TOSOH BIOSCIENCE

Separations Business Unit

TSK-GEL® Application Databook
Food & Nutraceuticals

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TOSOH

The Tosoh logo symbolizes the Corporate Philosophy or Tosoh's "vision of the ideal". The curved lines represent "the realization of happiness", reflecting Tosoh's management philosophy of putting people first. The square in the center expresses the advanced nature of Tosoh's technology and also represents the outstanding quality of Tosoh's products. The right-angle cut at the top portrays an image of contributing to society, Tosoh's basic stance towards the outside world. The red corporate color symbolizes the "Tosoh Spirit", which guides the ceaseless efforts to realize the ideal.

Nanyo Complex

Toyopearl resins are produced at the Tosoh Nanyo Complex – a sprawling 3 million square meter facility, that is Japan's largest chemical manufacturing complex.

Tokyo Research Center

Tosoh Bioscience is part of the Scientific Instruments division, located within Tosoh's Tokyo Research Center, Kanagawa-Ken, Japan.

TSK-GEL Application Databook Food & Nutraceuticals

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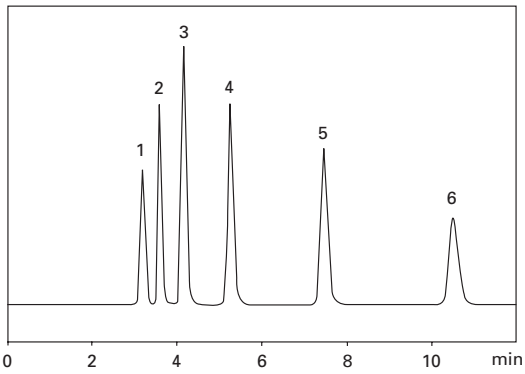
Vitamins

Introduction

With the marked advances in food manufacturing, processing and storage techniques, we have access to a wide variety of nutritional foods. However, the incidences of obesity and lifestyle diseases (adult diseases) are on the rise, while at the same time people pay more attention to a healthy lifestyle and have become more interested in food ingredients and food safety.
Advances in test methods for residual chemicals in food components and additives have led to more accurate and convenient methods. High performance liquid chromatography (HPLC) is one such method, and it is an established and widely used test method for many food items.
The present datasheet describes examples for the analysis of food ingredients and additives using TSK-GEL HPLC columns.

1 Separation of water soluble vitamins (1)

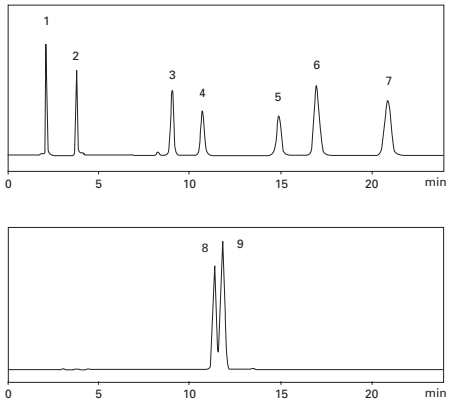
The TSKgel ODS-100V reversed phase column delivers an excellent separation of highly polar substances such as ascorbic acid, nicotinamide and nicotinic acid.



Column: TSKgel ODS-100V 5 µm, 4.6 mm ID X 15 cm L
Eluent: 0.1%TFA in H_2O/CH_3CN=99/1
Flow rate: 1.0 ml/min
Detection: UV @ 270 nm
Temp.: 25°C
Samples: 1: L-ascorbic acid (vitamin C) 1.0 g/l; 2: nicotinamide 0.5 g/l; 3: nicotinic acid 1.0 g/l; 4: thiamine (vitamin B1) 0.25 g/l; 5: pyridoxal (vitamin B6) 0.1 g/l; 6: pyridoxine (vitamin B6) 0.1 g/l
Inj. volume : 5 µl

2 Separation of fat soluble vitamins

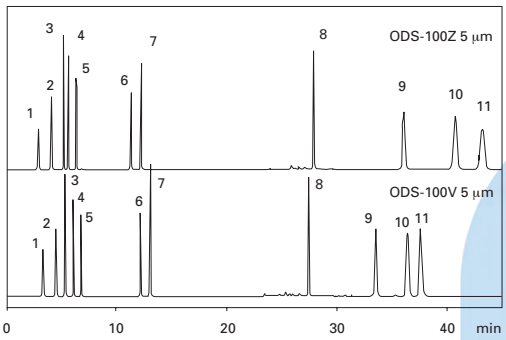
The hydrophobic TSKgel ODS-100Z column was used to achieve baseline separation of fat soluble vitamis with acetonitrile as mobile phase. Under the same conditions, it is also possible to separate vitamins D2 and D3, as shown in the lower chromatogram.



Column: TSKgel ODS-100Z 5 µm, 4.6 mm ID x 15 cm L
Eluent: CH_3CN
Flow rate: 1.0 ml/min
Detection: UV @ 280 nm
Temp.: 40°C
Samples: 1: menadione (vitamin K3) 0.5 g/l; 2: retinol (vitamin A) 1.0 g/l 3: menaquinone (vitamin K2) 0.5 g/l; 4: δ-tocopherol (vitamin E) 1.0 g/l; 5: α-tocopherol (vitamin E) 1.0 g/l; 6: α-tocopherol acetate 2.0 g/l; 7: phylloquinone (vitamin K1) 0.5 g/l; 8: ergocalciferol (vitamin D2) 0.25 g/l; 9: cholecalciferol (vitamin D3) 0.25 g/l
Inj. volume : 5 µl

3 Simultaneous separation of water soluble and fat soluble vitamins

Simultaneous separation of vitamins using TSKgel ODS-100V and TSKgel ODS-100Z columns. Highly hydrophobic vitamins were separated very well on TSKgel ODS-100Z.



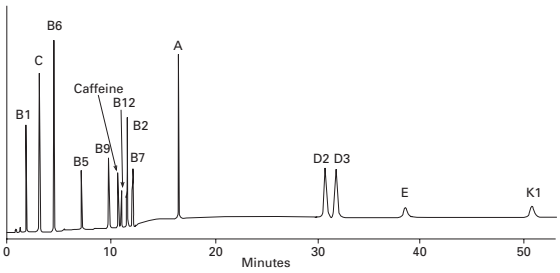
Column: TSKgel ODS-100Z, 5 µm, 4.6 mm ID x 15 cm L
TSKgel ODS-100V, 5 µm, 4.6 mm ID x 15 cm L
Eluent:A ; 0.1 % TFA in H_2O, B ; 0.1 % TFA in CH_3CN
Gradient:

0 min	0 % B
20 min	40 % B
22 min	100 % B
50 min	100 % B

Flow rate: 1.0 ml/min
Detection: UV @ 280 nm
Temp.: 25°C
Samples: 1: L-ascorbic acid (vitamin C) 1.0 g/l; 2: nicotinic acid 1.0 g/l; 3: thiamine (vitamin B1) 0.25 g/l; 4: pyridoxal (vitamin B6) 0.1 g/l; 5: pyridoxine (vitamin B6) 0.1 g/l; 6: caffeine 0.25 g/l; 7: riboflavin (vitamin B2) 0.1 g/l; 8: retinol (vitamin A) 2.0 g/l; 9: δ-tocopherol (vitamin E) 2.0 g/l; 10: α-tocopherol (vitamin E) 2.0 g/l; 11: α-tocopherol acetate 2.0 g/l
Inj. volume: 5 µl

Vitamins/Nucleic Acids, Nucleotides

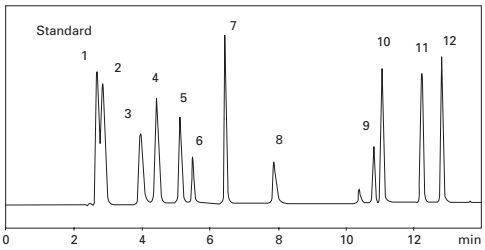
Simultaneous HPLC/ELSD analysis of water and fat soluble vitamins and caffeine

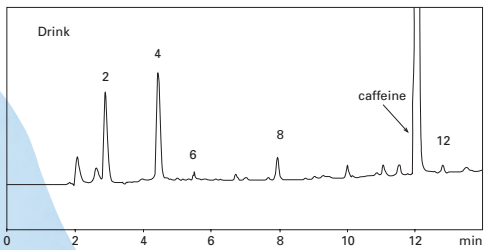


Column: TSKgel ODS-100Z, 5 µm, 4.6 mm ID x 15 cm L
Eluent: H_2O + 0.5% HCOOH (A) / CH_3CN (B), 0% B to 20% B in 12 min then from 20% B to 100% B in 2 min
Flow rate: 1 ml/min
Detector: SEDEX85LT, 40°C, 3.5 bar

4 Separation of water soluble vitamins in soft drinks

The sample was diluted with deionized water, filtered and then directly injected onto the column. Under the same conditions, it is also possible to determine caffeine in the presence of water soluble vitamins.

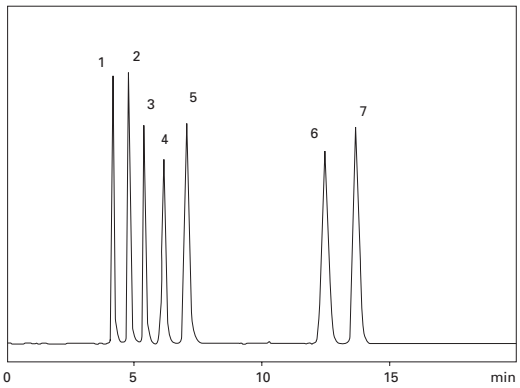




Column: TSKgel ODS-100V, 5 µm, 4.6 mm ID x 15 cm L
Eluent: A ; 10 mmol/l $HCOONH_4$ (pH 3.5)
B ; CH_3CN
Gradient: 0 min 0%B
15 min 30%B
Flow rate: 1.0 ml/min
Detection: UV @ 254 nm
Temp.: 40°C
Samples: Soft drink
Compounds: 1: pyridoxyamine; 2: L-ascorbic acid (vitamin C) 0.04 g/l; 3: thiamine (vitamin B1); 4: nicotinic acid 0.01 g/l; 5: pyridoxal (vitamin B6); 6: pyridoxine (vitamin B6) 0.003 g/l; 7: nicotinamide; 8: pantothenic acid 7.0 g/l; 9:folic acid; 10: riboflavin phosphate sodium salt; 11: cyanocobalamin (vitamin B12); 12: riboflavin (vitamin B2) 0.0003 g/l
Inj. volume : 5 µl

5 HILIC separation of water soluble vitamins (2)

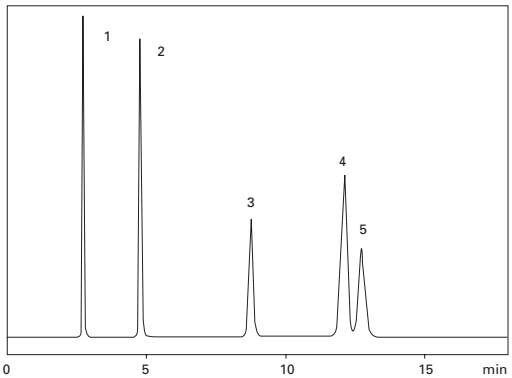
Hydrophilic interaction chromatography (HILIC) is widely used for highly polar substances, such as carbohydrates. Its selectivity differs from that of RPC (see data 1 on page 2). Water soluble vitamins were separated by HILIC using a 25 cm TSKgel Amide-80 5 µm column.



Column: TSKgel Amide-80, 5 µm, 4.6 mm ID x 25 cm L
Eluent: 20 mmol/l KH_2PO_4 (pH 2.0) /CH_3CN = 20/80
Flow rate: 1.0 ml/min
Detection: UV @ 260 nm
Temp.: 40°C
Samples 1: nicotinic acid 0.25 g/l; 2: nicotinamide 0.25 g/l
3: iso-ascorbic acid 1.0 g/l (erythorbic acid); 4: L-ascorbic acid (vitamin C) 1.0 g/l; 5: riboflavin (vitamin B2) 0.025 g/l
6: pyridoxal (vitamin B6) 0.05 g/l; 7: pyridoxine (vitamin B6) 0.05 g/l
Inj. volume : 5 µl

6 Separation of nucleic acid bases (1)

TSKgel ODS-100V has a high surface polarity. This allows the use of an aqueous mobile phase without organic solvent. Hence, TSKgel ODS-100V 5 µm is also suited for separation of highly hydrophilic nucleic acid bases, as shown below.

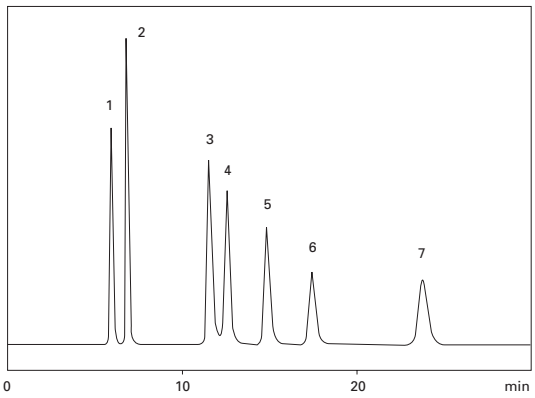


Column: TSKgel ODS-100V 5 µm; 4.6 mm ID x 15 cm L
Eluent: 20 mmol/l KH_2PO_4 (pH4.0)
Flow rate: 1.0 ml/min
Detection: UV @ 260 nm
Temp.: 25°C
Samples: 1: cytosine; 2: uracil; 3: guanine; 4: adenine; 5: thymine
Concentration: 0.1 g/l each
Inj. volume : 5 µl

Nucleic Acids, Nucleotides

7 HILIC Separation of nucleic acid bases (2)

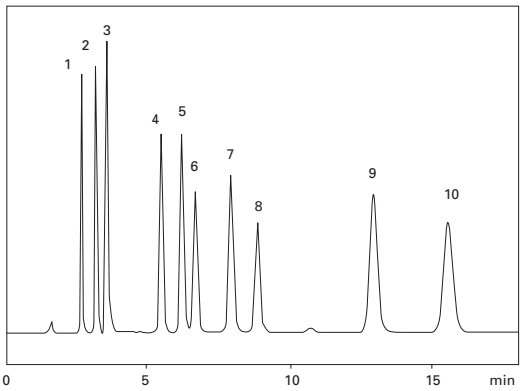
Separation of nucleic acid bases using TSKgel Amide-80 5 µm. When compared to RPC (TSKgel ODS-100V, see data 6 on page 3), retention is generally stronger and selectivity is different.



Column:	TSKgel Amide-80, 5 µm 4.6 mm ID x 25 cm L
Eluent:	20 mmol/l $HCOONH_4$ (pH 4.0) /CH_3CN = 10/90
Flow rate:	1.0 ml/min
Detection:	UV @ 260 nm
Temp.:	25°C
Samples:	1: thymine; 2: uracil; 3: adenine; 4: hypoxanthine; 5: xanthine; 6: cytosine; 7: guanine
Concentration:	0.04 g/l each
Inj. volume :	5 µl

8 Separation of nucleosides

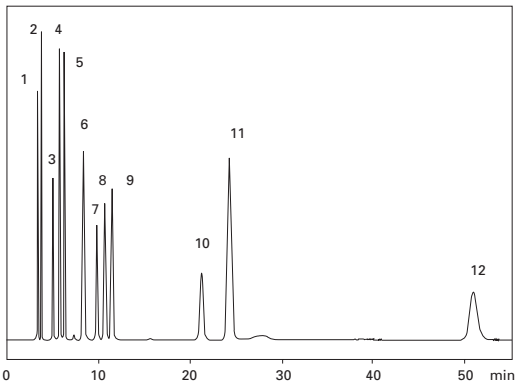
Separation of ribonucleosides and deoxyribonucleosides using TSKgel ODS-100V 5 µm.



Column:	TSKgel ODS-100V 5 µm; 4.6 mm ID x 15 cm L
Eluent:	20 mmol/l KH_2PO_4 (pH 4.0)/CH_3OH= 90/10
Flow rate:	1.0 ml/min
Detection:	UV @ 260 nm
Temp.:	25°C
Samples:	1: cytidine; 2: 2'-deoxycytidine; 3: uridine; 4: inosine; 5: guanosine; 6: 2'-deoxyinosine; 7: 2'-deoxyguanosine; 8: thymidine; 9: adenosine; 10: 2'-deoxyadenosine
Concentration:	0.05 g/l each
Inj. volume :	5 µl

9 Reversed phase separation of nucleotides (1)

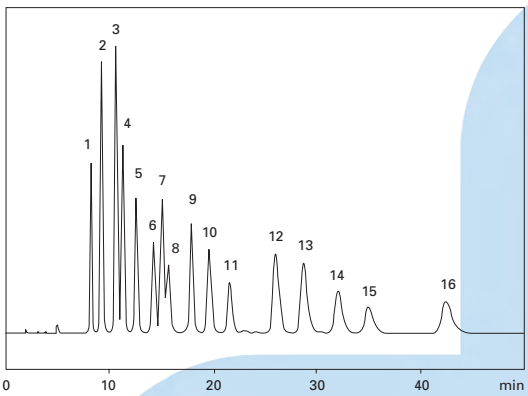
Separation of nucleotides using TSKgel ODS-100V 5 µm in a mobile phase consisting of phosphate buffer. If needed, ion pair reagent can be added to obtain longer retention of some of the early eluting nucleotides (see later).



Column:	TSKgel ODS-100V, 5 µm, 4.6 mm ID x 25 cm L
Eluent:	100 mmol/l KH_2PO_4 (pH 4.5)
Flow rate:	1.0 ml/min
Detection:	UV @ 260 nm
Temp.:	25°C
Samples:	1: UTP; 2: UDP; 3: CMP; 4: GDP; 5: UMP; 6: ATP; 7: TDP; 8: ADP; 9: IMP; 10: TMP; 11: AMP; 12: dAMP
Concentration:	0.04 g/l each
Inj. volume :	5 µl

10 HILIC separation of nucleotides (2)

Separation of nucleotides using TSKgel Amide-80 5 µm. When compared to RPC (TSKgel ODS-100V 5 µm, see data 9), retention is stronger for most nucleotides and selectivity is also favorable.

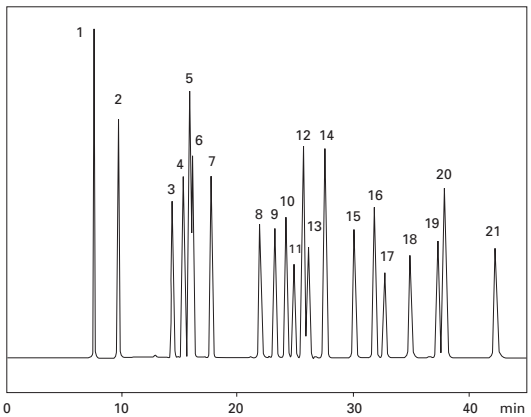


Column:	TSKgel Amide-80, 5 µm 4.6 mm ID x 25 cm L
Eluent:	75 mmol/l KH_2PO_4 (pH 4.5) /CH_3CN = 30/70
Flow rate:	1.0 ml/min
Detection:	UV @ 260 nm
Temp.:	25°C
Samples:	1: TMP; 2: dAMP; 3: AMP; 4: UMP; 5: IMP; 6: TDP; 7: GMP; 8: CMP; 9: ADP; 10: UDP; 11: IDP; 12: GDP; 13: ATP; 14: UTP; 15: ITP; 16: GTP
Concentration:	0.02 g/l each
Inj. volume:	5 µl

Nucleic Acids, Nucleotides/Organic Acids

11 Separation of nucleotides by ion pair chromatography (3)

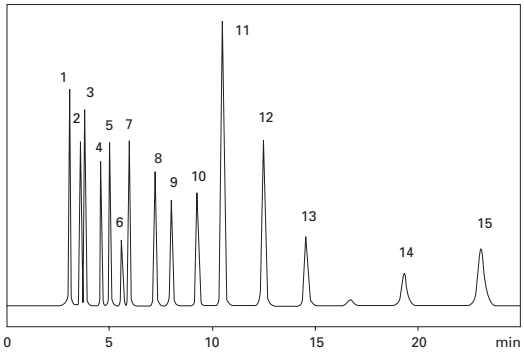
Separation of nucleotides by reversed phase ion pair chromatography by adding butylamine to the mobile phase. Retention and selectivity of analytes change markedly depending on amine concentration, amine structure, alkyl chain length, mobile phase pH, and column temperature.



Column: TSKgel ODS-100V, 5 µm, 4.6 mm ID x 25 cm L
Eluent: A: 20 mmol/l t-butylamine + H_3PO_4 (pH 6.8)
B: A/CH_3OH = 90/10
Gradient: 0 min 0 % B
35 min 100 % B
Flow rate: 1.0 ml/min
Detection: UV @ 260 nm
Temp.: 25°C
Samples: 1: CMP; 2: UMP; 3: CDP; 4: dUMP; 5: GMP; 6: IMP; 7: UDP; 8: CTP; 9: TMP; 10: GDP; 11: IDP; 12: AMP; 13: UTP; 14: dGMP; 15: TDP; 16: GTP; 17: ITP; 18: ADP; 19: TTP; 20: dAMP; 21: ATP
Concentration: 0.3 g/l each
Inj. volume: 2 µl

12 Separation of organic acids (1)

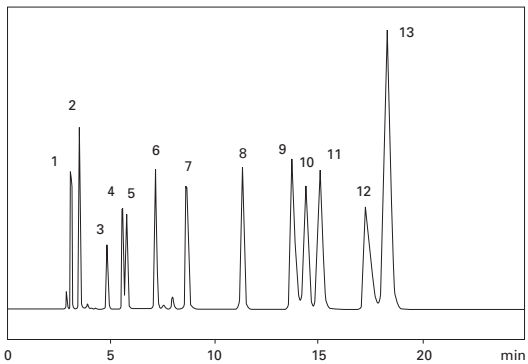
Separation of organic acids in a low pH phosphoric acid mobile phase. Excellent separation was achieved for both polar organic acids (oxalic, formic and ascorbic acid) as well as for more hydrophobic organic acis (propionic and itaconic acids).



Column: TSKgel ODS-100V, 5 µm, 4.6 mm ID x 25 cm L
Eluent: 0.1 % H_3PO_4
Flow rate: 1.0 ml/min
Detection: UV @ 210 nm
Temp.: 40°C
Samples: 1: oxalic acid 0.1 g/l; 2: L-tartanic acid 0.5 g/l; 3: formic acid 1.0 g/l; 4: L-malic acid 1.0 g/l; 5: L-ascorbic acid 0.1 g/l; 6: lactic acid 1.0 g/l; 7: acetic acid 1.0 g/l; 8: maleic acid 0.01 g/l; 9: citric acid 1.0 g/l; 10: succinic acid 1.0 g/l; 11: fumaric acid 0.025 g/l; 12: acrylic acid 0.1 g/l; 13: propionic acid 2.0 g/l; 14: glutaric acid 1.0 g/l; 15: itaconic acid 0.025 g/l
Inj. volume : 10 µl

13 Separation of organic acids (2)

Separation of organic acids using a mobile phase containing 10% acetonitrile. When compared to data 12 faster separation for less hydrophilic acids was achieved.

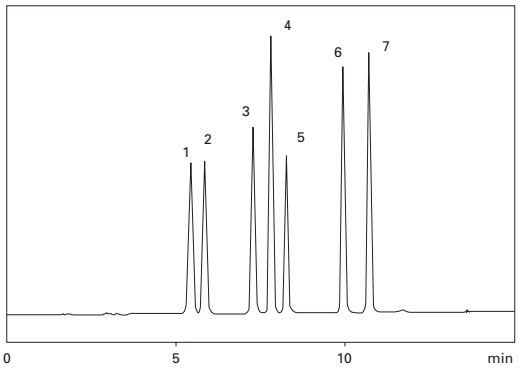


Column: TSKgel ODS-100V, 5 µm, 4.6 mm ID x 25 cm L
Eluent: 0.1 % H_3PO_4/CH_3CN = 90/10
Flow rate: 1.0 ml/min
Detection: UV @ 210 nm
Temp.: 40°C
Samples: 1: glycolic acid 1.0 g/l; 2: malonic acid 1.0 g/l; 3: t-aconit acid 0.02 g/l; 4: citraconic acid 0.01 g/l; 5: levulinic acid 1.0 g/l; 6: mesaconic acid 0.02 g/l; 7: adipic acid 2.0 g/l; 8: crotonic acid 0.05 g/l; 9: N-butyric acid 5.0 g/l; 10: methacrylic acid 0.02 g/l; 11: amygdalic acid 0.05 g/l; 12: pimelic acid 2.0 g/l; 13: phthalic acid 0.1 g/l
Inj. volume : 10 µl

Catechins

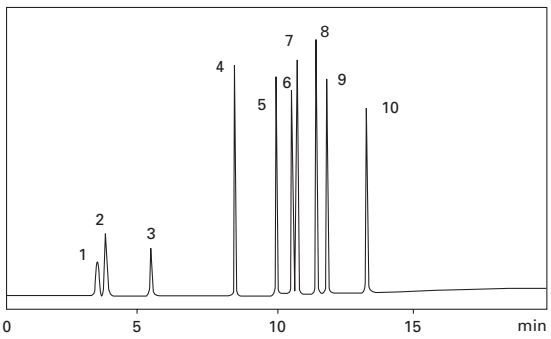
14 Separation of catechins

Catechins, which are found in large quantities in tea, are polyphenols and have extensively been studied for their antioxidant properties. Using TSKgel ODS-100Z 5 µm, excellent separation is achieved for six catechins in the presence of caffeine.



Column:	TSKgel ODS-100Z, 5 µm, 4.6 mm ID x 15 cm L
Eluent:	A: 10 mmol/l KH_2PO_4 (pH 2.5); B: CH_3OH
Gradient:	0 min 18 % B 15 min 60 % B
Flow rate:	1.0 ml/min
Detection:	UV @ 270 nm
Temp.:	40°C
Samples:	1: (-) -epigallocatechin 175 mg/l; 2: (-) -catechin 87 mg/l; 3: (-) -epigallocatechin gallate 43 mg/l; 4: caffeine 217 mg/l; 5: (+) -epicatechin 87 mg/l; 6: (-) -epicatechin gallate 43 mg/l; 7: (-) -catechin gallate 43 mg/l
Inj. volume :	5 µl

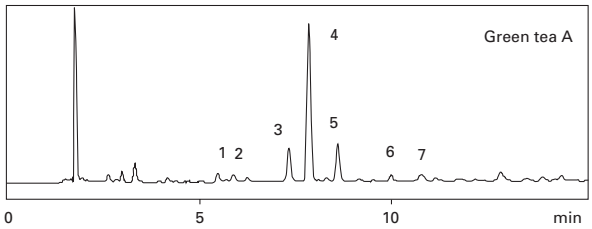
HPLC/ELSD Analysis of catechin derivatives and other components of interest

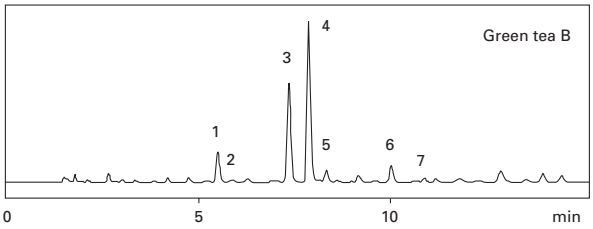


Column:	TSKgel ODS-100V, 3 µm, 2 mm ID x 15 cm L
Compounds:	1. ascorbic acid, 2. theanine, 3. citric acid, 4. theobromine, 5. epigallocatechin, 6. caffeine, 7. catechin, 8. epicatechin, 9. epigallocatechin gallate, 10. epicatechin gallate
Eluent:	H_2O + 0.5% HCOOH (A) / CH_3CN (B), 1%B to 50%B in 16 min
Flow rate:	0.2 ml/min
Detector:	SEDEX 85LT, 40°C, 3.5 Bar

15 Separation of catechins in green tea

Separation of catechins in two green tea samples. Samples were prepared by steeping green tea leaves, followed by dilution and filtration.



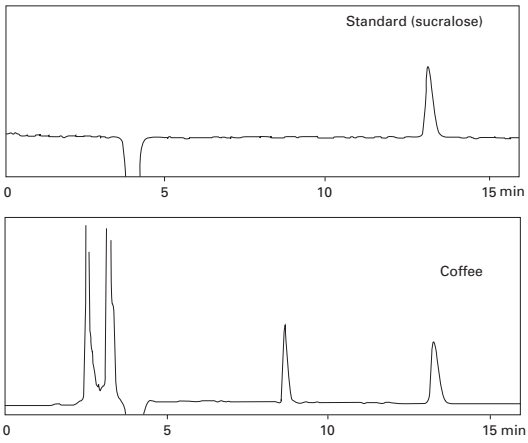


Column:	TSKgel ODS-100Z, 5 µm, 4.6 mm ID x 15 cm L		
Eluent:	A: 10 mmol/l KH_2PO_4 (pH 2.5); B: CH_3OH		
Gradient:	0 min 18 % B 15 min 60 % B		
Flow rate:	1.0 ml/min		
Detection:	UV @ 270 nm		
Temp.:	40°C		
Samples:	green tea A, B		
Compounds		green tea A	green tea B
	1: (-) -epigallocatechin	12.8 mg/l	439.5 mg/l
	2: (-) -catechin	6.5 mg/l	23.6 mg/l
	3: (-) -epigallocatechin gallate	12.4 mg/l	336.9 mg/l
	4: caffeine	222.3 mg/l	2087.3 mg/l
	5: (+) -epicatechin	3.1 mg/l	102.2 mg/l
	6: (-) -epicatechin gallate	2.2 mg/l	49.2 mg/l
	7: (-) -catechin gallate	2.8 mg/l	8.9 mg/l
Inj. volume :	5 µl		

Sweeteners

16 Separation of sweetener (sucralose) in coffee

Sucralose is a white powder which is generated by replacing three hydroxyl groups in sucrose to chlorine atoms. It is a zero calorie sweetener that is about 600 times sweeter than sucrose. In Japan, sucralose was designated as a food additive in 1999. Its sweetness and heat stability are similar to those of sugar. Therfore it is used in beverages and snacks. The chromatogram below shows the results of sucralose analyses conducted in accordance with the Standard Methods of Analysis for Hygienic Chemists: Food Additive Test Methods.



Column:	TSKgel ODS-100V, 5 µm, 4.6 mm ID x 25 cm L
Eluent:	H_2O/CH_3CN = 85/15
Flow rate:	1.0 ml/min
Detection:	RI
Temp.:	40°C
Samples:	coffee (sucralose 200 µg/g added)
Compound:	sucralose
Concentration:	standard 1 g/l; sample 740 mg/l
Inj. volume:	50 µl

Sample preparation:

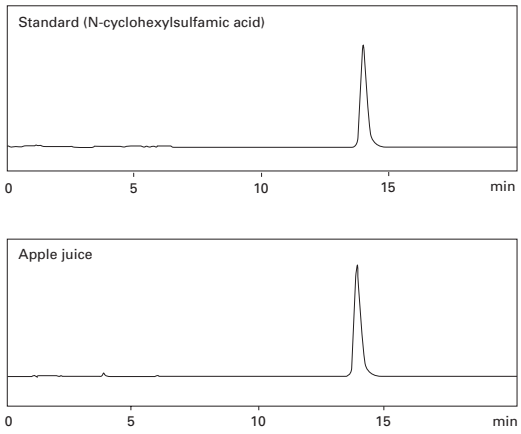
1) Mix 20 g sample with 20 ml of internal dialysis solution. Place it in dialysis membrane.
2) Put dialysis membrane in 200 ml cylinder and fill it with external dialysis solution to 200 ml.
3) Mix using a stirrer at RT for 24 hours.
4) Load 40 ml of external dialysis solution onto a SPE cartridge.
5) Wash SPE cartridge with 10 ml water, 5 ml 0.2 M NaOH and 10 ml water.
6) Elute sample with 5 ml methanol. Dry over N_2. Dissolve in 1 ml water.
7) Filter lysate using filter membrane (pore size: 0.45 µm) and inject

Internal dialysis solution: 0.01 M HCl +100 g/l NaCl
External dialysis solution: 0.01 M HCl

SPE cartridge: OnGuard-RP (Dionex), three cartridges in series.
Dialysis membrane: Visking tube, diameter 23.8 mm

17 Separation of sweetener (N-cyclohexylsulfamic acid) in apple juice

N-cyclohexylsulfamic acid (also called sodium cyclamate) was used as a sweetener in Japan from 1956 to 1969 when its carcinogenicity was called into question in the US. In China and Europe, the use of N-cyclohexylsulfamic acid is not banned. The chromatogram below shows the results of N-cyclohexylsulfamic acid analyses in accordance with the Standard Methods of Analysis for Hygienic Chemists: Food Additive Test Methods.



Column:	TSKgel ODS-100V, 5 µm, 4.6 mm ID x 25 cm L
Eluent:	H_2O/CH_3CN = 30/70
Flow rate:	1.0 ml/min
Detection:	UV @ 314 nm
Temp.:	40°C
Sample:	apple juice (N-cyclohexylsulfamic acid 500 µg/g added)
Compound:	N-cyclohexylsulfamic acid
Concentration:	standard 200 mg/l; sample 237 mg/l
Inj. volume:	10 µl

Sample preparation:

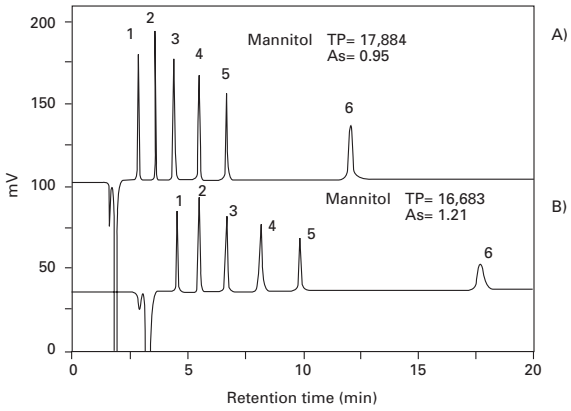
1) Mix 20 g sample with 25 ml auxiliary dialysis solution. Place it in dialysis membrane.
2) Put the dialysis membrane in a 200 ml cylinder, and fill with auxiliary dialysis solution up to 200 ml.
3) Mix using a stirrer at RT for 48 hours.
4) Take 25 ml of external dialysis solution and add 5 ml of 0.1 M TBA solution and 20 ml of 0.2 M phosphate buffer solution (pH 6.0) to prepare sample solutions.
5) Load onto SPE cartridge and wash with 30 ml water.
6) Elute with 10 ml acetonitrile/water (70/30) solution. Collect eluate in separatory funnel.

7) Add 2 ml 50% H_2SO_4, 5 ml hexane and 1 ml $NaClO_4$ and stir for one minute.
8) Eliminate aqueous layer. Add 25 ml 5% $NaHCO_3$ to hexane layer and stir.
9) Eliminate aqueous layer and subject hexane layer to HPLC.

Auxiliary dialysis solution: 0.02 M NaOH
SPE cartridge: Bond Elute Jr. C18 (Varian), four cartridges in series.
Dialysis membrane: Visking tube, diameter 23.8 mm

HILIC separation of sugar alcohols

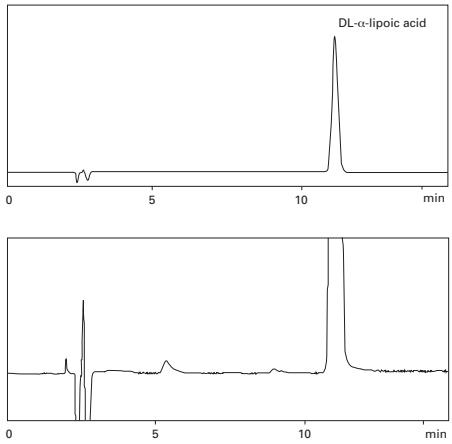
Sugar alcohols are common ingredients in many processed foods. Xylitol is a bulk sweetener which is reduced in calories and dentally safe. The chromatograms show the analysis of sugar alcohols on 3 µm and 5 µm Amide-80 HILIC columns. Overall the 3 µm column provides better resolution at reduced analysis time when compared to the 5 µm TSK-GEL Amide-80 column.



Column: A) TSKgel Amide-80, 3 µm, 4.6 mm ID x 15 cm L
B) TSKgel Amide-80, 5 µm, 4.6 mm ID x 25 cm L
Eluent: H_2O/CH_3CN = 25/75; isocratic
Flow rate: 1.0 ml/min
Detection: RI
Temperature: 25°C
Sample: 1: ethyleneglycol; 2: glycerin; 3: erythritol; 4: xylitol; 5: mannitol; 6: inositol
Injection vol.: 10 µl

18 Analysis of DL-α-lipoic acid by RPC (1)

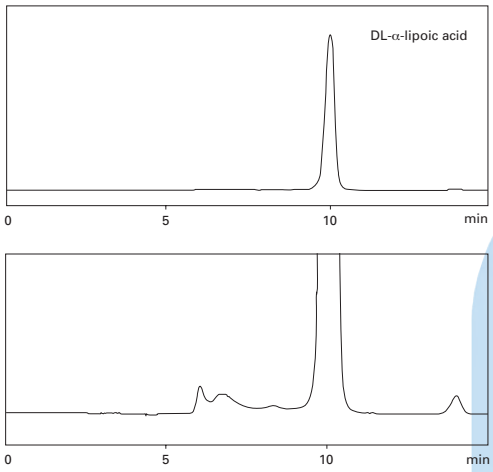
DL-α-lipoic acid (thiotic acid) has strong antioxidative properties and facilitates sugar metabolism. It is believed to play a positive role in weight loss. The magnified image of the chromatogram demonstrates the symmetrical peak shape of thiotic acid, when analysed on a TSKgel ODS-100Z RPC column.



Column: TSKgel ODS-100Z, 5 µm, 4.6 mm ID x 25 cm L
Eluent: 5 mmol/l NaH_2PO_4 (pH 3.0) /CH_3CN/CH_3OH = 920/180/1160
Flow rate: 1.2 ml/min
Detection: UV @ 215 nm
Temp.: 35°C
Sample: DL-α-lipoic acid (thiotic acid)
Concentration: 1 g/l
Inj. volume: 20 µl

19 Analysis of DL-α-lipoic acid by SEC (2)

The bottom figure is a magnified image of the top chromatogram using a TSKgel G1000HXL column and a mobile phase containing dimethylformamide (DMF) modified with lithium bromide and phosphoric acid.

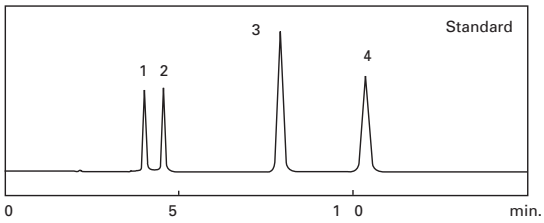


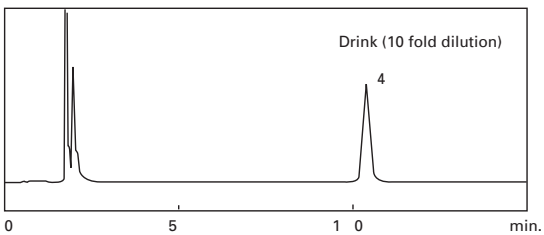
Column: TSKgel G1000HXL, 7.8 mm ID x 30 cm L +
TSKgel HXL-L Guardcolumn, 6.0 mm ID x 4 cm L
Eluent: 10 mmol/l LiBr, 20 mmol/l H_3PO_4 in DMF
Flow rate: 0.9 ml/min
Detection: UV @ 300 nm
Temp.: 40°C
Samples: DL-α-lipoic acid (thiotic acid)
Concentration: 30 g/l
Inj. volume: 10 µl

Food Additives

20 Separation of coenzyme Q10 in beverage

Coenzyme Q10 aids the body's energy production and is also known as vitamin Q, Co-Q10 and ubiquinone. Coenzyme Q10 has been examined closely for use as a food supplement. As it is fat soluble, it is retained strongly by RPC, and the below analysis was performed using a mobile phase containing a mixture of THF and acetonitrile. Under the same conditions, it is possible to simultaneously analyze coenzyme Q9 and tocopherol (vitamin E).

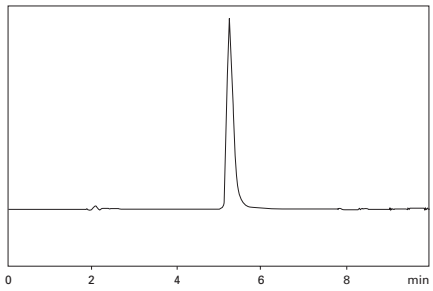




Column: TSKgel ODS-100V, 5 µm, 4.6 mm ID x 15 cm L
Eluent: THF/CH_3CN = 20/80
Flow rate: 1.0 ml/min
Detection: UV @ 270 nm
Temp.: 40°C
Samples: health drink; (RoyalQ10; CoQ10 30 g/50 l)
Compounds in Standard: 1: γ-tocopherol 0.06 g/l; 2: α-tocopherol; 0.06 g/l
3: ubiquinone-9; 0.25 g/l (Coenzyme Q9)
4: ubiquinone-10; 0.25 g/l (Coenzyme Q10)
Inj. volume : 10 µl

21 Separation of chlorhexidine gluconate

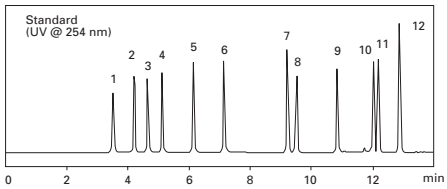
Chlorhexidine gluconate is an antibacterial agent with superior persistent germicidal properties. It is often used in skin refreshers and mouthwashes. The Standard Methods of Analysis for Hygienic Chemists: Cosmetic Test Methods describes a method where sodiumdodecylsulfate (SDS, an anionic surfactant) is added to a mobile phase, but in the chromatogram below, separation was carried out under acidic conditions without adding SDS. The absence of SDS should benefit the analyst in obtaining faster column equilibration.

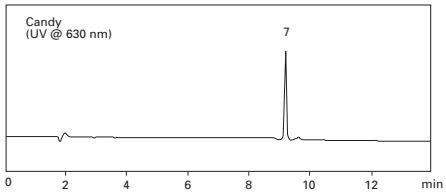


Column: TSKgel ODS-100V, 5 µm, 4.6 mm ID x 15 cm L
Eluent: 10 mmol/l $HCOONH_4$ (pH 3.5) /CH_3CN = 67/33
Flow rate: 1.0 ml/min
Detection: UV @ 258 nm
Temp.: 40°C
Samples: chlorhexidine gluconate
Concentration: 0.05 g/l
Inj. volume : 5 µl

22 Separation of food dyes in candy

Dyes can be roughly divided into synthetic and natural dyes, and most synthetic dyes are tar dyes. While more than 3,000 tar dyes have been developed, few are used in food items. In Japan, in 1960, 24 dyes were approved for use, but due to reasons such as potential carcinogenicity, only 12 such dyes are currently used. Here the results of analysis of a blue dye in a candy extract. Highly selective detection is possible by altering detection wavelength with the color of the various dyes.



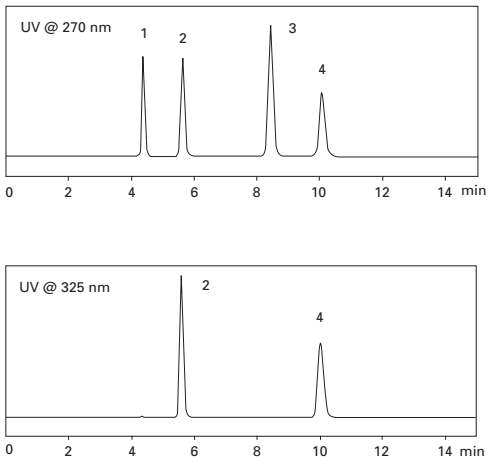


Column: TSKgel ODS-100V, 5 µm, 4.6 mm ID x 15 cm L
Eluent: A: 10 mmol/l CH_3COONH_4
B: CH_3CN
Gradient: 0 min 5 % B
20 min 70 % B
Flow rate: 1.0 ml/min
Detection: UV @ 254/630 nm
Temp.: 40°C
Samples: 1: Food Yellow No.4, 10 g/l; 2: Food Red No.2, 25 g/l;
3: Food Blue No.2, 20 g/l; 4: Food Red No.102, 25 g/l;
5: Food Yellow No.5, 25 g/l; 6: Food Red No.40, 25 g/l;
7: Food Green No.3, 100 g/l; 8: Food Blue No.1, 100 g/l;
9: Food Red No.3, 25 g/l; 10: Food Red No.104, 25 g/l;
11: Food Red No.106, 20 g/l; 12: Food Red No.105, 50 g/l
Inj. volume : 5 µl

Antioxidants/Isoflavones

23 Analysis of chlorogenic acid

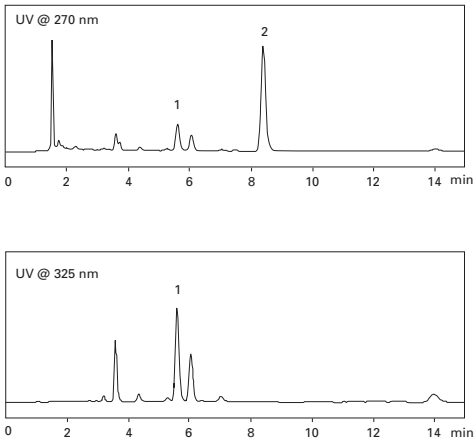
The antioxidant activity of chlorogenic acid (3-caffeoylquinic acid) is similar to that of catechins and flavonoids.



Column:	TSKgel ODS-100Z, 5 µm, 4.6 mm ID x 15 cm L
Eluent:	10 mmol/l $HCOONH_4$ (pH 3.5) / CH_3CN = 90/10
Flow rate:	1.0 ml/min
Detection:	UV @ 270/325 nm
Temp.:	40°C
Samples:	1: protocatechuric acid 0.05 g/l; 2: chlorogenic acid 0.2 g/l; 3: caffeine 0.1 g/l; 4: caffeic acid 0.1 g/l
Inj. volume :	5 µl

24 Separation of chlorogenic acid in coffee

Coffee beans contain large quantities of chlorogenic acid. Chlorogenic acid was extracted from coffee beans using boiling water. By selecting an appropriate wavelength with an UV-VIS detector, chlorogenic acid and coffeic acid can be selectively measured with high sensitivity.

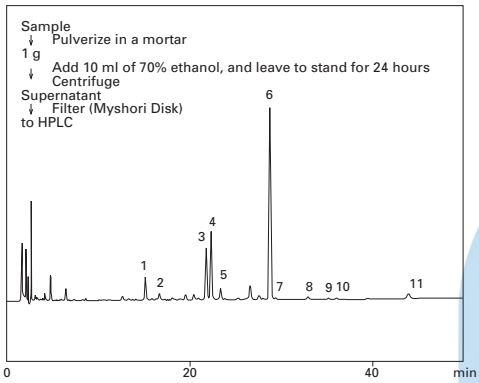


Column:	TSKgel ODS-100Z, 5 µm, 4.6 mm ID x 15 cm L
Eluent:	10 mmol/l $HCOONH_4$ (pH 3.5) / CH_3CN = 90/10
Flow rate:	1.0 ml/min
Detection:	UV @ 270/325 nm
Temp.:	40°C
Samples:	coffee 8 g
Compound:	1: chlorogenic acid 0.06 g/l; 2l caffeine 0.09 g/l
Inj. volume :	5 µl

*Sample preparation: 8 g of coffee beans were boiled in hot water for 10 minutes and diluted 10-fold with mobile phase.

25 Separation of isoflavones in soybean extract

Isoflavones, which are found in large quantities in soybeans, have similar functions as female hormones. Their effects on climacteric disorders and aging have been studied. Here the results of analysis of an alcohol extract of soybeans.



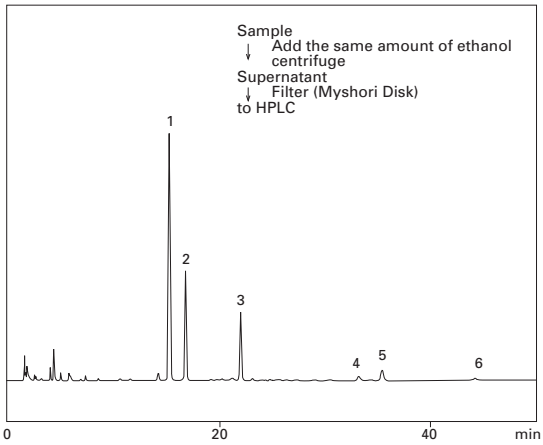
Column:	TSKgel ODS-100V, 5 µm, 4.6 mm ID x 15 cm L
Eluent:	A) 0.1 % acetic acid in 10 % CH_3CN
	B) 0.1 % acetic acid in 35 % CH_3CN
Gradient:	0 min 0 % B
	50 min 100 % B
Flow rate:	1.0 ml/min
Detection:	UV @ 260 nm
Temp.:	40°C
Samples:	1: daidzin 0.94 mg/l, 2:glycitin 0.35 mg/l, 3:genistin 2.06 mg/l, 4:malonyldaidzin 2.86 mg/l, 5:malonylglycitin 0.84 mg/l, 6:acetyldaidzin 8.79 mg/l, 7: malonylgenistin 0.08 mg/l, 8: daidzein 0.15 mg/l, 9: glycitein 0.05 mg/l, 10: acetylgenistin 0.07 mg/l, 11: genistein 0.20 mg/l
Inj. volume :	10 µl

*: Sample preparation (see figure)

Isoflavones

26 Separation of isoflavones in soybean drink

The chromatogram below shows the results of the gradient analysis of isoflavones in a soybean-containing health drink.

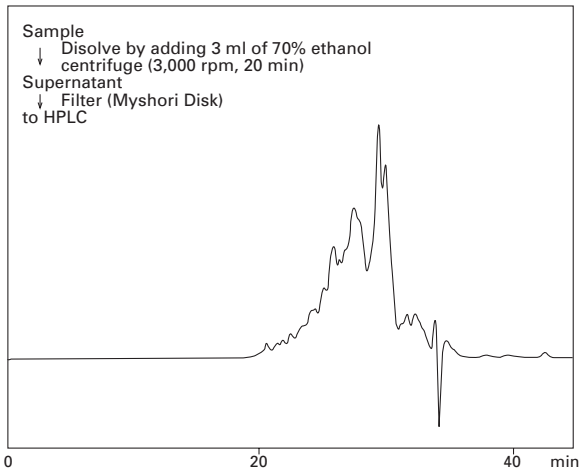


Column:	TSKgel ODS-100V, 5 µm, 4.6 mm ID x 15 cm L
Eluent:	A) 0.1 % acetic acid in 10 % CH_3CN
	B) 0.1 % acetic acid in 35 % CH_3CN
Gradient:	0 min 0 % B
	50 min 100 % B
Flow rate:	1.0 ml/min
Detection:	UV @ 260 nm
Temp.:	40°C
Sample:	soybean beverage
Compounds:	1: daidzin 23.66 mg/l, 2: glycitin 11.53 mg/l, 3: genistin 6.29 mg/l, 4: daidzein 0.54 mg/l, 5: glycitein 1.23 mg/l, 6: genistein 0.18 mg/l
Inj. volume :	10 µl

*: Sample preparation (see figure)

27 Separation of soybean peptide jelly extract

Soybean peptides are enzymatically digested soybean proteins and have various structures and molecular weight distributions. The chromatogram below shows the separation of a soybean peptide jelly extract by SEC. Three TSKgel G2000SWXL columns in series were used with a mobile phase containing water, acetonitrile, and TFA.



Column:	3 x TSKgel G2000SWXL, 5 µm, 7.8 mm ID x 30 cm L
Eluent:	0.1 % TFA + 45 % CH_3CN
Flow rate:	1.0 ml/min
Detection:	UV @ 215 nm
Temp.:	30°C
Sample:	soy peptide beverage
Compound:	soy peptide
Inj. volume :	50 µl

*: Sample preparation (see the figure)

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B07L05A