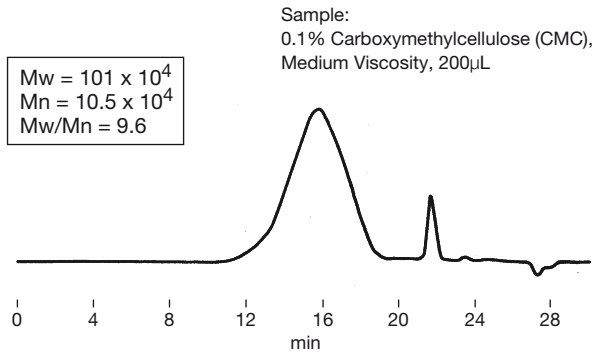
2-6. Polysaccharides

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.



Column : Shodex OHpak SB-806M HQ (8.0mmID x 300mm) x 2
Eluent : 0.1M $NaNO_3$ aq.
Flow Rate : 1.0mL/min
Detector : Shodex RI
Column Temp. : 40°C

Fig. 2-24 Sodium Alginate



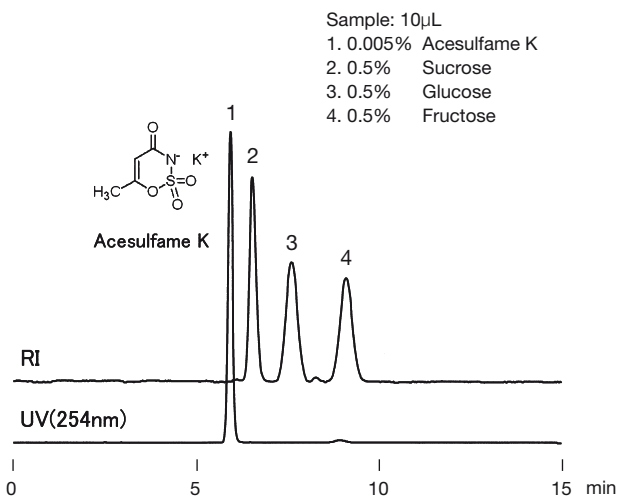
Carboxymethylcellulose was analyzed using OHpak SB-806M HQ (a column for GFC separation). Mw and Mn were calculated as pullulan.

Column : Shodex OHpak SB-806M HQ (8.0mmID x 300mm) x 2
Eluent : 0.1M NaCl aq.
Flow Rate : 1.0mL/min
Detector : Shodex RI
Column Temp. : 40°C

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.



The concentration of acesulfame K is at a trace level, therefore it is difficult to detect it by RI detection. Saccharides do not have UV-absorption, thus there is no peak by UV detection.

Column : Shodex SUGAR SC1011 (8.0mmID x 300mm)
Eluent : 10mM $CaSO_4$ aq.
Flow Rate : 1.0mL/min
Detector : Shodex RI, UV (254nm)
Column Temp. : 80°C

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).

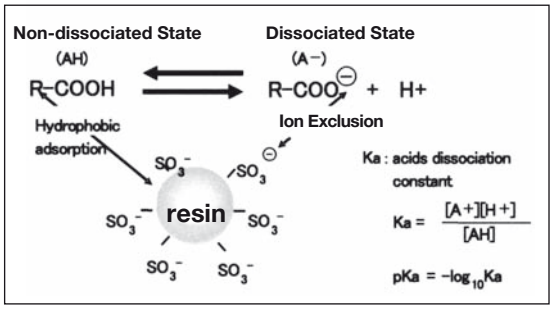


Fig. 3-1 Ion Exclusion Mode

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 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

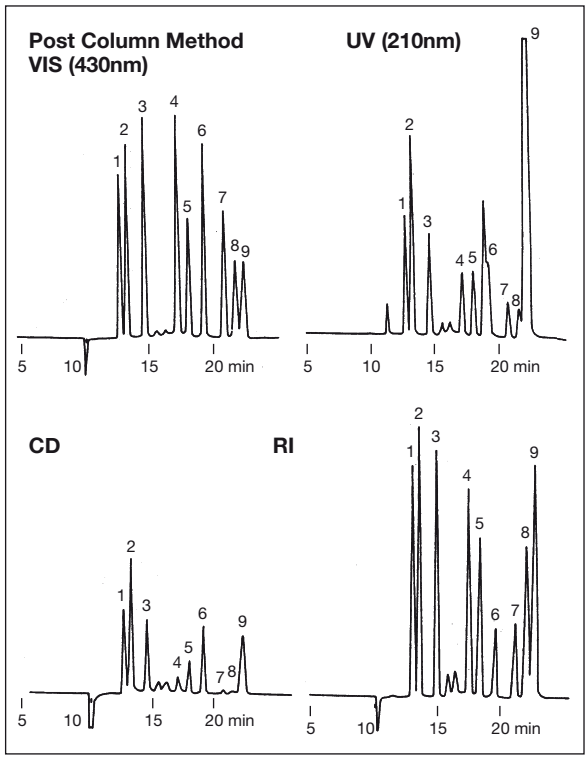
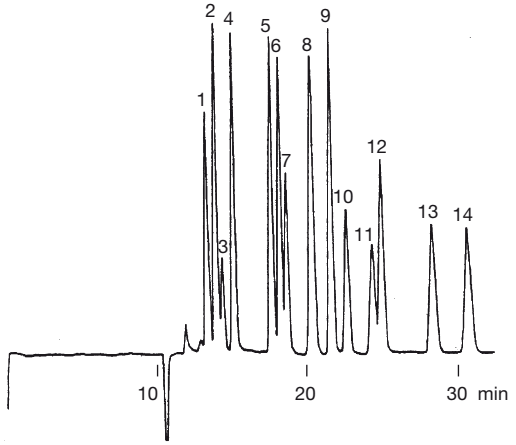


Fig. 3-2 Comparison of Chromatograms with Various Detectors

3-2. Organic Acids

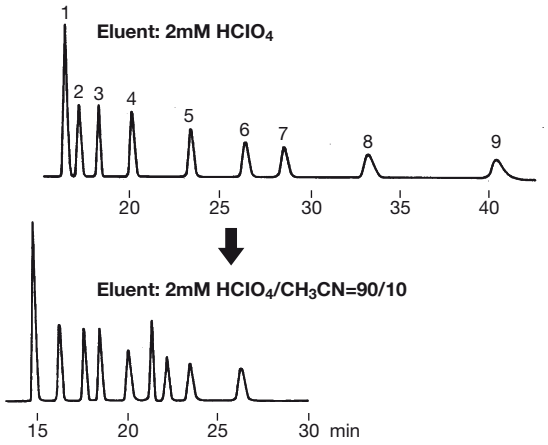


Sample:
1. Citric Acid
2. Tartaric Acid
3. Pyrubic Acid
4. Malic Acid
5. Succinic Acid
6. Glyconic Acid
7. Lactic Acid
8. Fumaric Acid
9. Acetic Acid
10. Levulinic Acid
11. Pyroglutamic Acid
12. Propionic Acid
13. Isobutyric Acid
14. n-Butyric Acid

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

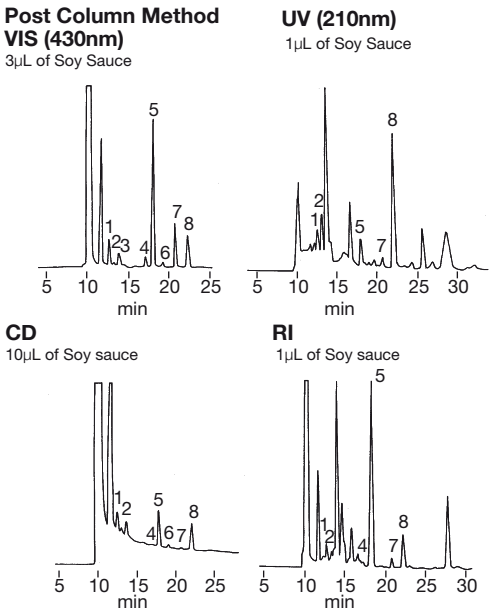


Sample:
1. Succinic Acid
2. Lactic Acid
3. Formic Acid
4. Acetic Acid
5. Propionic Acid
6. Isobutyric Acid
7. n-Butyric Acid
8. Isovaleric Acid
9. n-Valeric Acid

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

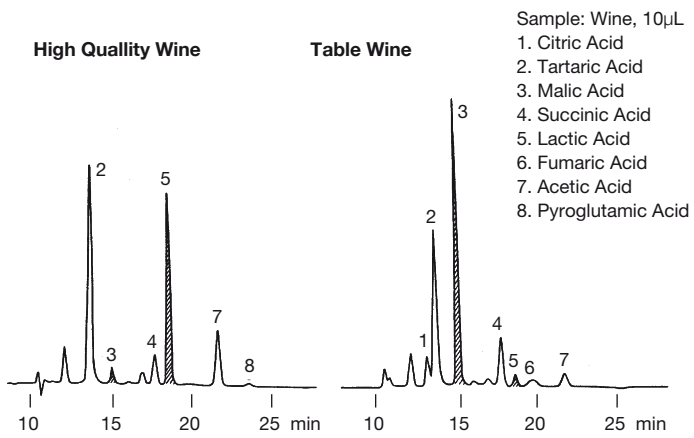


Sample: Soy Sauce
1. Citric Acid
2. Pyruvic Acid
3. Malic Acid
4. Succinic Acid
5. Lactic Acid
6. Formic Acid
7. Acetic Acid
8. Pyroglutamic Acid

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

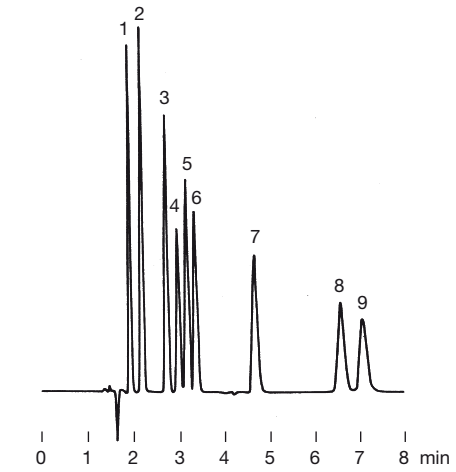


Sample: Wine, 10μL
1. Citric Acid
2. Tartaric Acid
3. Malic Acid
4. Succinic Acid
5. Lactic Acid
6. Fumaric Acid
7. Acetic Acid
8. Pyroglutamic Acid

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

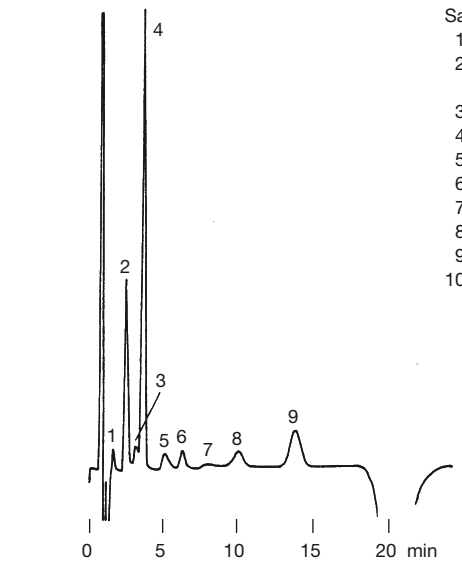


Sample:
1. Glyoxylic Acid, 1.78mg/mL
2. Tartaric Acid, 1.95mg/mL
3. Malic Acid, 2.06mg/mL
4. Lactic Acid, 2μL/mL
5. Malonic Acid, 1.95mg/mL
6. Acetic Acid, 2μL/mL
7. Succinic Acid, 2.05mg/mL
8. Levulinic Acid, 1.95mg/mL
9. Propionic Acid, 2μL/mL

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.

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.

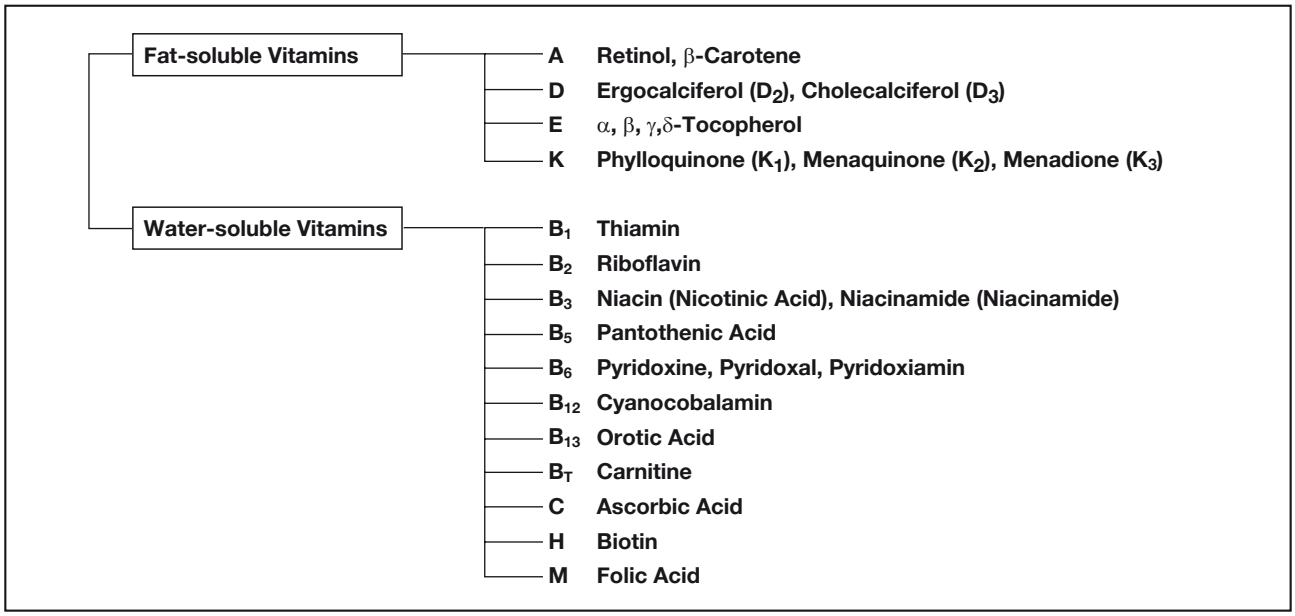


Fig. 4-1 Categories of Vitamins

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

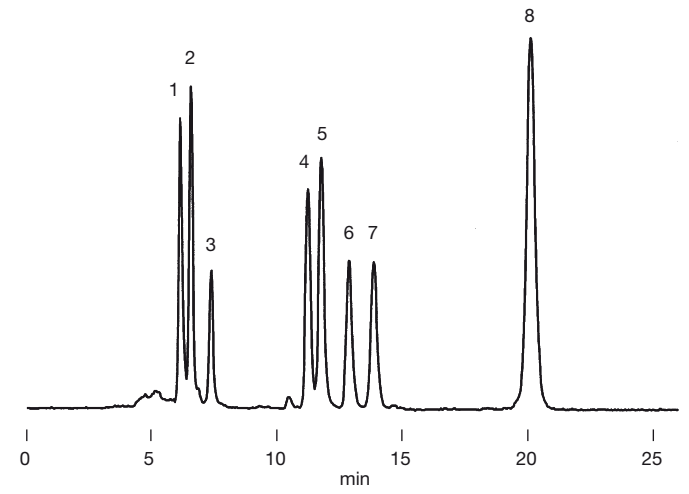
(Size Exclusion)

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

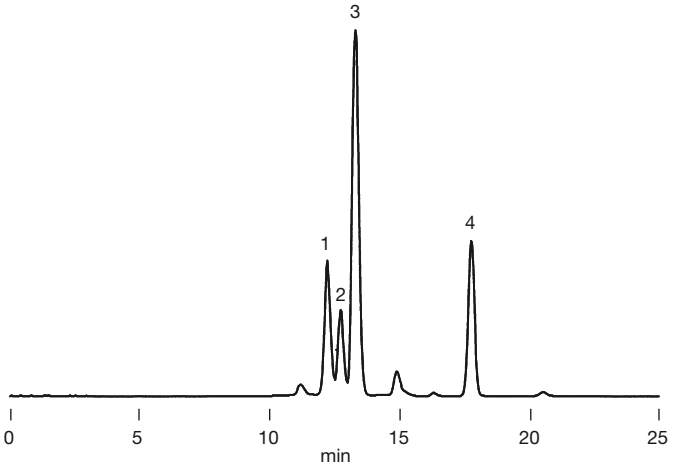
4-1. Fat-Soluble Vitamins



Sample: 20μL
1. Menadione (Vitamin K_3)
2. Retinol (Vitamin A)
3. Retinol acetate (Vitamin A Acetate)
4. Menadione (Vitamin D_2)
5. Cholecalciferol (Vitamin D_3)
6. α-Tocopherol Acetate (Vitamin E Acetate)
7. α-Tocopherol (Vitamin E)
8. Phylloquinone (Vitamin K_1)

Column : Shodex Asahipak ODP-50 4E (4.6mmID x 250mm)
Eluent : CH_3CN/CH_3OH=50/50
Flow Rate : 0.6mL/min
Detector : UV (280nm)
Column Temp. : 30℃

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

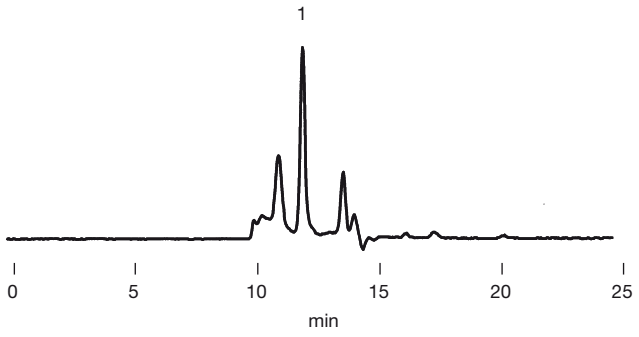


Sample: 100μL
1. Retinol Palmitate (Vitamin A Palmitate)
2. α-Tocopherol (Vitamin E)
3. Cholecalciferol (Vitamin D_3)
4. Menadione (Vitamin K_3)

Column : Shodex GPC KF-801 (8.0mmID x 300mm) x 2
Eluent : THF
Flow Rate : 1.0mL/min
Detector : UV (280nm)
Column Temp. : 40℃

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

Sample: 0.2% Margarine, 100μL
1. Retinol Palmitate
(Vitamin A Palmitate)

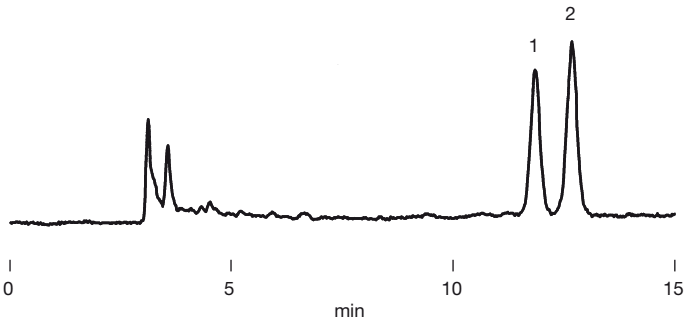


The Vitamin A palmitate was analyzed using GPC KF-801. A wave length of 325nm at which it has a maximum UV absorption was used for the detection.
Only dissolving the sample in the eluent (THF) and filtrating it using a disposable filter (0.45μm), vitamin A palmitate can be analyzed without any other pretreatment like saponification.

Column	: Shodex GPC KF-801 (8.0mmID x 300mm) x 2
Eluent	: THF
Flow Rate	: 1.0mL/min
Detector	: UV (325nm)
Column Temp.	: 40°C

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

Sample: 0.1μg/mL each, 100μL
1. Ergocalciferol (Vitamin D_2)
2. Cholecaciferol (Vitamin D_3)

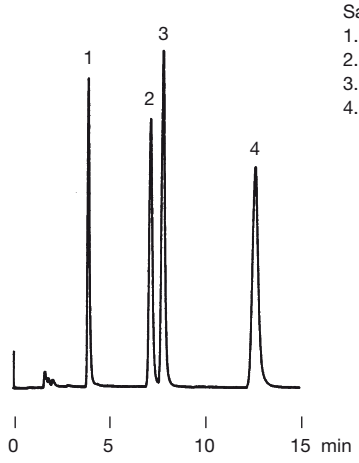


Vitamin D_2 and D_3 were separated using Asahipak ODP-50 6D (polymer-based column) by reversed phase mode. Since the usable pH range of the ODP-50 column is very wide and it can be washed with both acidic and alkaline solvents, it is very durable even if it is used for crude samples.

Column	: Shodex Asahipak ODP-50 6D (6.0mmID x 150mm)
Eluent	: CH_3CN/CH_3OH=90/10
Flow Rate	: 1.5mL/min
Detector	: UV (265nm)
Column Temp.	: 27°C

Fig. 4-5 Vitamin D_2 and D_3 (Reversed Phase Mode)

Sample: Tocopherol
1. α-Tocopherol
2. β-Tocopherol
3. γ-Tocopherol
4. δ-Tocopherol

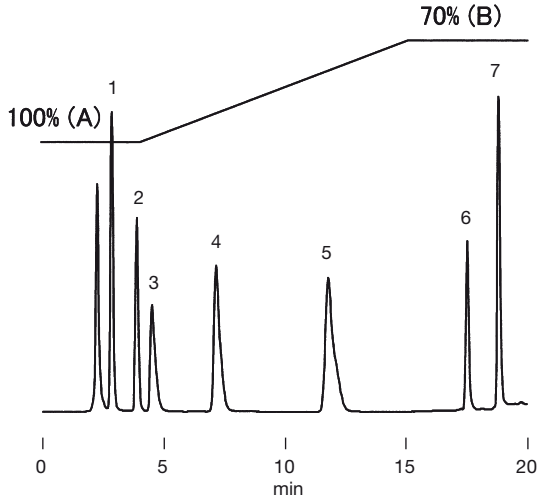


Alpha-, beta-, gamma- and delta-tocopherol were separated by normal phase mode using the column with a mixture of n-hexane/isopropanol as the eluent.

Column	: Shodex Silica 5SIL 4D (4.6mmID x 150mm)
Eluent	: n-Hexane/2-Propanol=99.5/0.5
Flow Rate	: 1.0mL/min
Detector	: UV (280nm)
Column Temp.	: 30°C

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

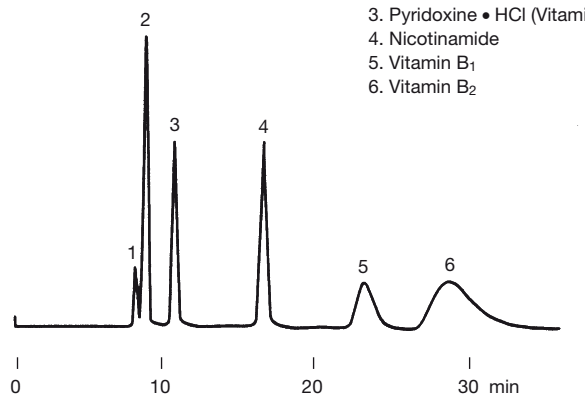
4-2. Water-soluble Vitamins



Sample: 20μL
1. Ascorbic Acid (Vitamin C)
2. Niacin (Vitamin B_3)
3. Pyridoxine (Vitamin B_6)
4. Niacinamide (Vitamin B_3)
5. Thiamine (Vitamin B_1)
6. Cyanocobalamin (Vitamin B_{12})
7. Riboflavin (Vitamin B_2)

Column	: Shodex RSpak DE-413 (4.6mmID x 150mm)
Eluent	: (A); 1/30M Potasium Phosphate Buffer (pH6.8) /CH_3CN=98/2 (B); H_2O/CH_3CN=50/50 0min to 4 min, 100% (A) 4min to 15 min, 100% (A) to 70% (B) After 15 min, 70% (B)
Flow Rate	: 0.6mL/min
Detector	: Shodex UV (254nm)
Column Temp.	: 35℃

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

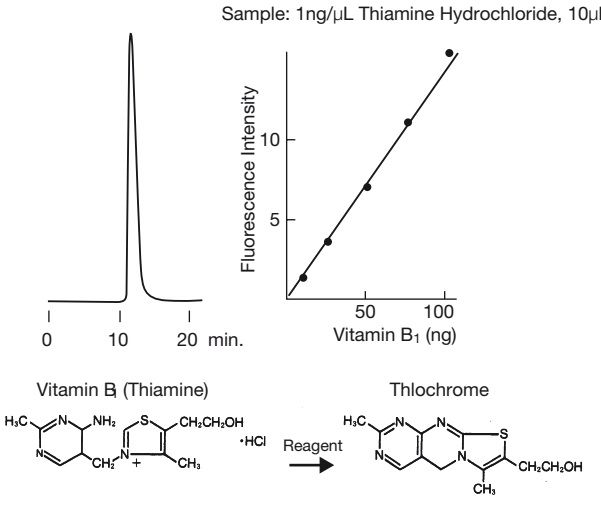


Sample: 0.25% each, 3μL
1. Pantothenic Acid
2. Orotic Acid
3. Pyridoxine • HCl (Vitamin B_6)
4. Nicotinamide
5. Vitamin B_1
6. Vitamin B_2

The NN-814 is a multimode column (reversed phase + cation exchange). Using NN-814, isocratic analysis can be carried out, and it does not require an ion pair reagent.

Column	: Shodex RSpak NN-814 (8.0mmID x 250mm)
Eluent	: 45mM KH_2PO_4 + 55mM Na_2HPO_4 + 7% CH_3OH
Flow Rate	: 1.0mL/min
Detector	: UV (210nm)
Column Temp.	: Room Temp.

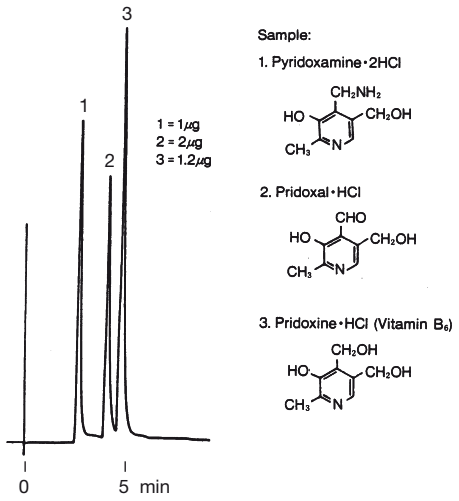
Fig. 4-8 Simultaneous Analysis of Water-soluble Vitamins (Multimode)



The total quantity of vitamin B_1 was measured using RSpak NN-614 with a phosphate buffer eluent. The vitamin B_1 separated by the column is converted into thiochrome using a postcolumn reagent and detected by a fluorescence detector.
This method was recommended by the Japanese standard, "Standard Methods of Analysis for Hygienic Chemists with Commentary" (1990).

Column	: Shodex RSpak NN-614 (6.0mmID x 150mm)
Eluent	: 45mM KH_2PO_4 + 55mM Na_2HPO_4 aq.
Reagent	: 0.01% $K_3Fe(CN)_6$ + 15% NaOH
Flow Rate	: 0.7mL/min
Detector	: Fluorescence (Ex. 375nm, Em. 450nm)
Column Temp.	: 25℃

Fig. 4-9 Vitamin B1 (Multimode)



Sample:

1. Pyridoxamine·2HCl

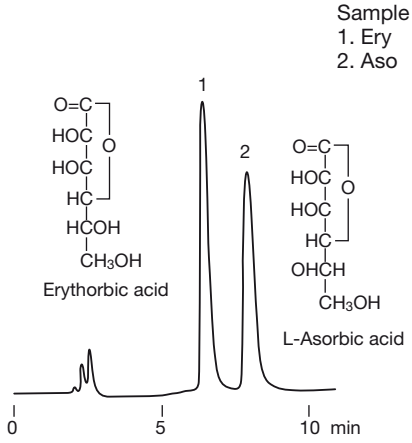
2. Pridoxal·HCl

3. Pridoxine·HCl (Vitamin B_6)

Members of the vitamin B_6 family are difficult to separate due to structural similarity. Although the usual method of separation employs an ion pair reagent, analysis in reversed-phase mode is possible on the basis of slight differences in hydrophobicity among individual samples, provided that the acidity is high, for example, pH2.0. Actually, three ingredients were separated in about 5 minutes using the Asahipak ODP-50 polymeric reversed-phase column, which is stable even at a pH of 2.0.

Column	:	Shodex Asahipak ODP-50 6D (6.0mmID x 150mm)
Eluent	:	50mM Sodium phosphate buffer (pH2.0)
Flow rate	:	1.0mL/min
Detector	:	UV (254nm)
Column temp.	:	30℃

Fig. 4-10 Vitamin B_6 (reversed phase mode)

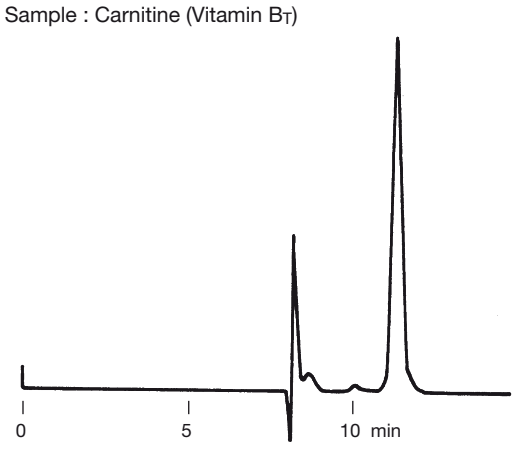


Amino columns or silica columns are usually used for the analysis of ascorbic acid. Using amino columns is particularly wide spread because isoascorbic acid (erythorbic acid) as well as ascorbic acid can be analyzed simultaneously. However, conventional silica-based amino columns are not chemically stable, so their life time is not long.
Asahipak NH2P-50 4E has long life time, since it is a polymer-based column and stable.

Column	:	Shodex Asahipak NH2P-50 4E (4.6mmID x 250mm)
Eluent	:	20mM NaH_2PO_4+30mM H_3PO_4 aq. /CH_3CN=20/80
Flow rate	:	1.0mL/min
Detector	:	UV(254nm)
Column temp.	:	30℃

Equilibration : After passing 60mM phosphoric acid in the column by 0.5mL/min for 2h, it replaces by eluent.

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



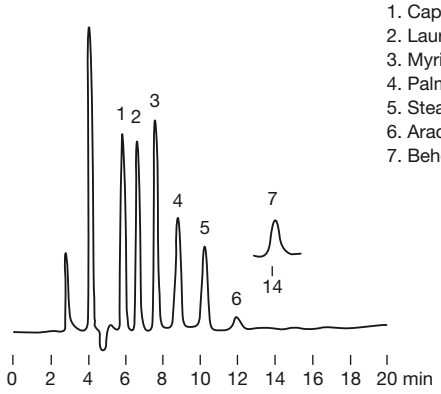
Carnitine (Vitamin B_T) standard was separated using RSpak NN-814 (multi-mode). Main peak was observed at 12min and impurity peaks were observed at 8 to 10min.

Column	:	Shodex RSpak NN-814 (8.0mmID x 250mm)
Eluent	:	0.1M KH_2PO_4 aq.
Flow rate	:	1.0mL/min
Detector	:	Shodex RI
Column temp.	:	Room temp.

Fig. 4-12 Vitamin B_T (multi- mode)

5. Other Ingredients in Foods

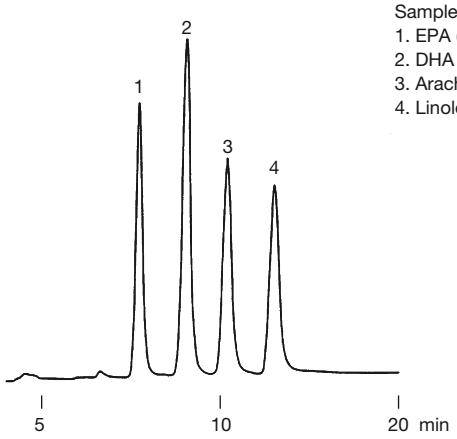
5-1. Higher Fatty Acids



Sample: $C_nH_{2n+1}COOH$, 40μL
1. Capric Acid (n=9)
2. Lauric Acid (n=11)
3. Myristic Acid (n=13)
4. Palmitic Acid (n=15)
5. Stearic acid (n=17)
6. Arachidic Acid, Eicosanoic Acid (n=19)
7. Behenic Acid, Docosanoic Acid (n=21)

Column	:	Shodex RSpak DE-413 (4.6mmID x 150mm)
Eluent	:	$CH_3CN/THF/H_2O$=45/20/35
Flow Rate	:	0.5mL/min
Detector	:	Shodex RI
Column Temp.	:	40℃

Fig. 5-1 Higher Fatty Acids (1)



Sample:
1. EPA (Eicosapentaenoic Acid)
2. DHA (Docosahexaenoic Acid)
3. Arachidonic Acid
4. Linoleic Acid

EPA and DHA, essential fatty acids rich in blue fish and the like, are reported to be effective in preventing thrombosis and enhancing brain function, respectively.

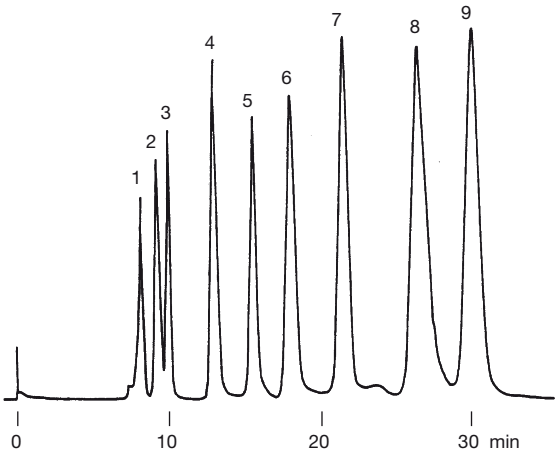
Column	:	Shodex ODSpak F-411 (4.6mmID x 150mm)
Eluent	:	H_2O/CH_3CN=20/80
Flow Rate	:	0.7mL/min
Detector	:	Shodex RI
Column Temp.	:	30℃

Fig. 5-2 Higher Fatty Acids (2)

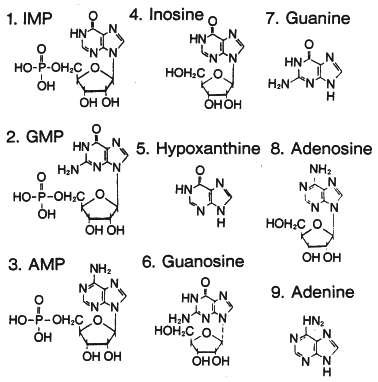
Table 5-1 Shodex Columns for Higher Fatty Acid Analysis (Absorption and Partition)

Product Code	Product Name	Theoretical Plate Number (TP/column)	Particle Size (μm)	Carbon Content (%)	ID x Length (mm)
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
F6604112	ODSpak F-411	≥8,000	5	14	4.6 x 150
F6605110	ODSpak F-511	≥14,000	5	14	4.6 x 250
F6604113	ODSpak F-411/S	≥10,000	3	14	4.6 x 100

5-2. Nucleic Acids as Taste Components



Sample:



IMP, GMP, and AMP (nucleotides), representative flavour ingredients (tasty nucleic acids), and all of their metabolites were analyzed by isocratic elution using the Asahipak GS-320 7E column. Not only the size exclusion mode (main mode), but also the reversed-phase and ion exchange modes are available therefore, the GS-320 7E column is capable of completely separating within ca. 30min a total of nine ingredients (corresponding nucleosides and some bases) from IMP, GMP, AMP and other nucleotides.

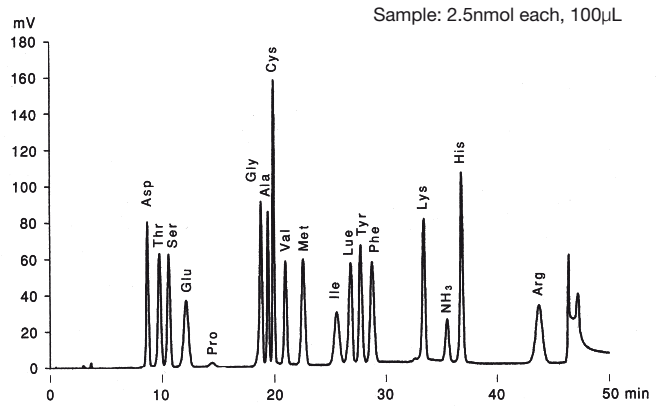
Column	:	Shodex Asahipak GS-320 7E (7.5mmID x 250mm)
Eluent	:	10mM Sodium Phosphate Buffer (pH5.0)
Flow Rate	:	1.0mL/min
Detector	:	UV (260nm)
Column Temp.	:	30˚C

Fig. 5-3 Nucleic Acids as Taste Components

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

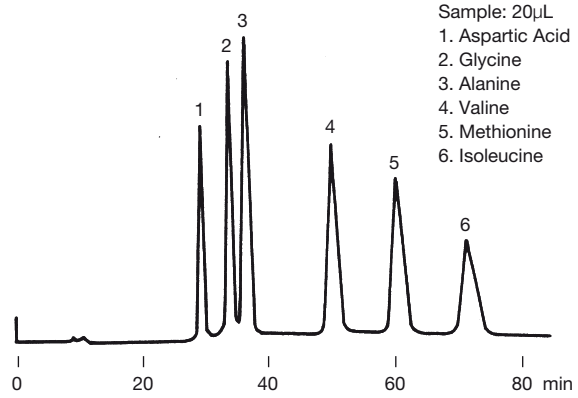
Product Code	Product Name	ID x Length (mm)
F7610005	Asahipak GS-320 7E	7.5 x 300
F6710019	Asahipak GS-2G 7B	7.5 x 50

5-3. Amino Acids



Column : Shodex CXpak P-421S (4.6mmID x 150mm)
Eluent : No.1; 0.12M Sodium Citrate Buffer (pH3.3)
No.2; 0.13M Sodium Citrate Buffer (pH3.2)
No.3; 0.11M Sodium Citrate Buffer (pH4.0)
No.4; 1.02M Sodium Citrate Buffer (pH4.9)
No.5; 0.2M NaOH + 10% C_2H_5OH (Rinse Solution)
Low Pressure Gradient:
0 min, No.1 1.2 min, No.2 10.0 min, No.3
21.2 min, No.4 40.0 min, No.5
Flow Rate : 0.5 mL/min
Detector : VIS (570nm) (Ninhydrin Reaction; 0.35 mL/min, 120℃)
Column Temp. : 63℃

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



Sample: 20µL
1. Aspartic Acid
2. Glycine
3. Alanine
4. Valine
5. Methionine
6. Isoleucine

Acidic amino acids, such as aspartic acid, are separated by ion exclusion mode and elute faster. Neutral substances are separated by reversed phase and alkaline amino acids are separated by a mixture of reversed phase (in case of hydrophobic structure) and ion exchange mode. Therefore, amino acids basically elute in the following order: first the acidic amino acids, second the neutral amino acids and finally the alkaline amino acids .

Column : Shodex RSpak NN-814
(8.0mmID x 250mm)
Eluent : 40mM H_3PO_4 aq.
Flow Rate : 1.0mL/min
Detector : Shodex RI
Column Temp. : 40℃

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 + Ion Exchange + Reversed Phase	10	8.0 x 250
F7008150	RSpak NN-614		10	6.0 x 150
F6700510	RSpak NN-G		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 ×2
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 : H_2O
Flow Rate : 1.0mL/min
Detector : Shodex RI
Column Temp. : 80℃

Column : Shodex SUGAR SZ5532 (6.0mmID x 150mm)
Eluent : H_2O/CH_3CN=25/75
Flow Rate : 1.0mL/min
Detector : Shodex RI
Column Temp. : 60℃

Column : Shodex SUGAR SC1211 (6.0mmID x 250mm)
Eluent : H_2O/CH_3CN=65/35
Flow Rate : 1.0mL/min
Detector : Shodex RI
Column Temp. : 70℃

Column : Shodex Asahipak NH2P-50 4E (4.6mmID x 250mm)
Eluent : H_2O/CH_3CN=25/75
Flow Rate : 1.0mL/min
Detector : Shodex RI
Column Temp. : 30℃

Column : Shodex Asahipak GS-220 HQ (7.5mmID x 300mm) x 2
Eluent : H_2O
Flow Rate : 0.5mL/min
Detector : Shodex RI
Column Temp. : 60℃

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-Pyroglutamic 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 H_2SO_4 aq.
Flow Rate : 0.6mL/min
Detector : Shodex RI
Column Temp. : 60℃

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-Hydroxygltaric 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-}	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 : $HClO_4$ 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-Hydroxygltaric 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
$CO3^{2-}$,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 $HClO_4$ aq.
Flow Rate : 1.0mL/min