LC6000 Column Choices Guide

Contents

A. HPLC Separation Modes

Liquid Chromatography uses liquid as an eluent, also known as mobile phase. It is an analytical method that separates a mixture of compounds based on their physical and chemical differences. High Performance Liquid Chromatography (HPLC) is a method that introduces the mobile phase under high pressure conditions, resulting in rapid and high-performance separations. The various interactions between the analyte, packing material of the analytical column (stationary phase) and mobile phase are key factors for successful separation. There are a wide variety of separation modes, which can be achieved by using specific combinations of both stationary and mobile phases. Table 1 details the characteristics of three different separation modes, commonly used within HPLC.

Table 1. LC separation modes and associated characteristics

Separation Mode	Characteristics
Reverse Phase Chromatography (RPC)	• Separation is based on the partition equilibration between stationary phase and mobile phase • The polarity of the stationary phase is lower than that of the mobile phase • Using a lower polarity mobile phase makes elution of target compounds quicker • The mobile phase typically contains a mixture of organic solvents (methanol, acetonitrile or THF) and aqueous solvents (water or buffer)
Normal Phase Chromatography (NPC)	• Separation is based on the partition equilibration between the stationary phase and the mobile phase • The polarity of the stationary phase is higher than that of the mobile phase • The mobile phase typically contains a mixture of organic solvents with different polarities such as hexane and isopropanol
Hydrophilic Interaction Chromatography (HILIC)	• Separation is based on hydrophilic interaction • A high polarity stationary phase is used • The mobile phase typically contains a mixture of organic solvents (acetonitrile) and aqueous solvents (water or buffer) • Recommended for the analysis of highly polar compounds

B. Column Selection Guide – Application Markets

Table 2 details the various different application markets, target compound types and associated separation modes. When selecting a column for a particular application, use the guide a reference for identifying the separation mode to be used. This is the first step in choosing the right column for the desired application.

Table 2. Application markets, target compound type and associated separation mode

Application Market	Compound Type	Separation Mode
Pharmaceutical		
Metabolites an Additives	Hydrophobic	RPC
	Hydrophilic	HILIC RPC
	Substances in biological fluid	RPC
Moisturisers	Polyalcohols	RPC
	Protein Hydrolysates	RPC HILIC
Emulsifiers	Surfactants	RPC
Preservatives	Parabens	RPC
Food		
Nutritional Ingredients	Monosaccharides, Disaccharides and 811 Alcohols	HILIC
	Oligosaccharides	HILIC
	Organic Acids	RPC
	Water Soluble Vitamins	RPC
	Fat Soluble Vitamins	RPC
	Fatty Acids	RPC
	Amino Acids	RPC HILIC
Food Safety	Food Additives	RPC HILIC
	Pesticides	RPC HILIC
	Mycotoxins	RPC
New Materials		
	Additive Oligomers	RPC
Biotechnology		
Genomics	Nucleobases, Nucleotides, Nucleosides	RPC
	Oligo Nucleic Acids	RPC HILIC
Proteomics	Amino Acids	RPC HILIC
	Peptide Proteins	RPC
Glycomics	Glycoproteins	RPC
	Sugar Chains	HILIC
	Monosaccharides	HILIC
Hormones	Amines	RPC

	Steroids	RPC HILIC
Environment		
Water Quality	Oxyhalides	HILIC
	Surfactants	RPC
	Pesticides	RPC HILIC
Soil	Organic Arsenic	RPC
	Pesticides	RPC HILIC
Bio-ethanols	Monosaccharides, Oligosaccharides	HILIC
	Saccharides, Organic Acids, Alcohols, Furfural	RPC
Biodiesels	Fatty Acid Methyl Esters	RPC

C. Column Base Material and Functional Groups

There are many different columns available depending on the separation type and target compounds for an analysis. Each column has specific base material and functional groups which can be utilised for method optimisation, target compound identification and quantification. Table 3 details the different base materials and functional groups for all available HPLC columns. If a HPLC column you require is not listed in the table below, please contact your SCION Instruments representative.

Table 3. Separation type, column product name, column base material and modified functional group

Separation Type	Product Name	Base Material	Functional Group
Reverse Phase, Normal Phase & HILIC Silica Based	C18X	Silica	Octadecyl
	C18M	Silica	Octadecyl
	C18P	Silica	Octadecyl
	5SIL	Silica	-
	5C8	Silica	Octyl
	5CN	Silica	Cyanopropyl
	5NH	Silica	Aminopropyl
Reverse Phase & HILIC Polymer Based	Asahipak ODP-40	Polyvinyl alcohol	octadecyl
	Asahipak ODP-50	Polyvinyl alcohol	octadecyl
	Asahipak ODP2	Polyhydroxy methacrylate	-
	Asahipak C8P	Polyvinyl alcohol	Octyl
	Asahipak C4P	Polyvinyl alcohol	Butyl
	Asahipak ET-RP1	Polyvinyl alcohol	Octadecyl
	Asahipak NH2P	Polyvinyl alcohol	Amino
	RSpak RP18	Styrene divinylbenzene copolymer	-
	RSpak DS-413	Styrene divinylbenzene copolymer	-
	RSpak DS-613	Styrene divinylbenzene copolymer	-
	RSpak DE-213	Polymethacrylate	-
	RSpak DE-413	Polymethacrylate	-
	RSpak DM-614	Polyhydroxy methacrylate	-
	RSpak NN-414	Polyhydroxy methacrylate	Sulfo
	RSpak NN-614	Polyhydroxy methacrylate	Sulfo
	RSpak NN-814	Polyhydroxy methacrylate	Sulfo
	RSpak NN-G	Polyhydroxy methacrylate	Sulfo

	RSpak JJ-50	Polyvinyl alcohol	Quaternary Ammonium
	HILICpak VG	Polyvinyl alcohol	Amino
	HILICpak VT	Polyvinyl alcohol	Quaternary Ammonium
	HILICpak VN	Polyvinyl alcohol	Diol
	HILICpak VC	Polyvinyl alcohol	Carboxyl

The following sections detail all features of the available HPLC columns, for the different separation modes.

D. Column Details and Features

D1 Comparison of Reverse Phase Chromatography Column Features

ODS columns are the most popular reverse phase columns that are packed with silica-based octadecyl groups. Polymer based reverse phase columns with different functional groups are also available. The following descriptions detail the column features, as should be used as a guideline to select a suitable column for the desired applications. Table 4 details the features of all reverse phase chromatography columns in the SCION Instruments portfolio.

Table 4. Features of all reverse phase chromatography columns

Features	
ODP2	• Provides a large theoretical plate number compared to general polymer based reverse phase columns • Offers enhances retention of high polar substances compared to ODS columns • Suitable for the analysis of small molecules such as pharmaceuticals in the presence of protein matrix • Ideal for the analysis of high polarity compounds • Fulfils USP L39 requirements
ODP-50 **C8P-50** **C4P-50**	• Relatively large pore size • Suitable for the analysis of amino acids, peptides and proteins • Wide pH range from 2 to 13 • Compatible with 100% water and buffer mobile phase • Best used for the analysis of basic compounds
ODP-40	• Higher performance than ODP-50 • Fulfils USP L67 requirements
DS-413 **DS-613**	• Suitable for the analysis of highly hydrophobic substances that are not well retained by ODS columns • Fulfils USP L21 requirements
DE-213 **DE-413** **DE-613**	• General purpose polymer based column • Similar polarity to ODS columns • Wide working pH range from 2 to 12 • Usable in 100% water and buffer mobile phase • Fulfils USP L71 requirements
DM-614	• Suitable for the analysis of amino acids and water soluble vitamins • Fulfils USP L39 requirements
NN-414 **NN-614**	• The packing material is modified with Sulfo groups • Supports multimode analysis; reverse phase and cation exchange

NN-814	• Ideal for the analysis of complex samples containing neutral and ionic compounds
JJ-50	• The packing material is modified with trace amounts of quaternary ammonium groups • Supports multimode analysis; reverse phase and anion exchange • Ideal for the analysis of complex samples containing neutral and ionic substances
C18X	• Fully end capped ODS column • Fulfils USP L1 requirements
C18M	• Fully end capped, monomeric ODS column • High purity silica (>99.99%) • Fulfils USP L1 requirements
C18P	• Fully end capped, polymeric ODS column • High purity silica (>99.99%) • Advantageous for separation planar and nonplanar compounds • Fulfils USP L1 requirements
5C8	• Used when the retention capacity of C18 is too strong • Rapid mass transfer and fast equilibration • Can be used in ion-pair chromatography • Fulfils USP L7 requirements
5CN	• Utilises reverse phase interactions and π-electron interaction to separate regioisomers, which cannot be separate with ODS or C8 columns • Fulfils USP L10 requirements
5NPE	• Utilises several types of interactions based on π-electrons to separate structural isomers

Tables 5-7 details the product name, plate number, particle size, pore size, column size and associated part number for polymer based reverse phase chromatography columns.

Table 5. Product details for polymer based reverse phase chromatography columns (ODP2)

Part Number	Product Name	Plate Number	Particle Size (µm)	Pore Size (Å)	Column Size (mm) I.D x Length
LC20220047	ODP2 HP-4B	≥3,500	5	40	4.6 x 50
LC20220048	ODP2 HP-4D	≥10,000	5	40	4.6 x 150
LC20220049	ODP2 HP-4E	≥17,000	5	40	4.6 x 250
LC20220050	ODP2 HPG-4A	Guard Column	5	-	4.6 x 10
LC20220051	ODP2 HP-2B	≥3,000	5	40	2.0 x 50
LC20220052	ODP2 HP-2D	≥7,000	5	40	2.0 x 150
LC20220053	ODP2 HPG-2A	Guard Column	5	-	2.0 x 10

Table 6. Product details for polymer based reverse phase chromatography columns (Asahipak)

Part Number	Product Name	Plate Number	Particle Size (µm)	Pore Size (Å)	Column Size (mm) I.D x Length
Functional Group: Octadecyl					
LC20220036	ODP-40 4D	≥11,000	4	250	4.6 x 150
LC20220037	ODP-40 4E	≥17,000	4	250	4.6 x 250
LC20220038	ODP-50 6D	≥9,000	5	250	6.0 x 150
LC20220039	ODP-50 6E	≥14,000	5	250	6.0 x 250
LC20220040	ODP-50G 6A	Guard Column	5	-	6.0 x 10
LC20220041	ODP-50 4B	≥2,500	5	250	4.6 x 50
LC20220042	ODP-50 4D	≥9,000	5	250	4.6 x 150
LC20220043	ODP-50 4E	≥14,000	5	250	4.6 x 250
LC20220044	ODP-50G 4A	Guard Column	5	-	4.6 x 10
LC20220045	ODP-50 2D	≥5,000	5	250	2.0 x 150
LC20220046	ODP- 50G 2A	Guard Column	5	-	2.0 x 10
Functional Group: Octyl					
LC2022054	C8P-50 4D	≥7,000	5	250	4.6 x 150
LC2022055	C8P-50 4E	≥11,000	5	250	4.6 x 250
LC2022056	C8P-50G 4A	Guard Column	5	-	4.6 x 10
Functional Group: Butyl					
LC2022057	C4P-50 4D	≥6,000	5	250	4.6 x 150
LC2022058	C4P-50 4E	≥9,000	5	250	4.6 x 250
LC2022059	C4P-50G 4A	Guard Column	5	-	4.6 x 10

Table 7. Product details for polymer based reverse phase chromatography columns (RSpak)

Part Number	Product Name	Plate Number	Particle Size (µm)	Pore Size (Å)	Column Size (mm) I.D x Length
Base Material: Styrene divinylbenzene copolymer					
LC20220019	RP18-415	≥5,000	6	450	4.6 x 150
LC20220020	RP18-G	Guard Column	6	-	4.6 x 10
LC20220021	DS-613	≥6,500	6	200	6.0 x 150
LC20220022	DS-413	≥11,000	3.5	200	4.6 x 150
Base Material: Polymethacrylate					
LC20220023	DE-613	≥7,000	6	25	6 x 150
LC20220024	DE-413	≥11,000	4	25	4.6 x 150
LC20220025	DE-413L	≥17,000	4	25	4.6 x 250
LC20220026	DE-213	≥8,000	4	25	2 x 150
LC20220027	DE-G 4A	Guard Column	10	-	4.6 x 10
LC20220028	DE-G 2A	Guard Column	6	-	2 x 10
Base Material: Polyhydroxy methacrylate					
LC20220029	DM-614	≥4,500	10	200	6 x 150
LC20220030	NN-814	≥9,000	10	200	8.0 x 250
LC20220031	NN-614	≥4,000	10	200	6.0 x 150
LC20220032	NN-414	≥6,000	10	200	4.6 x 150
LC20220033	NN-G	Guard Column	10	-	6.0 x 50

Base Material: Polyvinyl Alcohol					
LC20220034	JJ-50 4D	≥4,500	5	100	4.6 x 150
LC20220035	JJ-50 2D	≥3,500	5	100	2 x 150

D2. Comparison of Polymer Based Hydrophilic Interaction Chromatography (HILIC) Column Features

Table 8 details the features of all available HILICPak chromatography columns, suitable for use with HILIC separations.

Table 8. Features of HILICPak polymer columns

Features	
VG-50	• Ideal for saccharide analysis using HILIC mode • Polymer base packing material • Excellent chemical stability with minimum deterioration over an extended time period • Easily regenerated by washing in alkaline solution • Suited to ELSD, corona charged aerosol detectors and MS
VT-50	• Suitable for anionic substances using HILIC mode • Use when target compounds are phosphate based • Can be adapted to ion exchange mode depending on mobile phase • Polymer based packing material • Excellent chemical stability with minimum deterioration oven an extended time period • Suited for MS analysis
VC-50	• Modified carboxyl group • Suited for cationic compound analysis including amines • Dominant separation is for reverse phase
VN-50	• Modified Diol groups for HILIC mode • Suited for oligosaccharide and oligonucleotide separation

Table 9 details the product name, plate number, particle size, pore size, column size and associated part numbers of all HILICPak polymer based columns.

Table 9. Product details for polymer based HILICPak columns

Part Number	Product Name	Plate Number	Particle Size (µm)	Pore Size (Å)	Column Size (mm) I.D x Length
Functional Group: Tertiary Amino					
LC20220073	VG-50 4D	≥5,500	5	100	4.6 x 150
LC20220074	VG-50 4E	≥7,500	5	100	4.6 x 250
LC20220075	VG-50G 4A	Guard Column	5	100	4.6 x 10
LC20220076	VG-50 2D	≥3,500	5	100	2 x 150
LC20220077	VG-50G 2A	Guard Column	5	100	2 x 10
Functional Group: Quaternary Ammonium					
LC20220078	VT- 50 2D	≥4,500	5	100	2 x 150
LC20220079	VT- 50G 2A	Guard Column	5	100	2 x 10
Functional Group: Diol					
LC20220080	VN- 50 4D	≥10,000	5	100	4.6 x 150
LC20220081	VN- 50G 4A	Guard Column	5	100	4.6 x 10
LC20220082	VN-50 2D	≥3,500	5	100	2 x 150
LC20220083	VN- 50G 2A	Guard Column	5	100	2 x 10

Functional Group: Carboxyl					
LC20220084	VC- 50 2D	≥3,500	5	100	2 x 150
LC20220085	VC – 50G 2A	Guard Column	5	100	2 x 10

Table 10 details the features of Asahipak, HILIC separation columns. All columns are polymer based.

Table 10. Features of polymer based Asahipak HILIC columns

Features	
NH2P-50	• Suitable for saccharide analysis using HILIC mode • Polymer based packing material • Excellent chemical stability and minimum deterioration over an extended time period • Easily regenerated by washing in an alkaline solution • Suited for ELSD, corona charged aerosol detector and MS • Fulfils USP L82 requirements
NH2P-40	• Higher theoretical plate number than NH2P-50 series • Polymer based packing material • Excellent chemical stability and minimum deterioration over an extended time period • Easily regenerate by washing in an alkaline solution • Fulfils USP L82 requirements

Table 11 details the product name, plate number, particle size, pore size, column size and associated part numbers of all Asahipak HILIC polymer based columns

Table 11. Product details for polymer based Asahipak HILIC columns

Part Number	Product Name	Plate Number	Particle Size (µm)	Pore Size (Å)	Column Size (mm) I.D x Length
Functional Group: Amino					
LC20220061	NH2P-50 4B	≥1,500	5	100	4.6 x 150
LC20220062	NH2P-50 4D	≥5,500	5	100	4.6 x 150
LC20220063	NH2P-50 4E	≥7,500	5	100	4.6 x 250
LC20220064	NH2P-50G 4A	Guard Column	5	-	4.6 x 10
LC20220065	NH2P-50 2D	≥3,500	5	100	2 x 150
LC20220066	NH2P-50G 2A	Guard Column	5	-	2 x 10
LC20220067	NH2P-40 3E	≥8,500	4	100	3 x 250
LC20220068	NH2P-50G 3A	Guard Column	5	-	3 x 10
LC20220069	NH2P-LF	Line Filter	-	-	8.0 x 75
LC20220070	NH2P-40 2B	≥2,000	4	100	2 x 50
LC20220071	NH2P-40 2D	≥5,500	4	100	2 x 150
LC20220072	NH2P-40 2E	≥7,000	4	100	2 x 250

D3. Comparison of Silica Based Reverse Phase Chromatography Columns (ODS) Features

Table 12 details the features of all ODS, silica based reverse phase chromatography columns. These are the most versatile C18 column available and a typically used for method development and validation.

Table 12. Features of silica based reverse phase chromatography columns (ODS)

Features	
C18X	• Fully end capped ODS column • Fulfils USP L1 requirements
C18M	• Fully end capped, monomeric ODS column • High purity silica (>99.99%) • Fulfils USP L1 requirements
C18P	• Fully end capped, polymeric ODS column • High purity silica (>99.99%) • Advantageous for separation planar and nonplanar compounds • Fulfils USP L1 requirements

Table 13 details the product name, plate number, particle size, pore size, column size and associated part numbers of all ODS silica based reverse phase chromatography columns.

Table 13. Product details for silica based chromatography columns (ODS)

Part Number	Product Name	Plate Number	Particle Size (µm)	Carbon Load (%)	Pore Size (Å)	Column Size (mm) I.D x Length
Functional Group: Octadecyl						
LC20220000	C18X – 4D	≥13,000	5	17	120	4.6 x 150
LC20220001	C18X – 4E	≥21,000	5	17	120	4.6 x 150
LC20220002	C18M – 4D	≥10,000	5	16	100	4.6 x 150
LC20220003	C18M – 4E	≥16,000	5	16	100	4.6 x 250
LC20220004	C18M – 2D	≥9,000	5	16	100	2 x 150
LC20220005	C18P – 4D	≥10,000	5	17	100	4 x 150
LC20220006	C18P – 4E	≥16,000	5	17	100	4 x 250
LC20220007	C18P – 2D	≥9,000	5	17	100	2 x 150

D4. Comparison of Silica Based Reverse Phase Chromatography Columns (Others) Features

Table 14 details the features of all other ODS, silica based reverse phase chromatography columns. These columns are recommended when C18 columns provide too much retention. The Other ODS column range also includes modified cyanopropyl and nitrophenylethyl columns.

Table 14. Features of silica based reverse phase chromatography columns (ODS)

Features	
5C8	• Used when the retention capacity of C18 is too strong • Rapid mass transfer and fast equilibration • Can be used in ion-pair chromatography • Fulfils USP L7 requirements
5CN	• Utilises reverse phase interactions and π-electron interaction to separate regioisomers, which cannot be separate with ODS or C8 columns • Fulfils USP L10 requirements
5NPE	• Utilises several types of interactions based on π-electrons to separate structural isomers

Table 15 details the product name, plate number, particle size, pore size, column size and associated part numbers of all other ODS silica based reverse phase chromatography columns.

Table 15. Product details for silica based chromatography columns (Other)

Part Number	Product Name	Plate Number	Particle Size (µm)	Carbon Load (%)	Pore Size (Å)	Column Size (mm) I.D x Length
Functional Group: Octyl						
LC20220012	5C8 – 4D	≥9,000	5	10	100	4.6 x 150
LC20220013	5C8 - 4E	≥15,000	5	10	100	4.6 x 250
Functional Group: Cyanopropyl						
LC20220014	5CN – 4D	≥7,000	5	-	100	4.6 x 150
LC20220015	5CN – 4E	≥12,000	5	-	100	4.6 x 250
Functional Group: Nitrophenylethyl						
LC20220018	5NPE – 4D	≥8,000	5	-	100	4.6 x 150

D5. Comparison of Silica Based Normal Phase Chromatography and HILIC Columns

Table 16 details HILIC columns which can be used in HILIC mode. These include a high purity silica column and a modified aminopropyl column.

Table 16. Features of silica based normal phase chromatography and HILIC Columns

Features	
5SIL	• High purity silica (>99.99%) • Suited for nonpolar organic solvents with normal phase analysis • Fulfils USP L3 requirements
5NH	• Modified with aminopropyl functional groups • Suited for saccharides analysis using HILIC mode • Fulfils USP L8 requirements

Table 17 details the product name, plate number, particle size, pore size, column size and associated part numbers of all silica based normal phase chromatography and HILIC Columns.

Table 17. Product details for normal phase chromatography and HILIC Columns

Part Number	Product Name	Plate Number	Particle Size (µm)	Pore Size (Å)	Column Size (mm) I.D x Length
LC20220010	5SIL – 4D	≥9,000	5	100	4.6 x 150
LC20220011	5SIL – 4E	≥15,000	5	100	4.6 x 250
Functional Group: Aminopropyl					
LC20220016	5NH – 4D	≥5,000	5	100	4.6 x 150
LC20220017	5NH – 4E	≥8,000	5	100	4.6 x 250

E. Column Cleaning Guidelines

Changes in peak shape, retention time and elevated column pressure may be resolved by cleaning the column. The following guidelines describe general indications of column deterioration and column cleaning procedures. For detailed column cleaning procedures, refer to the operating manual packaged with each column.

Typical indicators of column deterioration:

- Elevated column pressure
- Abnormal peak shape (broadening, fronting or tailing)
- Split peaks
- Change in retention time
- Unstable baseline

RPC Columns

- Always use a strong organic solvent such as methanol, acetonitrile or THF for cleaning
- If a buffered mobile phase has been used, check the miscibility of the buffer solution and the organic solvent before cleaning the column
- Disconnect any guard column
- Reverse the direction of the column and disconnect the outlet from the detector
- Set the flow rate to 0.5mL/min or half of method flow rate
- Wash the column 3 to 5 times the column volume with 100% organic solvent
- Wash the column with 3 to 5 times the column volume with original mobile phase
- Repeat the 100% organic step if enhanced cleaning is required

Sugar Analysis Columns (NH2P and VG-50 Series)

In cases where an acidic substance has been bound to the amino functional group, flush with solvents in the following sequence:

- 100% water
- 0.1M perchloric acid (aqueous)
- 100% water
- 0.1M NaOH (aqueous)
- 100% water
- New mobile phase

HILIC Chromatography Columns

- Always use a strong organic solvent such as IPA, methanol or THF for cleaning
- If a buffered mobile phase has been used, check the miscibility of the buffer solution and the organic solvent before cleaning the column
- Prepare a 50:50 cleaning solution of solvent and Water
- Disconnect any guard column
- Reverse the direction of the column and disconnect the outlet from the detector
- Set the flow rate to 0.5mL/min or half of method flow rate
- Wash the column 3 to 5 times the column volume with the cleaning solution
- Wash the column with 3 to 5 times the column volume with original mobile phase
- Strong buffers may be required to remove ionic contaminants

F. Guidelines for Column Handling

For best column performance, precautions must be taken when handing the HPLC column.

Column Installation

- Before installing a HPLC column, replace the mobile phase within the full HPLC system with new mobile phase (always check the miscibility of a buffer with solvent)
- Using the flow direction arrow on the column, connect the column inlet to the end of the column
- Gradually increase the flow rate of the mobile phase throughout the column
- When heating the column, be sure to pump the mobile phase at a low flow rate until the specified temperature is reached
- Once column flow is established and the column is properly flushed, connect the column outlet to the detector inlet

Column Removal

- Flush the column with 3 to 5 times volume of 100% organic solvent (50:50% organic/water for HILIC columns)
- If the column oven is heated, turn off the heater and reduce the flow rate to 1/3rd of the regular flow
- When the column oven is at room temperature, turn off pump flow
- Remove the inlet and outlet from the column and fit the shipping caps
- Gently tighten the caps to be finger tight

Column Storage

- Securely store the column in its original packaging
- Keep the column in a cool, dark space away from sunlight and extreme temperatures

G. Reference USP Column List

Table 18 details the USP requirements including packing material and recommended column for use, when following USP methods.

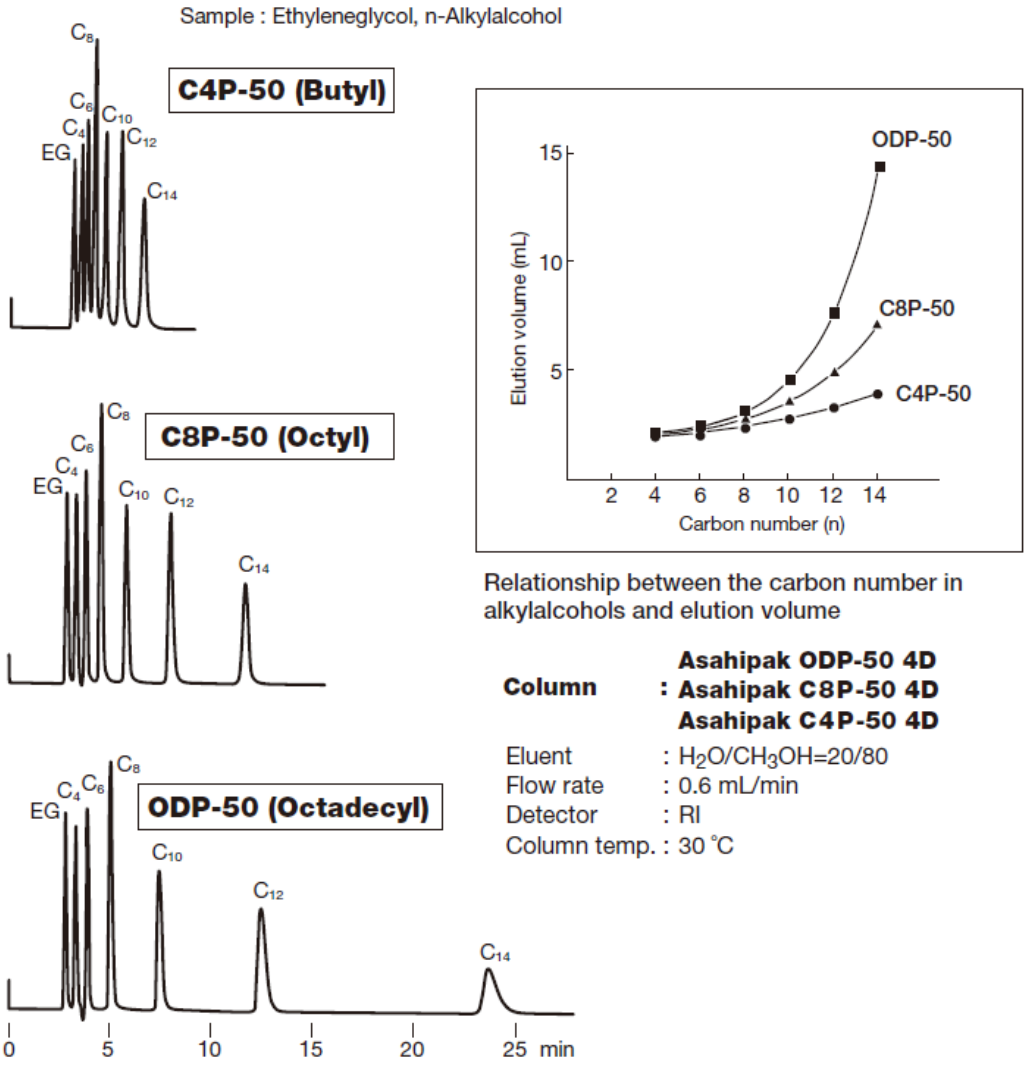
Table 18. USP requirements, packing material and recommended columns

USP	Packing Material	Recommended Column
L1	Octadecyl silane chemically bonded to silica	C18X, C18M, C18P
L3	Porous silica particles	5SIL
L7	Octylsilane chemically bonded to silica	5C8
L8	Monomolecular aminopropylsilane chemically bonded to totally porous silica gel support	5NH
L10	Nitrile groups chemically bonded to porous silica particles	5CN
L21	A rigid, spherical styrene-divinylbenzene copolymer	RP18-415, DS-613, DS-413
L39	A hydrophilic polyhyroxymethacrylate gel of totally porous spherical resin	ODP2-HP, DM-614
L67	Porous vinyl alcohol copolymer with a C18 alkyl group attached to the hydroxyl group of the polymer	ODP-40, ODP-50, ET-RP1
L71	A rigid, spherical polymetacrylate	DE-613, DE-413, DE-213
L82	Polyamine chemically bonded to cross-lined polyvinyl alcohol polymer	NH2P-50, NH2P-40

Appendix

The following appendix show example data and chromatograms for a variety of SCION Instrument HPLC columns. These are for illustrative and demonstrative purposes only.

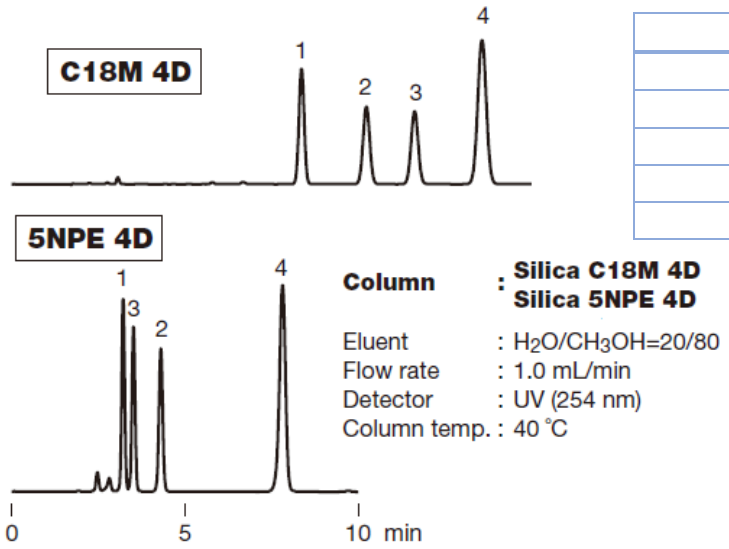
a1. Comparison of different functional groups on the separation of alkylalcohols



Relationship between the carbon number in alkylalcohols and elution volume

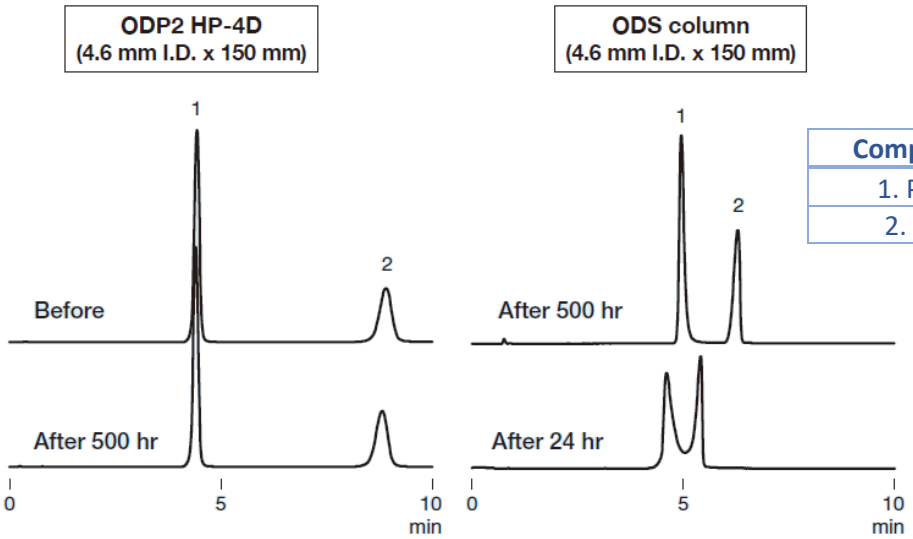
Column	:	**Asahipak ODP-50 4D** **Asahipak C8P-50 4D** **Asahipak C4P-50 4D**
Eluent	:	H_2O/CH_3OH=20/80
Flow rate	:	0.6 mL/min
Detector	:	RI
Column temp.	:	30 ℃

a2. Effect of steric selectivity differences using silica based columns



Compounds
Sample: 5µL
1. n-Butylbenzene
2. n-Amylbenzene
3. o-Terphenyl
4. Triphenylene

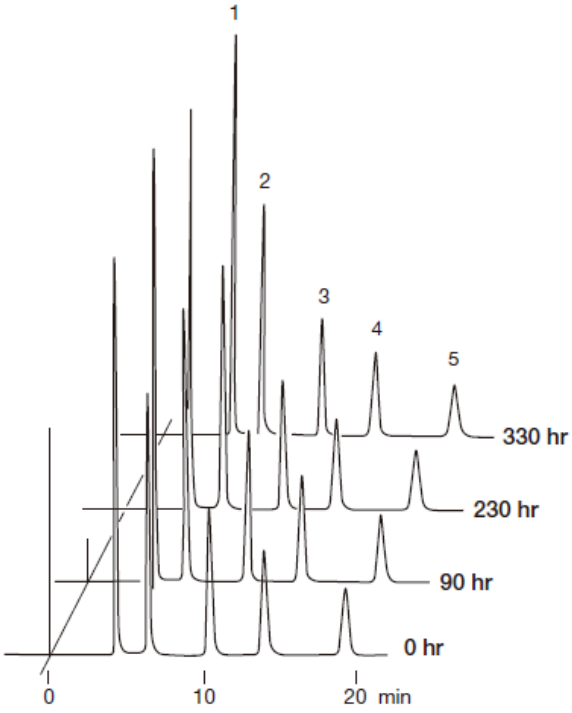
a3. Comparison between ODP2-HP 4D and a general ODS column for their alkaline intolerances



Compound	Concentration
1. Pyridine	200µg/mL
2. Phenol	430µg/mL

Analysis Conditions:
Columns: ODP2 HP-4D and general ODS
Eluent: H_20/ CH_3OH = 70/30
Flow rate: 1mL/min
Detector: UV (254nm)
Column Temp: 40°C

a4. Alkaline tolerance of ODP-50

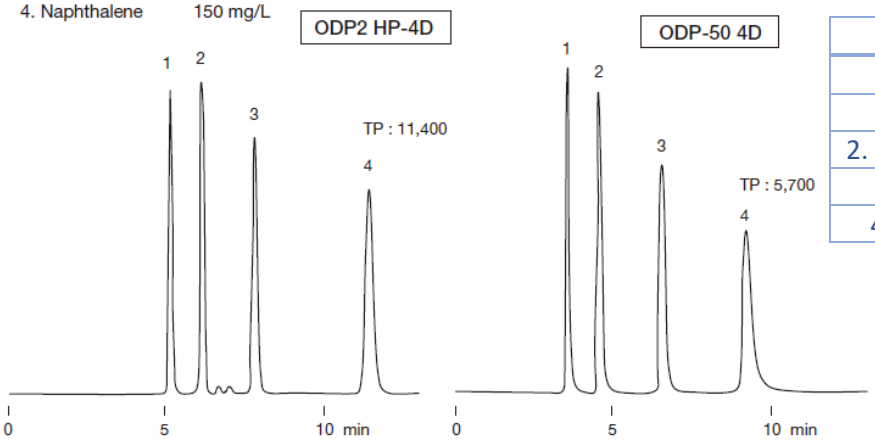


Compounds
1. Acetophenone
2. Butyrophenone
3. Hexanophenone
4. Heptanophenone
5. Octanophenone

Column : **Asahipak ODP-50 4D**
Eluent : 10 mM NaOH aq. (pH 12.0)/CH_3CN=35/65
Flow rate : 0.6 mL/min
Detector : UV (254 nm)
Column temp. : 30 ℃

a5. Comparison between ODP2-HP and ODP-50

Sample : 5 µL
1. Phenol 300 mg/L
2. Methyl benzoate 350 mg/L
3. Toluene 1000 mg/L
4. Naphthalene 150 mg/L

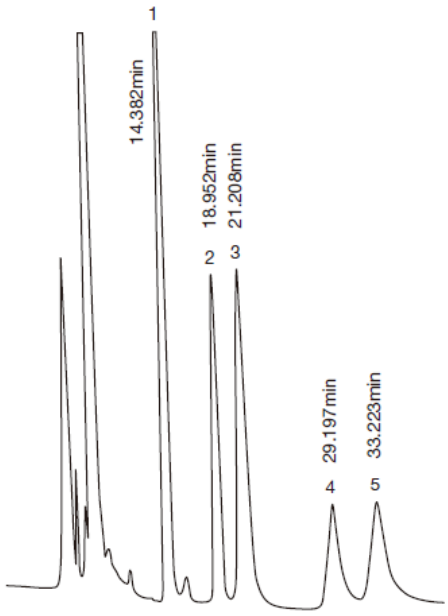


Compound	Concentration
Sample: 5µL	
1. Phenol	300mg/L
2. Methyl Benzoate	350mg/L
3. Toluene	1000mg/L
4. Naphthalene	150mg/L

Column : **ODP2 HP-4D**
Eluent : H_2O/CH_3CN=55/45
Flow rate : 0.6 mL/min
Detector : UV (254 nm)
Column temp. : 40 ℃

Column : **Asahipak ODP-50 4D**
Eluent : H_2O/CH_3CN=35/65
Flow rate : 0.6 mL/min
Detector : UV (254 nm)
Column temp. : 40 ℃

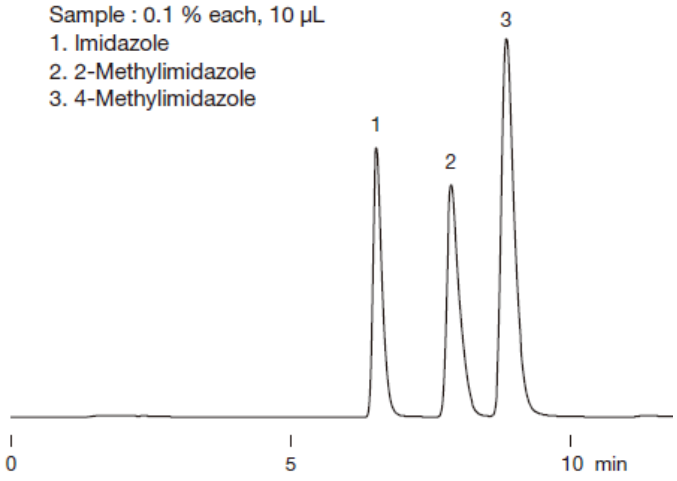
a6.Analysis of Imidazoles using ODP2 HP-4E column



Compound	Concentration
Sample: 20µL	
1. 2-Acetaminophenol (I.S)	10µg/mL
2. Zonisamide	13µg/mL
3. Phenobarbital	19µg/mL
4. Carbamazepine	4.5µg/mL
5. Phenytoin	9µg/mL

Column : **ODP2 HP-4E**
Eluent : 25 mM Sodium phosphate buffer (pH 5.2)/CH_3CN = 68 / 32
Flow rate : 0.35 mL/min
Detector : UV (210 nm)
Column temp. : 40 ˚C

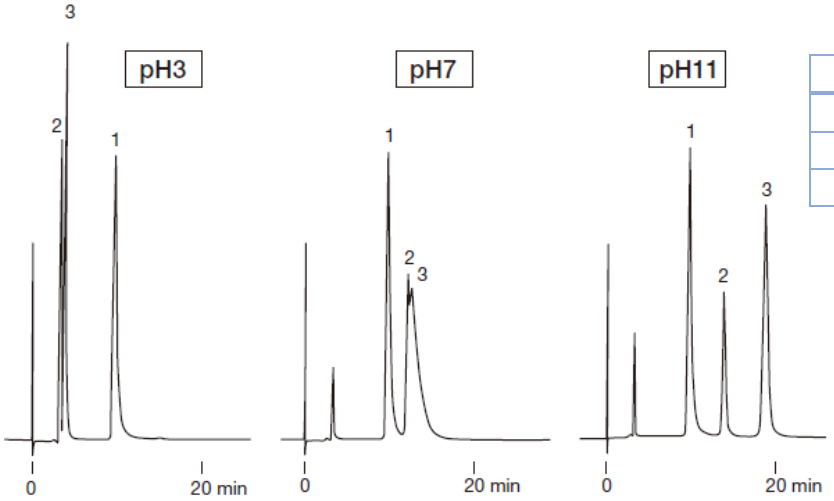
a7. Analysis of anticonvulsant drugs in serum



Compound
Sample: 0.1%, 10µL
1. Imidazole
2. 2-Methyl Imidazole
3. 4-Methyl Imidazole

Column : **ODP2 HP-4E**
Eluent : 10 mM Na_2HPO_4 aq./CH_3CN=90/10
Flow rate : 0.8 mL/min
Detector : UV (220 nm)
Column temp. : 40 ˚C

a8. Analysis of local anaesthetic drugs

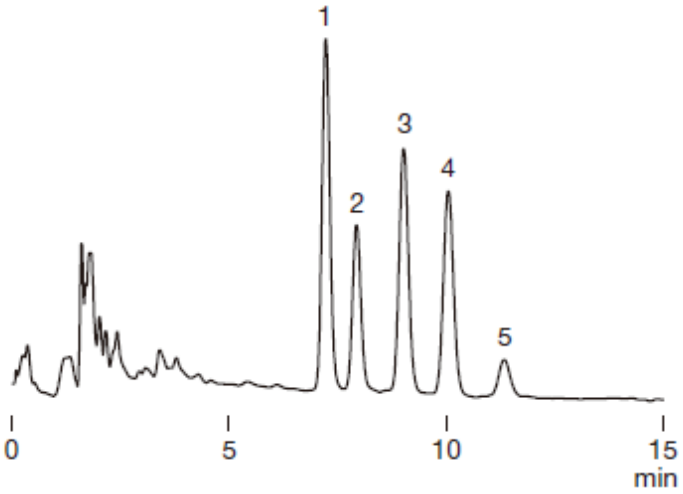


Compound
1. Benzocaine
2. Lidocaine
3. Tetracaine

Column : **Asahipak ODP-50 4D**
Eluent : 25 mM Phosphate buffer/CH_3CN=60/40
Flow rate : 0.6 mL/min
Detector : UV (254 nm)
Column temp. : 30 ℃

Dissociation of tertiary amino groups in basic drugs can be suppressed by making pH of the eluent higher than pKa of the amino groups. This increases the relative hydrophobicity of the basic drugs, thereby allowing the column to retain the drug stronger and provide baseline separation of them.

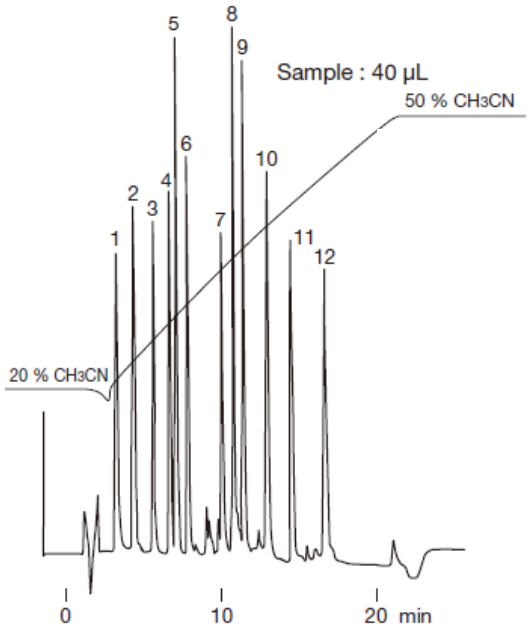
a9. Analysis of unsaturated fatty acids



Compound
Sample: 0.002%, 5µL
1. Eicosapentaenoic Acid
2. α-Linolenic Acid
3. Docosahexaenoic Acid
4. Arachidonic Acid
5. Linoleic Acid

Column : **Asahipak ODP-50 4D**
Eluent : 0.1 % H_3PO_4 in (H_2O/CH_3CN=30/70)
Flow rate : 1.0 mL/min
Detector : UV (215 nm)
Column temp. : 40 ℃

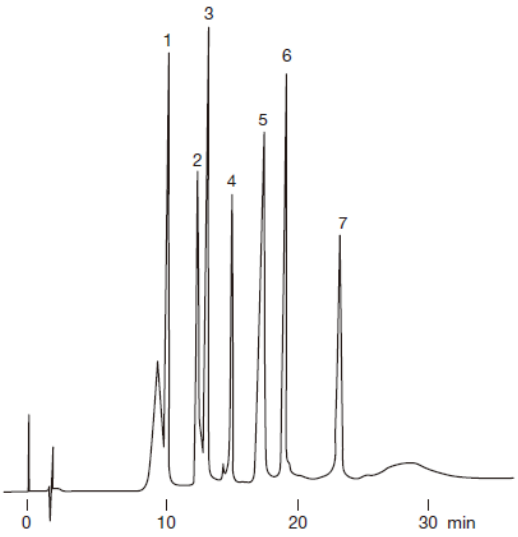
a10. Gradient analysis of proteins and peptides



Sample	MW	Recovery (%)
1. L-Bradykinin	1188	97
2. Bradykinin	1060	92
3. M-Enkephalin	574	97
4. Neurotensin	1073	99
5. L-Enkephalin	556	100
6. Substance P	1348	93
7. Bacitracin	1450	81
8. Insulin	5750	95
9. Insulin B Chain	3496	91
10. Lysozyme	14300	69
11. Mastoparan	1479	96
12. Myoglobin	17500	83

Column : **Asahipak ODP-50 6D**
Eluent : (A); 0.05 % TFA aq./CH_3CN=80/20
(B); 0.05 % TFA aq./CH_3CN=50/50
Linear gradient; (A) to (B), 20 min
Flow rate : 1.0 mL/min
Detector : UV (220 nm)
Column temp. : 30 ℃

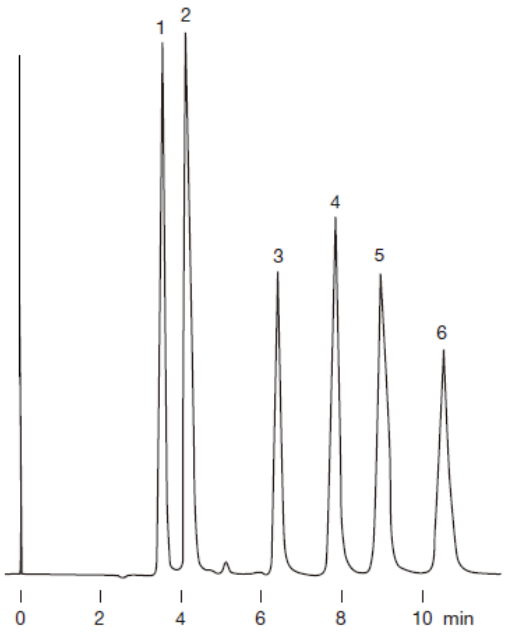
a11. Separation and recovery of standard proteins



Recovery (%)	
1. Ribonuclease	93
2. Insulin	98
3. Cytochrome C	100
4. Lysozyme	100
5. BSA	98
6. Myoglobin	108
7. Ovalbumin	-

Column : **RSpak RP18-415**
Eluent : (A); 0.1 % TFA aq./CH_3CN=99/1
(B); 0.1 % TFA aq./CH_3CN=5/95
Linear gradient; (B %) 20 % to 60 %, 20 min
Flow rate : 1.0 mL/min
Detector : UV (220 nm)
Column temp. : Room temp.

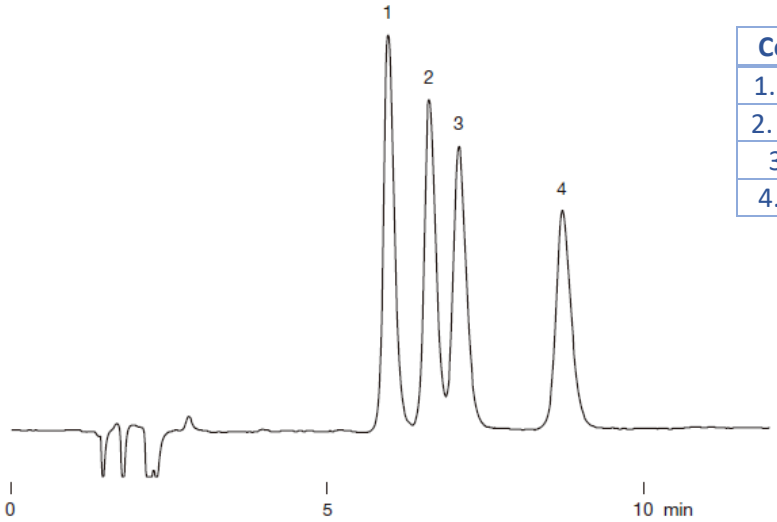
a12. Analysis of alkylbenzenes



Compound	Concentration (%)
1. m-Cresol	0.1
2. 2,4-xylenol	0.1
3. Benzene	0.5
4. Toluene	0.5
5. Ethylbenzene	0.5
6. n-propylbenzene	108

Column : **RSpak DS-613**
Eluent : $H_2O/CH_3CN/THF=30/40/30$
Flow rate : 1.0 mL/min
Detector : UV (254 nm)
Column temp. : 40 ℃

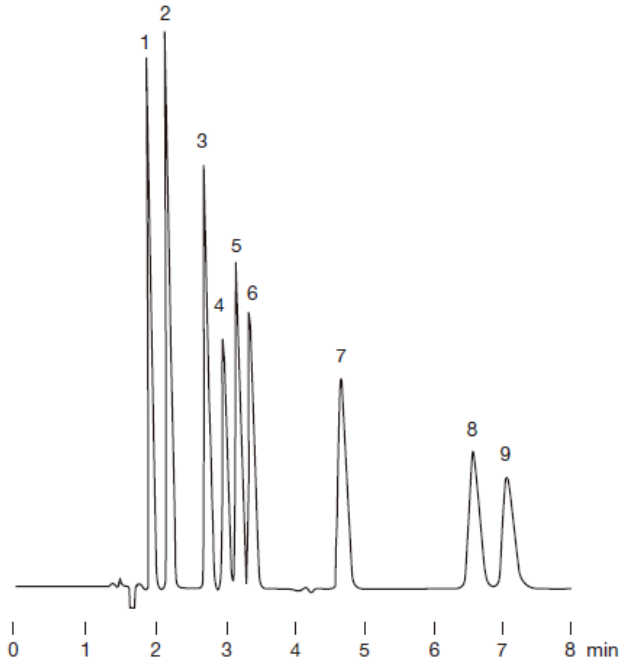
a13. Analysis of Fatty Acid Methyl Esters



Compound	Concentration (%)
1. Methyl Lineolate	0.2
2. Methyl Palmitate	0.2
3. Methyl Oleate	0.2
4. Methyl Stearate	0.2

Column : **RSpak DS-413**
Eluent : $H_2O/CH_3CN/THF=25/45/30$
Flow rate : 1.0 mL/min
Detetor : RI
Column temp. : 40 ℃

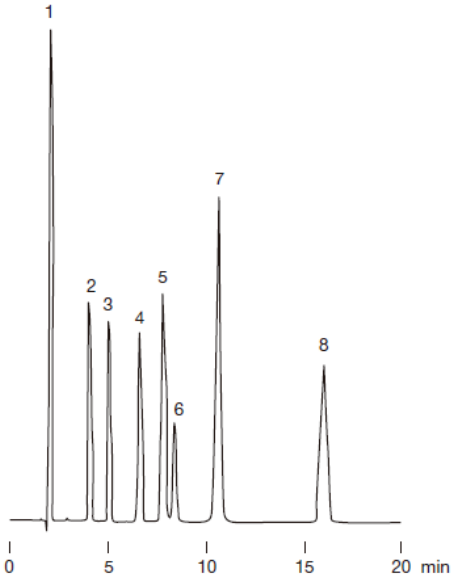
a14. Analysis of Organic Acids



Compound	Concentration
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

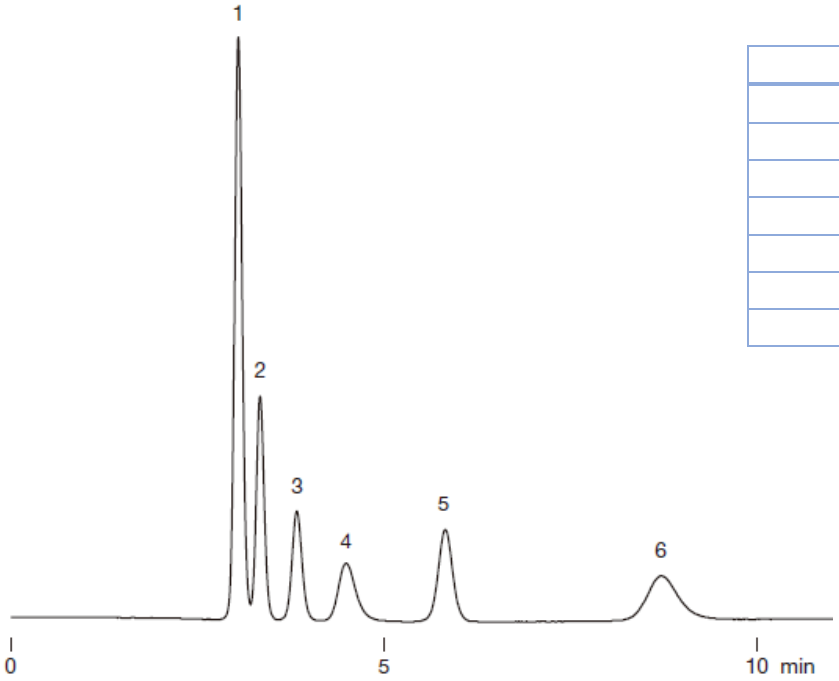
Column : **RSpak DE-413**
Eluent : 10 mM H_3PO_4 aq.
Flow rate : 1.0 mL/min
Detector : RI
Column temp. : 50 ℃

a15. Analysis of Food Additives (Preservatives)



Column : **RSpak DE-413**
Eluent : 50 mM KH_2PO_4 + 0.1 % H_3PO_4 aq./CH_3CN =65/35
Flow rate : 1.0 mL/min
Detector : UV (210 nm)
Column temp. : 40 ℃
Sample : 10 µL

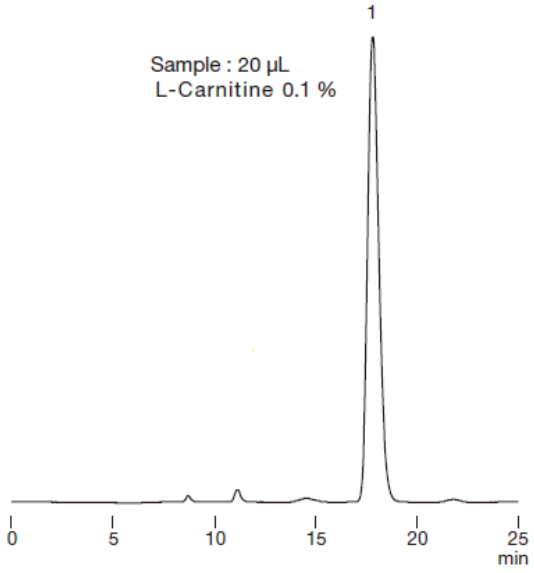
a16. Analysis of Vitamins



Compounds
Sample: 0.1%, 20µL
1. Aspartic Acid
2. Glycine
3. Alanine
4. Valine
5. Methionine
6. Isoleucine

Column : **RSpak DM-614**
Eluent : 0.055 M Na_2HPO_4 + 0.045 M KH_2PO_4 aq.
Flow rate : 1.0 mL/min
Detector : UV (254 nm)
Column temp. : 30 ℃

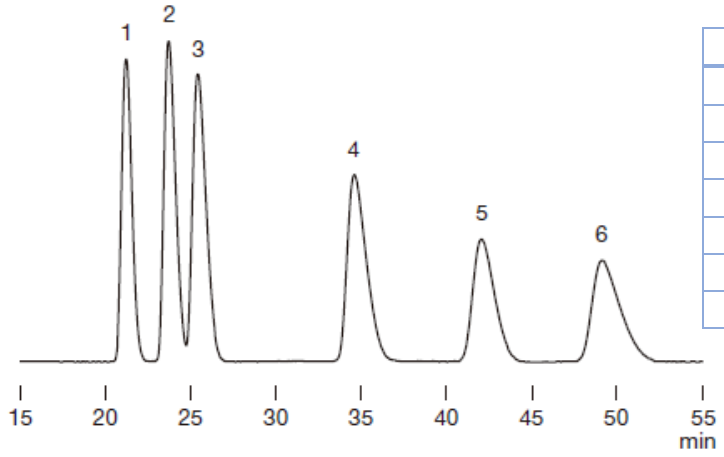
a17. Analysis of Carnitine



Compounds
Sample: 0.1%, 20µL
1. L-Carnitine

Column : **RSpak NN-814**
Eluent : 0.1 M H_3PO_4 aq.
Flow rate : 1.0 mL/min
Detector : UV (210 nm)
Column temp. : 25 ℃

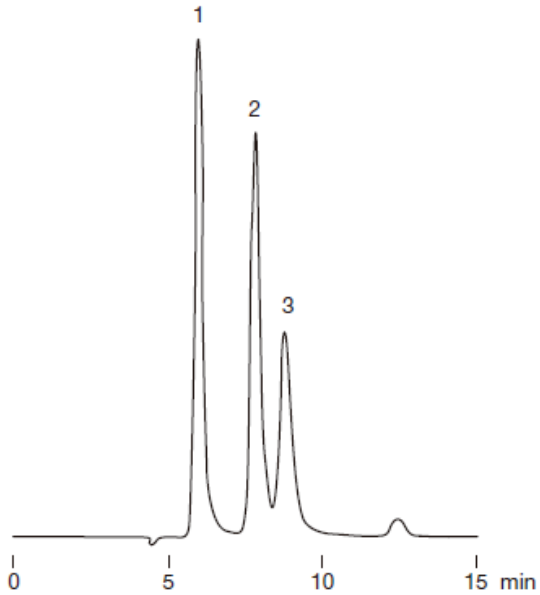
a18. Analysis of Amino Acids



Compounds
Sample: 0.1%, 20µL
1. Aspartic Acid
2. Glycine
3. Alanine
4. Valine
5. Methionine
6. Isoleucine

Column : **RSpak NN-814**
Eluent : 40 mM H_3PO_4 aq.
Flow rate : 1.0 mL/min
Detector : RI
Column temp. : 40 ℃

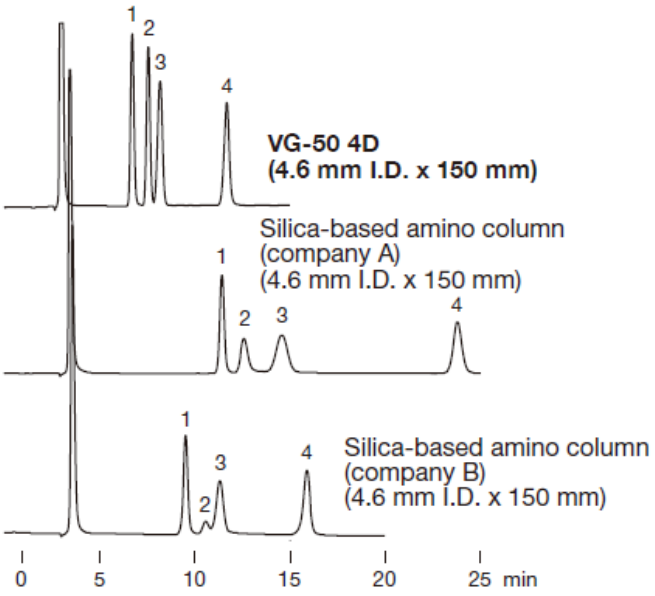
a19. Analysis of Amino Acids



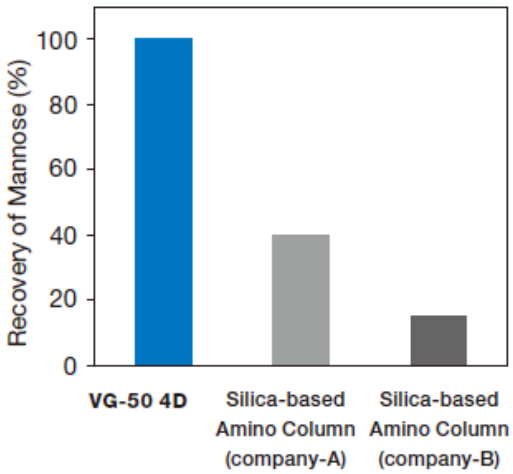
Compounds
Sample: 100mg/L, 20µL
1. n-Phenylenediamine
2. m-Phenylenediamine
3. o-Phenylenediamine

Column : **RSpak JJ-50 4D**
Eluent : 25 mM Ammonium acetate buffer (pH 9.2)/CH_3CN=70/30
Flow rate : 0.4 mL/min
Detector : UV (254 nm)
Column temp. : 30 ℃

a20. Recovery of Sugars



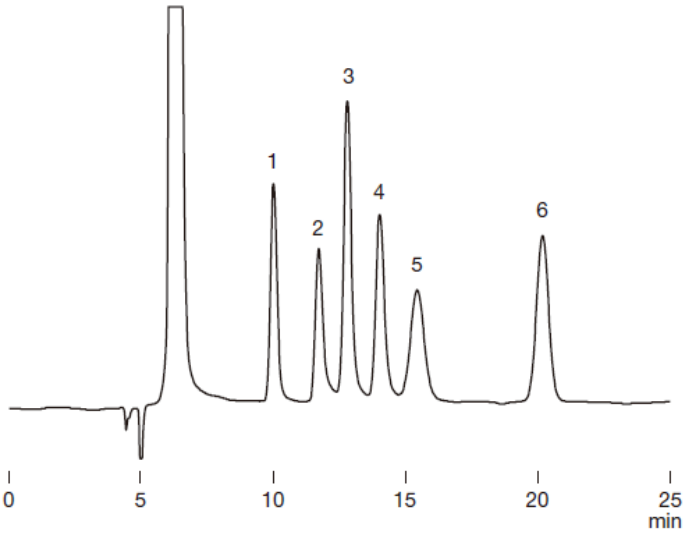
Compounds
Sample: 5mg/mL, 5µL
1. Fructose
2. Mannose
3. Glucose
4. Sucrose



Column : **HILICpak VG-50 4D**
Silica based amino columns from other manufacturers
Eluent : H_2O/CH_3CN=20/80
Flow rate : 0.6 mL/min (VG-50 4D)
1.0 mL/min (Silica based amino column)
Detector : RI
Column temp. : 40 ˚C

- When an amino column is used for analysing saccharides, the recovery ratio of reducing saccharides such as mannose, arabinose or xylose is low.
- HILICpak VG-50 is an amino column with improved saccharide recovery ratios, which results in enhanced sensitivity of results.

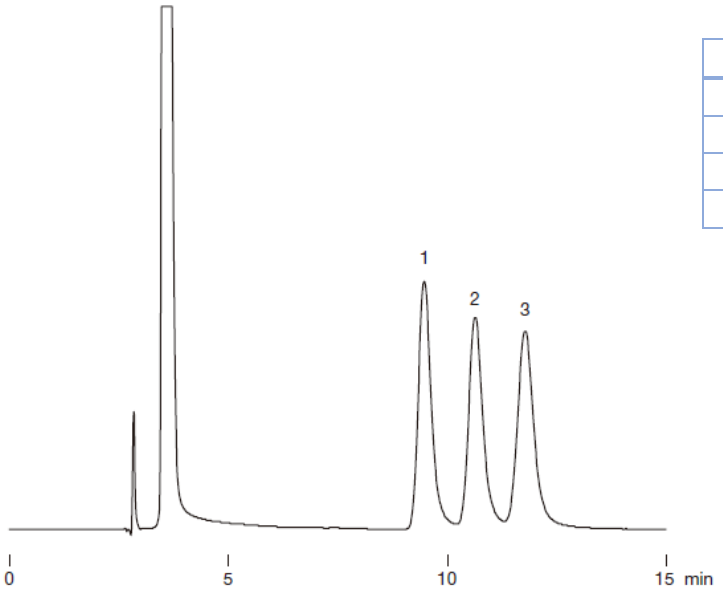
a21. Analysis of Sugars



Compounds
Sample: 0.2%, 10µL
1. L-Ribose
2. D-Psicose
3. D-Xylitol
4. D-Tagatose
5. D-Allose
6. L-Glucose

Column : **HILICpak VG-50 4E**
Eluent : $H_2O/CH_3CN/CH_3OH$=5/85/10
Flow rate : 0.6 mL/min
Detector : RI
Column temp. : 50 ˚C

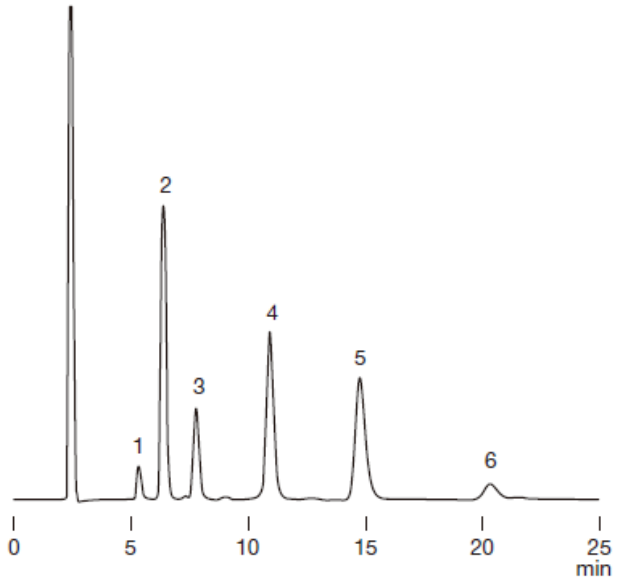
a22. Analysis of Lactose, Epilactose and Lactulose



Compounds
Sample: 5mg/mL, 5µL
1. Lactulose
2. Epilactose
3. Lactose

Column : **HILICpak VG-50 4E**
Eluent : $H_2O/CH_3CN/CH_3OH=5/75/20$
Flow rate : 1.0 mL/min
Detector : RI
Column temp. : 40 ℃

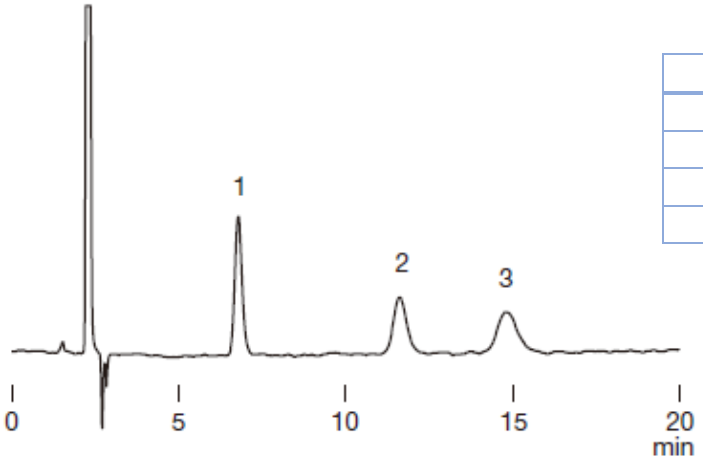
a23. Analysis of Fructo-Oligosaccharide Syrup



Compounds
Sample: 2.5%, 20µL
1. Fructose
2. Glucose
3. Sucrose
4. 1-Kestose
5. Nystose
6. 1-Fructofuranosyl-D-nystose

Column : **Asahipak NH2P-50 4E**
Eluent : $H_2O/CH_3CN=30/70$
Flow rate : 1.0 mL/min
Detector : RI
Column temp. : 25 ℃

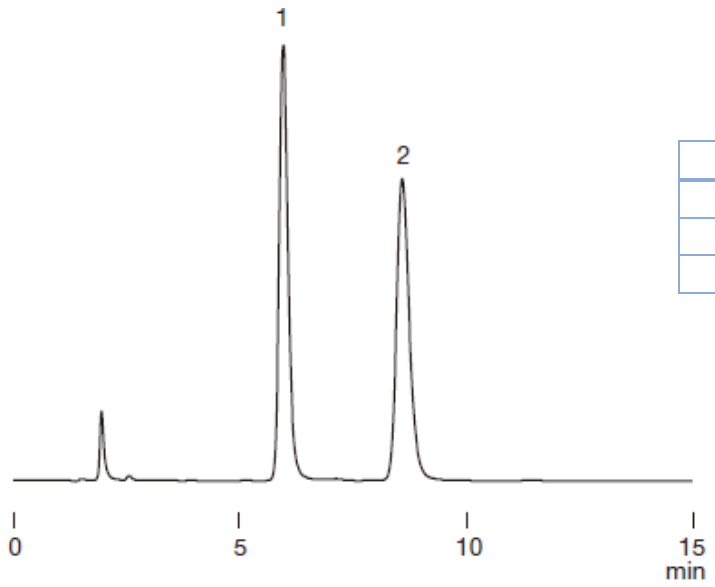
a24. Analysis of Cyclodextrins



Compounds
Sample: 250µg, 20µL
1. α-Cyclodextrin
2. γ-Cyclodextrin
3. β-Cyclodextrin

Column : Asahipak NH2P-50 4E
Eluent : H_2O/CH_3CN=40/60
Flow rate : 1.0 mL/min
Detector : RI
Column temp. : 40 ℃

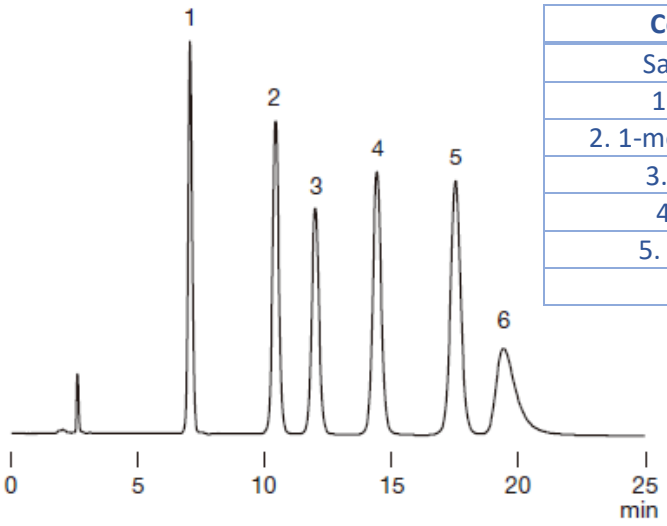
a25. Analysis of Stevioside and Rebaudioside A



Compounds
Sample: 0.05%, 20µL
1. Stevioside
2. Rebaudioside A

Column : Asahipak NH2P-50 4E
Eluent : H_2O/CH_3CN=25/75
Flow rate : 1.0 mL/min
Detector : UV (210 nm)
Column temp. : 30 ℃

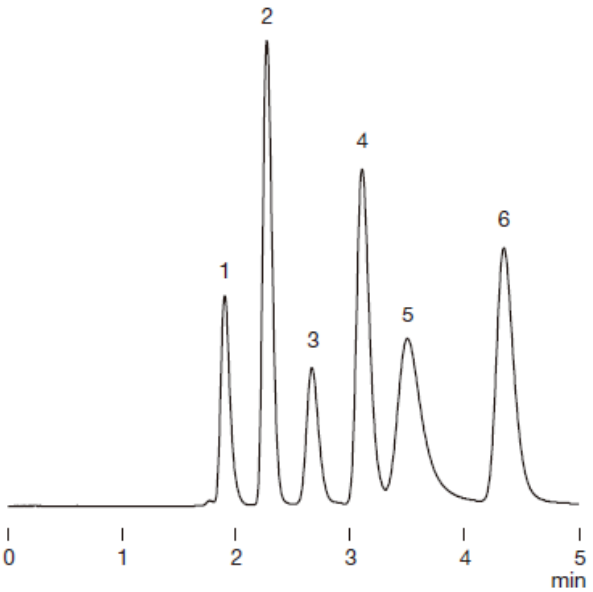
a26. Analysis of Imidazole Dipeptides



Compounds	Concentration
Sample: 20µL	-
1. β-Alanine	200µg/mL
2. 1-methyl-L-histidine	2µg/mL
3. L-Anserine	5µg/mL
4. Histidine	5µg/mL
5. L-Carnosine	5µg/mL
6. Nitrate	-

Column : **Asahipak NH2P-50 4E**
Eluent : 50 mM NaH_2PO_4 aq./CH_3CN=40/60
Flow rate : 1.0 mL/min
Detector : UV (210 nm)
Column temp. : 40 ˚C

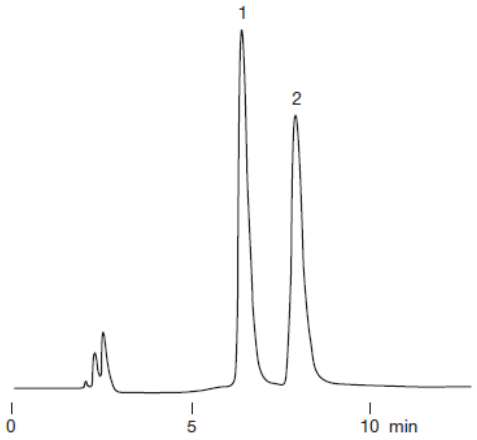
a27. Analysis of Water Soluble Vitamins



Compounds	Concentration
Sample: 20µL	-
1. Vitamin B_6	50µg/mL
2. Nicotinamide	10µg/mL
3. Vitamin B_{12}	10µg/mL
4. Nicotinic Acid	10µg/mL
5. Folic Acid	10µg/mL
6. Vitamin C	10µg/mL

Column : **Asahipak NH2P-50 4E**
Eluent : 40 mM H_3PO_4 aq./CH_3CN=45/55
Flow rate : 1.0 mL/min
Detector : UV (254 nm)
Column temp. : 40 ˚C

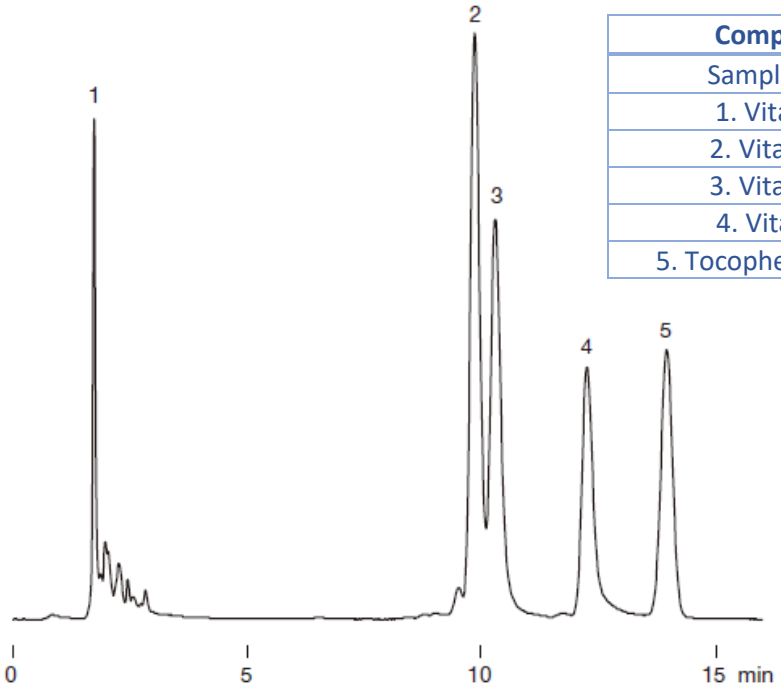
a28. Analysis of Ascorbic Acid and Erythorbic Acid



Compounds
Sample: 5µg/mL, 10µL
1. Erythorbic Acid
2. Ascorbic Acid

Column : **Asahipak NH2P-50 4E**
Eluent : 20 mM NaH_2PO_4 + 30 mM H_3PO_4 aq. /CH_3CN=20/80
Flow rate : 1.0 mL/min
Detector : UV (254 nm)
Column temp. : 30 ˚C

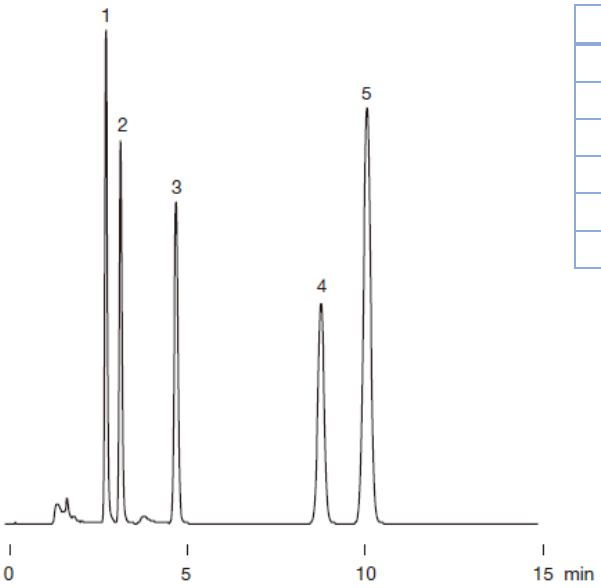
a29. Analysis of Fat Soluble Vitamins



Compounds	Concentration
Sample: 20µL	-
1. Vitamin A	50µg/mL
2. Vitamin D_2	25µg/mL
3. Vitamin D_3	25µg/mL
4. Vitamin E	100µg/mL
5. Tocopherol Acetate	100µg/mL

Column : **Silica C18-4D**
Eluent : CH_3CN
Flow rate : 1.0 mL/min
Detector : UV (280 nm)
Column temp. : 40 ˚C

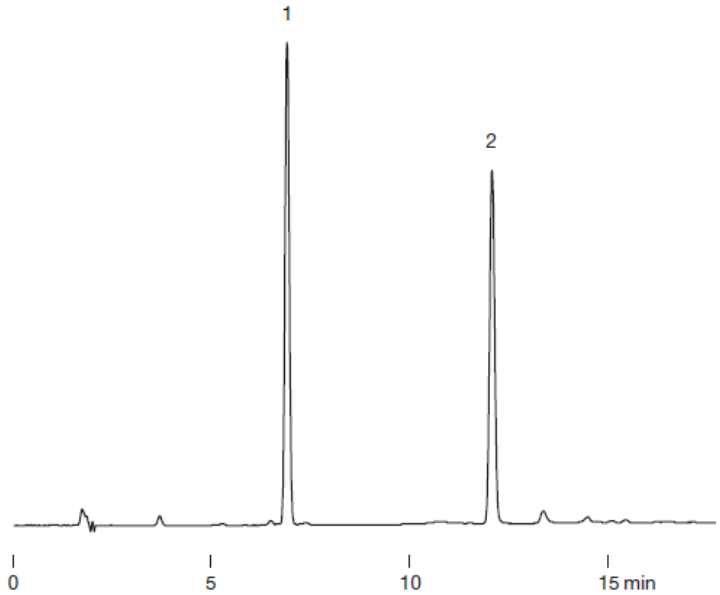
a30. Analysis of Anticonvulsants



Compounds	Concentration
Sample: 20µL	-
1. Ethosuximide	100µg/mL
2. Phenytoin	10µg/mL
3. Phenobarbital	10µg/mL
4. Primidone	10µg/mL
5. Carbamazepine	10µg/mL

Column : **Silica C18-4D**
Eluent : 100 mM Phosphate buffer (pH 2.1) /CH_3OH/CH_3CN=4/2/1
Flow rate : 1.0 mL/min
Detector : UV (210 nm)
Column temp. : 40 ˚C

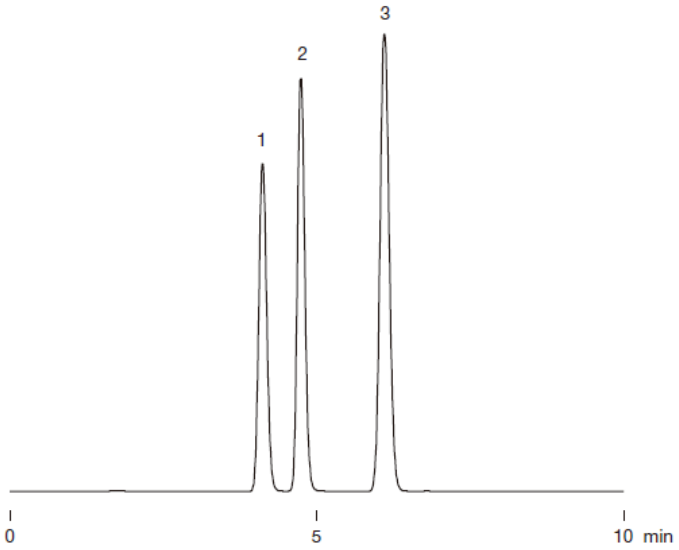
a31. Analysis of Gingerol and Shogaol



Compounds
Sample: 0.1mg/mL, 10µL
1. 6-Gingerol
2. 6-Shogaol

Column : **Silica C18-4D**
Eluent : (A) ; H_2O/(B) ; CH_3CN
Linear gradient : (B %) 40 % to 70 % (15 min)
Flow rate : 1.0 mL/min
Detector : UV (280 nm)
Column temp. : 40 ˚C

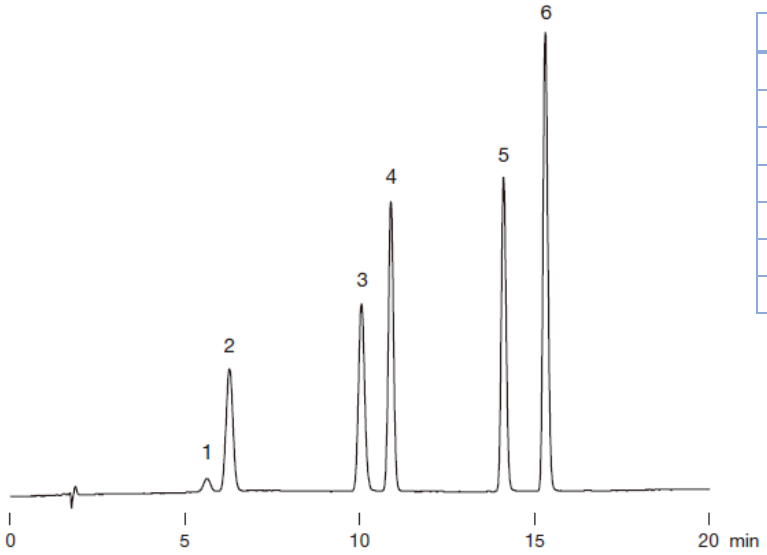
a32. Analysis of Chlorogenic Acid, Caffeine and Caffeic Acid



Compounds
Sample: 0.1mg/mL, 10µL
1. Chlorogenic Acid
2. Caffeine
3. Caffeic Acid

Column : **Silica C18M-4D**
Eluent : 20 mM H_3PO_4 aq. /CH_3OH=70/30
Flow rate : 1.0 mL/min
Detector : UV (280 nm)
Column temp. : 30 ˚C

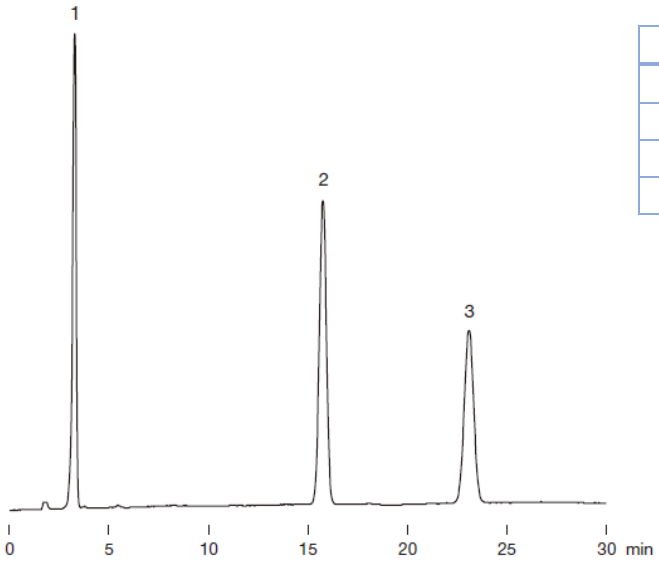
a33. Analysis of Catechins



Compounds
Sample: 10µg/mL, 10µL
1. Epigallocatechin
2. Catechin
3. Epigallocatechin Gallate
4. Epicatechin
5. Epicatechin Gallate
6. Catechin Gallate

Column : **Silica C18P-4D**
Eluent : (A) ; 20 mM H_3PO_4 aq./(B) ; CH_3CN
Linear gradient:
(B %) 20 % (0 to 5 min), 20 to 40 % (5 to 15 min),
40 % (15 to 20 min)
Flow rate : 1.0 mL/min
Detector : UV (280 nm)
Column temp. : 30 ˚C

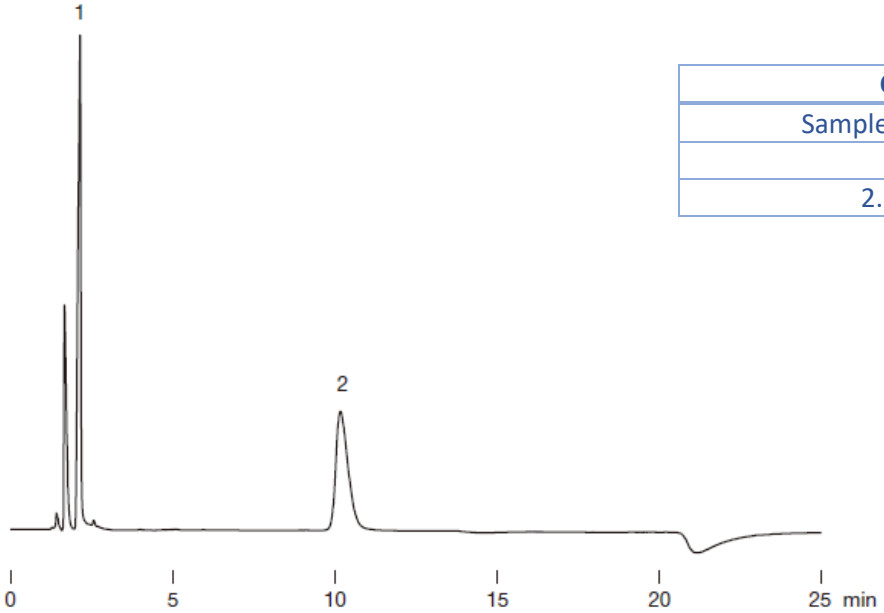
a34. Analysis of Estrogens



Compounds
Sample: 0.1mg/mL, 10µL
1. Estriol
2. 17β-Estradiol
3. Estrone

Column : **Silica C18P-4D**
Eluent : H_2O/CH_3CN=65/35
Flow rate : 1.0 mL/min
Detector : UV (280 nm)
Column temp. : 30 ˚C

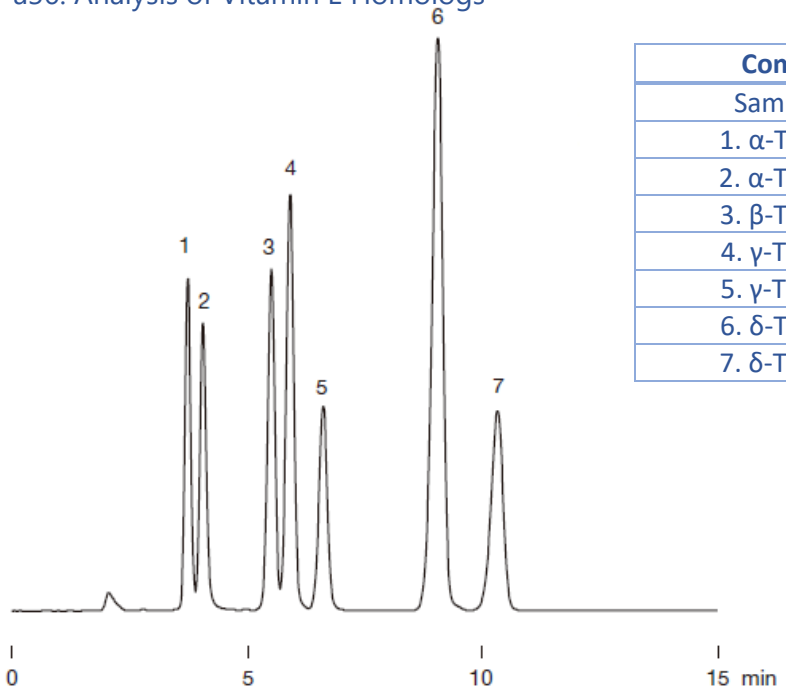
a35. Analysis of Glucosamine



Compounds
Sample: 3.8mg/mL, 10µL
1. Cl^-
2. Glucosamine

Column : **Silica 5NH 4D**
Eluent : *Buffer (pH 7.5)/CH_3CN=30/70
*Buffer ; in a 1-L volumetric flask, dissolve 3.5 g K_2HPO_4 in water. Add 0.25 mL Ammonium hydroxide (25 %), dilute with water to volume, and mix. Adjusted with H_3PO_4 to a pH 7.5
Flow rate : 1.1 mL/min
Detector : UV (195 nm)
Column temp. : 35 ˚C

a36. Analysis of Vitamin E Homologs



Compounds	Concentration
Sample: 20µL	-
1. α-Tocopherol	5µg/mL
2. α-Tocotrienol	10µg/mL
3. β-Tocopherol	5µg/mL
4. γ-Tocopherol	5µg/mL
5. γ-Tocotrienol	10µg/mL
6. δ-Tocopherol	5µg/mL
7. δ-Tocotrienol	10µg/mL

Column : **Silica 5SIL 4D**
Eluent : n-Hexane/Isopropanol/Acetic acid=1000/6/5
Flow rate : 1.0 mL/min
Detector : Fluorescence (Ex. : 298 nm, Em. : 325 nm)
Column temp. : 30 ˚C

SCION Instruments

UK

Livingston Business Centre

Kirkton South Road, Livingston

West Lothian EH54 7FA

Scotland, UK

Phone +44 (0)1506 300 200

sales-eu@scioninstruments.com

The Netherlands

Amundsenweg 22-24

4462 GP Goes,

The Netherlands

Phone +31 (0)113 287 600

sales-eu@scioninstruments.com