nacalai tesque
The quality for certainty.

COSMOSIL
COSMOGEL

High Performance Liquid Chromatography

CORPORATE PROFILE

NACALAI TESQUE, Inc. is committed to the well-being of all humanity through enhancing the research capabilities of the worldwide scientific community. We strive to connect countries through long-term business relationships based on trust and friendship.

The name Nacalai carries with it hard earned respect and tradition in the Japanese scientific community. In the ancient time when Kyoto was the capital of Japan, the ancestors of Nakarai Takatoshi, our present-day president supplied traditional Japanese and Chinese medicines to the Imperial Family. The documented history of our company began in 1846 when the company's founder opened Nakarai Mansuke Shoten, Ltd. Our more than 160 year-long presence in Kyoto, in walking distance from the preserved Imperial Household grounds is a place of honor and prestige in Japan. In 1958 the company's reagent department spun off and became Nakarai Chemicals, Inc. In a move to propel the company to the future the name was changed to Nacalai Tesque, Inc. in 1988.

Centering around research chemicals, the active fields of Nacalai Tesque include fine chemicals, diagnostics, consumables and kits for biotechnology, and related laboratory equipments and supplies. We import and distribute to Japanese research laboratories, universities, food and beverage industry and pharmaceutical companies commodities from the top biotechnology manufacturers of the world and export our own products.

Nacalai Tesque established its own Nacalai Research Institute as part of our long-term strategy to keep us at the frontier of science. With the technology and expertise that developed "TANAKA Plot" known as a column evaluation method, our highly qualified researchers are continuously inventing new products and steadily improving our conventional commodities. These constant efforts and great achievements are peaked in our HPLC packing materials and columns. We are proud of the excellence of our HPLC columns in accuracy, endurance, reasonable price and strong technical support throughout. We packed in our HPLC columns all of our expertise, the best available materials and innovations, culminating in our specialty columns.

Nacalai Tesque has realized that the company's future depends on the complete satisfaction of our precious customers. We are keen to provide you with the best service and support and hope that you kindly review the content of this catalogue to get acquainted with our products. Please contact us with any inquiries. Your comments will be highly appreciated.

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Ⅱ LIQUID CHROMATOGRAPHY RELATED PRODUCTS

Ⅲ TECHNICAL NOTE

Ⅳ APPLICATION DATA

General Information

General Ordering Information

When placing an order with us or making an enquiry, please contact our International Business Development Group or your local distributor. Please clearly identify the product in question when submitting your enquiry.

The speed of innovation is accelerating. We always have brand new or improved columns not listed here. There are also many other products Nacalai Tesque can supply. Therefore we urge you to make enquiries.

Product Description and Availability

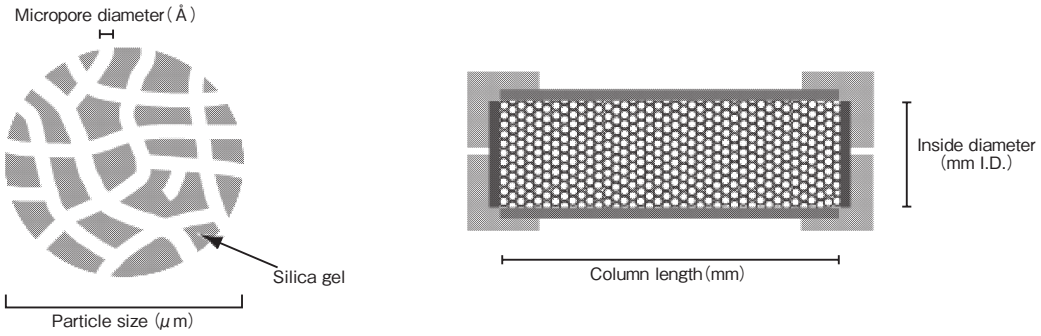
Please note that the product specifications are subjected to changes and the manufacturing of some product may be stopped. Please consult the table on page 13 for cross-reference information on old products and their newer and better equivalents.

Column Identification

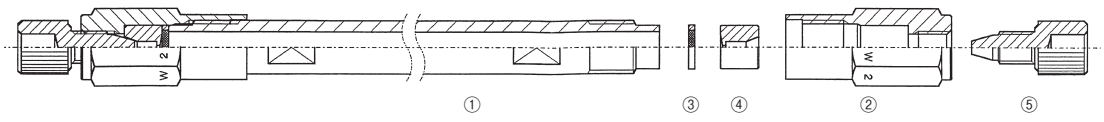
At the end of each section, the COSMOSIL and COSMOGEL packed columns are listed in a way that the particle size, stationary phase, column size of the packing material can be easily determined.

37913-81 (1) COSMOSIL (2) 5 (3) C_{18}-MS-Ⅱ (4) 4.6 mm I.D. × 150 mm (5) Waters (6)

When placing an order, please clearly indicate the product number (1), product name (2), particle size (3), type of stationary phase (4), column size (5) and the end fitting type (6).



The Structure of the Screw-in Type Columns



①Screw pipe
②Column End
③Filter
④Fitting Piece
⑤Plug

End-fitting Types

Columns in the recent COSMOSIL line-up are shipped with Waters type end fitting. Upon request different end fitting types may be available. Please enquire!

Warranty Claims

The manufacturer will replace defective columns if notified within 2 weeks of receipt of the product by the customer under the following conditions:

1) Column abnormalities are due to accidents in shipping or rough handling.
2) The number of effective plates of the column is considerably lower than the minimum guaranteed theoretical plate number documented in the inspection report that accompanies each column.

Please contact the International Business Development Group of Nacalai Tesque or your local distributor for additional information.

Terms and Conditions of Sale

Terms are subject to conditions set forth by the authorized Nacalai Tesque dealers in each country.

Not for Clinical Use

Nacalai Tesque products are not intended for clinical use. While clinical applications may be shown, these products are not validated for clinical use.

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COSMOSIL HPLC COLUMN

1. COSMOSIL / COSMOGEL
2. Reversed Phase Chromatography
   Octadecyl types
3. Reversed Phase Chromatography
   Specialty columns
4. Reversed Phase Chromatography
   Alkyl chains columns
5. Reversed Phase Chromatography
   Phenyl types・Cyano types
6. Silica Based Preparative Columns
7. Normal Phase Chromatography
8. Hydrophilic Interaction Chromatography
9. Saccharide Analysis
10. Protein Separation Wide Pore Columns
11. Specialty Columns for Fullerene

1. COSMOSIL / COSMOGEL

General description of the COSMOSIL / COSMOGEL packing materials

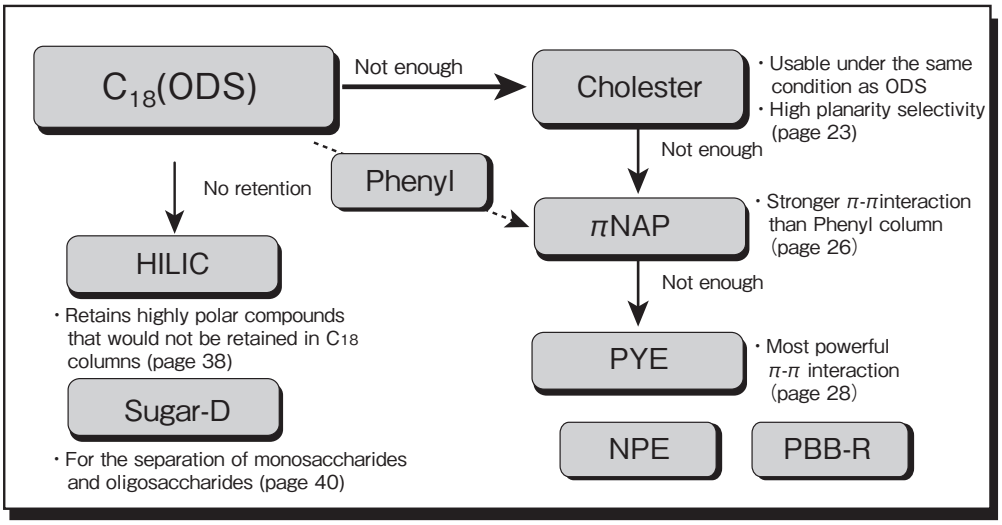
Sample	Separation mode	Packing material	Stationary phase	Special features and applications	Page
Organic compounds (low M.W.)	Reversed phase	C_{18}-MS-Ⅱ	Octadecyl group	Multi-purpose C_{18} column. Monofunctional silylation on ultra-pure silica gel for separation of the widest range of compounds	16
		C_{18}-AR-Ⅱ		Multi-purpose C_{18} column using ultra-pure silica gel. Features strong acid resistance and suitable for a wide range of separation	18
		C_{18}-PAQ		Reversed phase column, compatible with 100% water based mobile phases	20
		Cholester	Cholesteryl group	Usable under the same condition as C_{18} Unique rigid cholesteryl structure improves separation	23
		πNAP	Naphthylethyl group	Stronger π-π interaction than Phenyl column	26
		PYE	Pyrenylethyl group	Most powerful π-π interaction	28
		NPE	Nitrophenylethyl group	Separation utilizing π-π interaction and Dipole-dipole interaction	30
		PBB-R	Pentabromobenzyl group	Separation utilizing dispersion force	31
		C_{22}-AR-Ⅱ	Docosyl group	Alkyl chain columns except C_{18} column	32
		C_8-MS	Octyl group		
		C_4-MS	Butyl group		
		TMS-MS	Trimethyl group		
		PE-MS	Phenylethyl group	π-π interaction	34
		CN-MS	Cyanopropyl group		
	Normal phase	SL-Ⅱ		Normal Phase chromatography with non-polar organic solvents	36
	Hydrophilic interaction	HILIC	Triazole	Retains highly polar compounds that would not be retained in C_{18} column	38
Monosaccharide Oligosaccharide	Hydrophilic interaction	Sugar-D	Secondary/Tertiary amine	A novel stationary phase for saccharide separation. Extended column life and increased stability. Alternative to aminopropyl type	40
		NH_2-MS	Aminopropyl group	Primary amine bonded column	43
Proteins	Reversed phase	Protein-R	Octadecyl group	The most suitable reverse phased column for proteins	44
		C_{18}-AR-300	Octadecyl group	Wide pore type reversed phase columns with high acid resistance recommended for the separation of proteins, polypeptides, nucleic acids and other large molecules	46
		C_8-AR-300	Octyl group		
		C_4-AR-300	Butyl group		
		Ph-AR-300	Phenyl group		
	Gel permeation	Diol-120-Ⅱ	Diol group	Silica-based gel filtration column for high speed separation of proteins and water soluble polymer	48
		Diol-300-Ⅱ			
	Ion-exchange	DEAE	Diethylaminoethyl type	Weak anion-exchange	50
		QA	Quarternary ammonium type	Strong anion-exchange	
		CM	Carboxymethyl type	Weak cation-exchange	
		SP	Sulfopropyl type	Strong cation-exchange	
	Hydrophobic interaction	HIC	＊＊	Hydrophobic interaction charomatography column for protein separation	53
Fullerenes	＊＊	Buckyprep	Pyrenylpropyl group	Standard column for fullerenes separation	56
		Buckyprep-M	Phenothiazinyl group	Designed to separate metallofullerenes	57
		PBB	Pentabromobenzyl group	Designed for the preparative separation of fullerenes using carbon disulphide, *o*-dichlorobenzene and toluene	58
		PYE	Pyrenylethyl group	Separation of Fullerene and structural isomer	59
		NPE	Nitrophenylethyl group	Separation of derivatived fullerene	

Column Selection Guide

● Organic compounds (Low M.W.)

Octadecyl group bonded column (C_{18},ODS) are recommended as first-choice columns for separations of organic compounds (Low M.W.). If there is not enough separation or no retention using COSMOSIL C_{18} columns, COSMOSIL series offer many kinds of specialty columns.

Selection guide



For C_{18}-series selection guide, please refer to page 15.

● Saccharides

・COSMOSIL Sugar-D is recommended for the separation of monosaccharides and oligosaccharides as a first-choice column.

・For the separation of sugar derivatives, COSMOSIL C_{18}-PAQ is suitable as well.

● Proteins

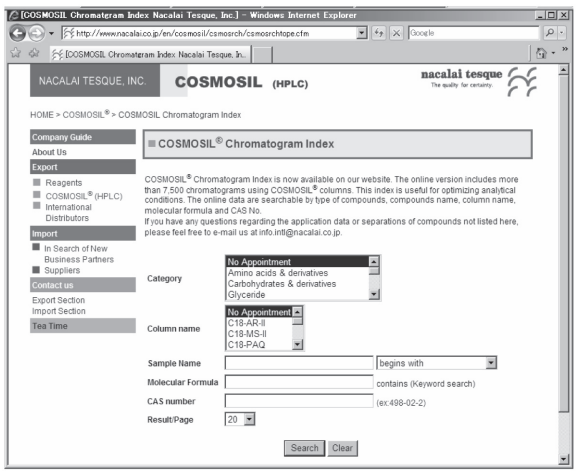
・Please select based on the separation mode.

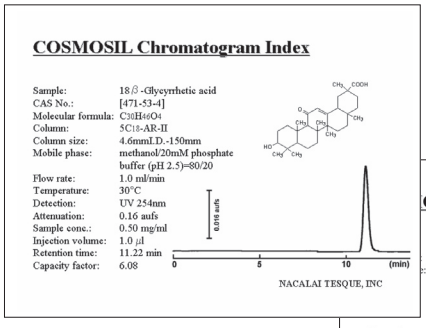
● Fullerenes

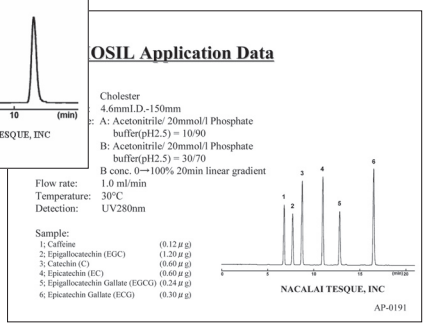
・COSMOSIL Buckyprep is most suitable for the separation of fullerenes.

COSMOSIL Chromatogram Index/Application Data

COSMOSIL Chromatogram Index and COSMOSIL Application Data are now available on our website (website : http://www.nacalai.com). The online version includes more than 5,700 chromatograms and more than 1,000 application data using COSMOSIL columns. These indexes are useful for optimizing analytical conditions. If you have any questions regarding the application data or separations of compounds not listed here, please feel free to e-mail us at info.intl@nacalai.co.jp.







COSMOSIL Silica Packing Material

Introduction

Superior HPLC columns can be produced only with excellent packing materials and superb packing technique. COSMOSIL columns are well known for their high efficiency and high-resolution separations. Based on spherical, totally porous silica, COSMOSIL columns provide enhanced chemical and mechanical stability as well as very high surface coverage.

The selection of the C_{18} chemistries available enables the chromatographer to tailor separation to special applications. The ultra pure silica based MS-Ⅱ series with widely extended pH range are developed for improved separation of basic compounds. The C_{18} AR-Ⅱ phase provides increased acid resistance.

Four unique bonded chemistries are available for Cosmosil specialty columns: Cholester, πNAP, PYE and HILIC. These specialty columns may improve the separation compared with conventional columns.

Five highly effective phases for fullerene separation are also available: Buckyprep, Buckyprep-M, PBB, PYE and NPE.

COSMOGEL packing materials are non-silica based and provide superior performance in ion exchange columns.

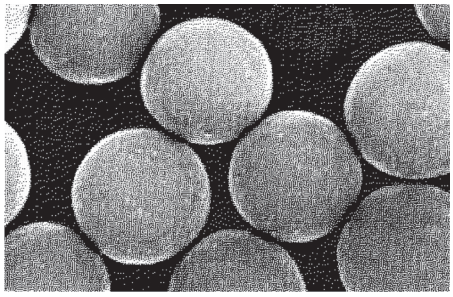
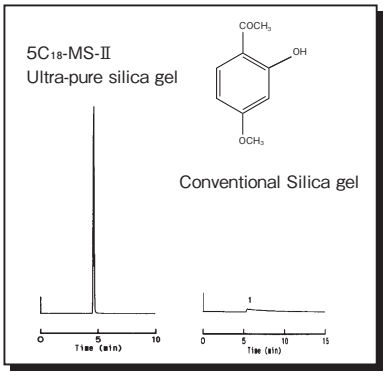


Figure. Microscopic photograph of the silica gel

Raw material silica gel

COMSOSIL is based on ultra pure porous spherical silica gel (purity: 99.99% or higher). Low-purity silica gel contains metal impurity which may cause interference in the separation, especially for metal coordination compounds.



Column size	4.6 mm I.D. × 150 mm
Mobile phase	acetonitrile : water = 50 : 50
Flow rate	1.0 ml/min
Temperature	30℃
Detection	UV 254 nm
Sample	Paeonol

Metal coordination compounds

The compounds, which have 2 or more hydroxyl groups, carboxyl groups, carbonyl groups and/or amino groups in the adjacent positions, can complex with metal impurity, which results in peak tailing.

Table. Metal content percentage (ppm)

Packing material	Al	Ca	Fe	Mg	Na	Ti	Total
Ultra pure silica gel	4	10	11	3	12	< 1	41
Normal silica gel	32	450	26	88	56	134	786

Stationary phase construction

While C_{18} columns are most widely used in reversed phase HPLC, it is important to distinguish between two very different bonded phase formats.

Monomeric type C_{18} format incorporates the bonding of the C_{18} alkyl chain to a single silica atom on the silica gel backbone. Monomeric type columns such as the COSMOSIL C_{18}-MS-Ⅱ and the MS series have excellent synthesis reproducibility, very good lot-to-lot reproducibility and short mobile phase equilibration times.

On the other hand, the polymeric C_{18} format incorporates a tri-functional silylation procedure whereby the octadecyl group is bonded to 2 or 3 silica atoms on the silica gel backbone. This increases silylation results in far greater column stability particularly in acidic mobile phase conditions. Stereo recognition capability is also greater than that of the monofunctional silylation type C_{18} columns. The polymeric format is offered in the AR-Ⅱ and the entire AR-300 series of COSMOSIL columns. Please refer to product descriptions and application chromatograms for selection guidance.

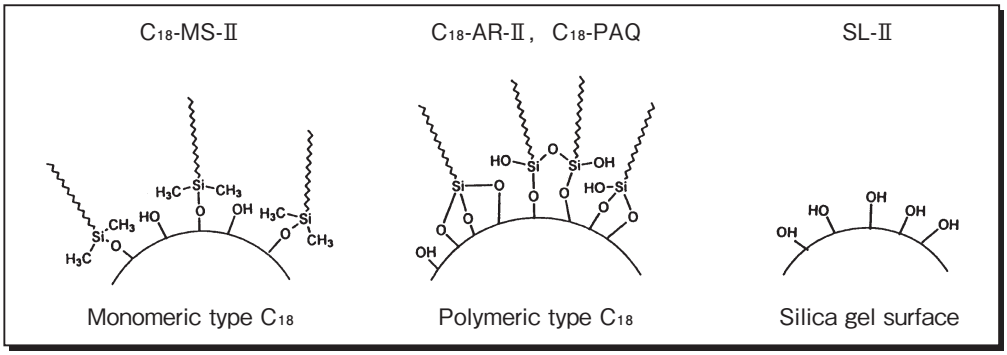
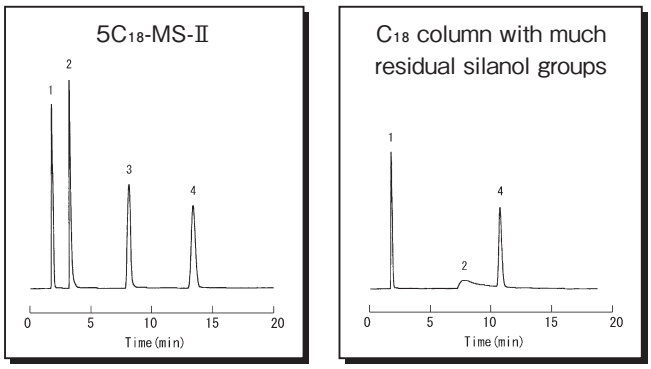


Figure. Diagrams of different stationary phase constructions (before end-capping treatment)

End-capping treatment

The silanols (Si-OH groups) on the silica surface provided bonding site for stationary phases. However, part of the silanol groups remain un-capped as residual silanol groups even after the end-capping treatment, they cause peak tailing for basic compounds. COSMOSIL packing materials for reversed phase chromatography are of near-perfectly end-capped residual silanol groups.

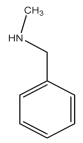


$R\text{-}NH^+$ (Basic compounds)

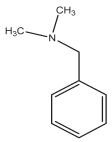
$Si\text{-}O^-$ (Free silanol group)

Basic compound can form ionic bonds with residual free silanols. The ionic bonding causes peak tailing of basic compounds if a silica-based column is not perfectly end-capped.

Column size	4.6 mm I.D. × 150 mm
Mobile phase	methanol : 20 mmol/l phosphate buffer (pH7) = 20 : 80
Flow rate	1.0 ml/min
Temperature	30℃
Detection	UV254 nm (0.16AUFS)
Sample	1. Uracil
	2. *N*-Methylbenzylamine
	3. *N,N*-Dimethylbenzylamine
	4. Benzylalcohol



2. *N*-Methyl Benzylamine

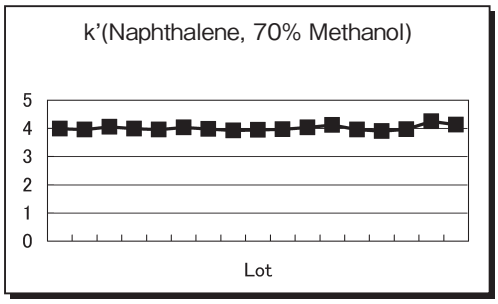


3. *N,N*-Dimethyl Benzylamine

Synthesis Reproducibility

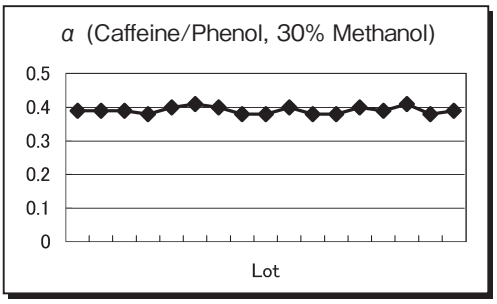
By using strictly selected silica gel and constant synthesis conditions, the chemically bonded type column retains a variance of the capacity factor (k') between synthetic lots of within ±10% and a variance of the separation factor (α) of within ± 5%.

The figures below show in graphic form the lot inspection results of synthesized packing material (COSMOSIL 5C18-MS-Ⅱ). Figure 1 shows the variance of stationary phase (octadecyl group) introduced volume which is the basic indicator of the quality of the packing material. Figure 2 shows the end-capping efficiency of the packing material. The variance among the lots is reduced to the minimum in the COSMOSIL packed columns.



k'Nap=k'value of Naphthalene in the 70% methanol.

Figure 1. Variance of the combining volume between silica gel and C18.



α C/P=k'(Caffeine)/k'(Phenol) in 30% methanol.

Figure 2. Variance of end-capping efficiency of the packing material.

Performance Guarantee

Quality guarantee of packing material (Validation)

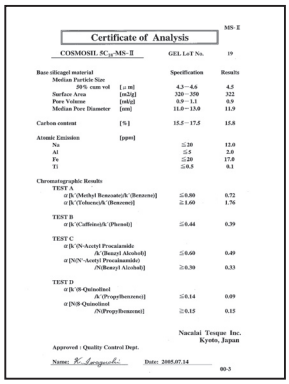
The strict quality control system of Nacalai Tesque supports the customers with an individual "Inspection Report" which accompanies each and every COSMOSIL and COSMOGEL Packed Column (except guard columns) and an additional "Certificate of Analysis" for the COSMOSIL 5C$_{18}$-MS-Ⅱ and 5C$_{18}$-AR-Ⅱ (4.6 mm I.D. × 150 mm and 4.6 mm I.D. × 250 mm).

・Validated columns

Product number	Product name	Column size
38019-81	COSMOSIL 5C$_{18}$-MS-Ⅱ	4.6 mm I.D. × 150 mm
38020-41	COSMOSIL 5C$_{18}$-MS-Ⅱ	4.6 mm I.D. × 250 mm
38144-31	COSMOSIL 5C$_{18}$-AR-Ⅱ	4.6 mm I.D. × 150 mm
38145-21	COSMOSIL 5C$_{18}$-AR-Ⅱ	4.6 mm I.D. × 250 mm

・COSMOSIL certificate of analysis

Validate terms of the physical properties of the silica gel, the carbon content, polar selectivity, hydrophobicity, silanol capacity, steric selectivity, inactive degree to basic and chelating compounds.


Certificate of Analysis

COSMOSIL 5C18-MS-II GEL LoT No. 19

Base silicagel material		Specification	Results
Median Particle Size			
50% cum vol	[μ m]	4.3 – 4.6	4.5
Surface Area	[m2/g]	320 – 350	322
Pore Volume	[ml/g]	0.9 – 1.1	0.9
Median Pore Diameter	[nm]	11.0 – 13.0	11.9
Carbon content	[%]	15.5 – 17.5	15.8
Atomic Emission	[ppm]		
Na		≦20	12.0
Al		≦5	2.0
Fe		≦20	17.0
Ti		≦0.5	0.1
Chromatographic Results			
TEST A			
α [k'(Methyl Benzoate)/k'(Benzene)]		≦0.80	0.72
β [k'(Toluene)/k'(Benzene)]		≧1.60	1.76
TEST B			
α [k'(Caffeine)/k'(Phenol)]		≦0.44	0.39
TEST C			
α [k'(N-Acetyl Procainamide /k'(Benzyl Alcohol)]		≦0.60	0.49
α [N(N'-Acetyl Procainamide) /N(Benzyl Alcohol)]		≧0.30	0.33
TEST D			
α [k'(8-Quinolinol /k'(Propylbenzene)]		≦0.14	0.09
α [N(8-Quinolinol /N(Propylbenzene)]		≧0.15	0.15

Nacalai Tesque Inc.
Kyoto, Japan

Approved : Quality Control Dept.

Name: K. Taniguchi Date: 2005.07.14

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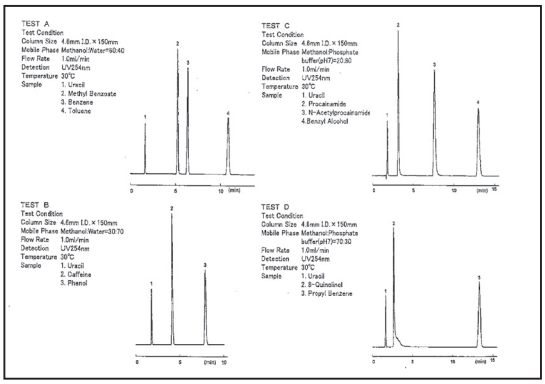


Validated terms	5C$_{18}$-MS-Ⅱ	5C$_{18}$-AR-Ⅱ
1) Basic silica gel material	Yes	Yes
2) Carbon content	Yes	Yes
3) Polar selectivity Separation factor of Methyl benzoate to Benzene	Yes	Yes
4) Hydrophobicity Separation factor of Toluene to Benzene	Yes	Yes
5) Silanol capacity Separation factor of Caffeine to Phenol	Yes	Yes
6) Steric selectivity Separation factor of Triphenylene to o-Terphenyl	No	Yes
7) Inactive degree to basic compounds Separation factor and effective plate number ratio of *N*-Acetylprocainamide to Benzylalcohol	Yes	No
8) Inactive degree to chelating compounds Separation factor and effective plate number ratio of *8*-Quinolinol to Propylbenzene	Yes	Yes

[Description of Validated terms]

1) Physical property of basic silica gel material
particle size, specific surface area, pore capacity, pore size

2) Carbon content
The bonding rate of C_{18} groups is shown. The higher it is, the higher the carbon content is.

3) Polar selectivity
The selectivity of polar group is shown.

4) Hydrophobicity
The hydrophobicity of packing material is shown. The higher the separation factor is.

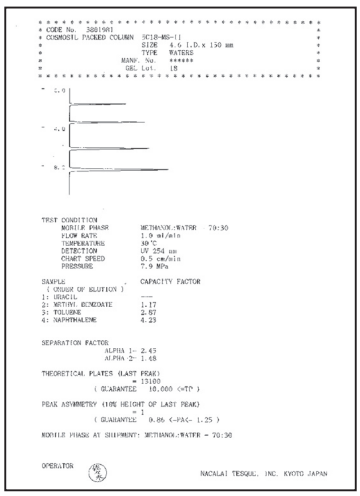
5) Silanol capacity
The rate of residual silanol on the silica surface is shown. The lower it is, the ealier the elution of caffeine.

6) Steric selectivity
Steric selectivity of the silica gel is characterized by this factor, as the bulkier is retained longer.

7) Inactive degree to basic compounds and 8) Inactive degree to chelating compounds
The lower the separation factor is and the higher the effective number ratio is, the better the basic compounds and chelating compounds are separated.

Quality guarantee of COSMOSIL packed column

Inspection report contains data of number of theoretical plates (N), peak asymmetry (s), capacity factor (k'), separation factor (α) and pressure.


CODE No. 3801501
COSMOSIL PACKED COLUMN 5C18-MS-II
SIZE 4.6 I.D. x 150 mm
TYPE WATERS
MANF. No. ******
GEL Lot. 18

TEST CONDITION
MOBILE PHASE METHANOL:WATER = 70:30
FLOW RATE 1.0 ml/min
TEMPERATURE 30°C
DETECTION UV 254 nm
CHART SPEED 0.5 cm/min
PRESSURE 7.9 MPa

SAMPLE CAPACITY FACTOR
(ORDER OF ELUTION)
1: URACIL ---
2: METHYL BENZOATE 1.17
3: TOLUENE 2.97
4: NAPHTHALENE 4.23

SEPARATION FACTOR
ALPHA 1- 2.43
ALPHA 2- 1.68

THEORETICAL PLATES (LAST PEAK)
= 13100
(GUARANTEE 10,000 <=TP)

PEAK ASYMMETRY (10% HEIGHT OF LAST PEAK)
= 1
(GUARANTEE 0.86 <=AS<= 1.25)

MOBILE PHASE AT SHIPMENT: METHANOL:WATER = 70:30

OPERATOR

NACALAI TESQUE, INC. KYOTO JAPAN

Inspection report

Conventional Columns versus High Performance Columns

A period of more than 27 years has passed since the first COSMOSIL $5C_{18}$ columns were developed and offered for sale. Continuous technical improvement has made many of these columns obsolete and not of the highest quality and performance available any more. However, many long-term users continue to employ these older conventional columns for routine analysis and quality control. Nevertheless, the manufacture of these older columns will eventually cease and we strongly urge customers to replace the conventional columns with their higher performance equivalents outlined in the table below. For additional information, contact the manufacturer or your local distributor directly.

Conventional columns (old)	High performance columns (new)
$5C_{18}$-AR	$5C_{18}$-AR-Ⅱ
$5C_{18}$	$5C_{18}$-MS-Ⅱ
$5C_{18}$-MS	$5C_{18}$-MS-Ⅱ
$5C_{18}$-P	$5C_{18}$-PAQ
$5C_{18}$-P-MS	$5C_{18}$-PAQ
$5C_8$	$5C_8$-MS
5TMS	5TMS-MS
5PE	5PE-MS
5CN-R	5CN-MS
$5NH_2$	$5NH_2$-MS
$5C_{18}$-300	$5C_{18}$-AR-300
$5C_8$-300	$5C_8$-AR-300
$5C_4$-300	$5C_4$-AR-300
5SL	5SL-Ⅱ

USP specifications

The USP (U.S. Pharmacopeia) specifications are listed below with the recommended COSMOSIL HPLC columns.

USP Code	Description	Recommended columns
L1	Octadecyl silane chemically bonded to porous silica or ceramic micro-particles, 1.7 to 10 μm in diameter, or a monolithic rod	COSMOSIL C_{18}-MS-Ⅱ COSMOSIL C_{18}-AR-Ⅱ COSMOSIL C_{18}-PAQ COSMOSIL C_{18}-AR-300
L3	Porous silica particles, 5 to 10 μm in diameter	COSMOSIL SL-Ⅱ
L7	Octylsilane chemically bonded to totally porous silica particles, 1.7 to 10 μ m in diameter	COSMOSIL C_8-MS COSMOSIL C_8-AR-300
L10	Nitrile groups chemically bonded to porous silica particles, 3 to 10 μm in diameter	COSMOSIL CN-MS
L11	Phenyl groups chemically bonded to porous silica particles, 1.7 to 10 μm in diameter	COSMOSIL PE-MS COSMOSIL Ph-AR-300
L13	Trimethylsilane chemically bonded to porous silica particles, 3 to 10 μm in diameter	COSMOSIL TMS-MS
L20	Dihydroxypropane groups chemically bonded to porous silica particles, 5 to 10 μm in diameter	COSMOSIL Diol-120-Ⅱ COSMOSIL Diol-300-Ⅱ
L23	An anion-exchange resin made of porous polymethacrylate or polyacrylate gel with quartenary ammonium groups, about 10 μm in size	COSMOGEL QA
L26	Butyl silane chemically bonded to totally porous silica particles, 3 to 10 μ m in diameter	COSMOSIL C_4-MS COSMOSIL C_4-AR-300

2. Reversed Phase Chromatography – Octadecyl types

Introduction

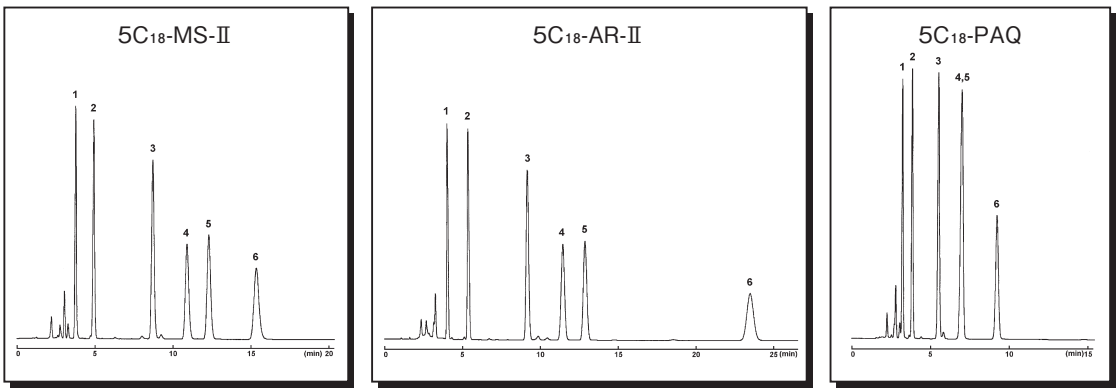
The reversed phase HPLC column is most commonly used because of the high theoretical plate number, excellent separation characteristics, reproducibility, affordable cost and ease of use. Columns packed with the octadecyl group bonded type silica gel (C_{18}, ODS) are the most widely employed. We offer three types of octadecyl group bonded columns: COSMOSIL C_{18}-MS-Ⅱ, C_{18}-AR-Ⅱ and C_{18}-PAQ, each of which has a different separation property.

Material characteristics

Packing material	C_{18}-MS-Ⅱ	C_{18}-AR-Ⅱ	C_{18}-PAQ
Silica gel	high purity porous spherical silica		
Average particle size	3・5・15 μm		
Average pore size	approx. 120 Å		
Specific surface area	approx. 300 m²/g		
Stationary phase			
	octadecyl group		
Bonding type	monomeric	polymeric	
Main interaction	hydrophobic interaction		
End-capping treatment	near-perfect treatment		
Carbon content	approx. 16%	approx. 17%	approx. 11%
pH range	2～10※	1.5～7.5※	2～7.5
Feature	This phase is recommended for most applications but particularly effective for basic compounds.	This phase is recommended for separations requiring acidic mobile phase conditions. It also shows superior molecular shape selectivity to monomeric type C_{18} columns.	This phase is designed to offer superior retention of polar compounds and excellent reproducibility in highly aqueous mobile phases, even in 100% aqueous.

※ Optimum pH range of columns based on silica gel is between 2 and 7.5.

Difference of separation property



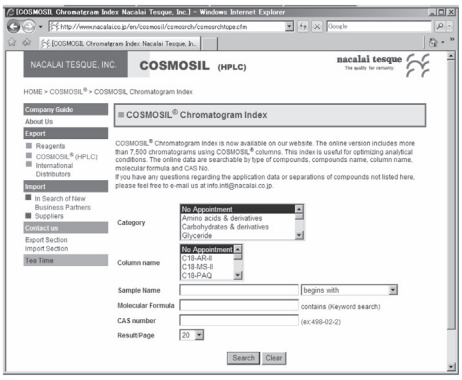
Column size	4.6 mm I.D. × 150 mm	Sample	1. Valerophenone	(0.17 μg)
Mobile phase	methanol : water = 80 : 20		2. Butyl Benzoate	(0.17 μg)
Flow rate	1.0 ml/min		3. Butylbenzene	(8.0 μg)
Temperature	30℃		4. *o*-Terphenyl	(0.17 μg)
Detection	UV 254 nm		5. Amylbenzene	(8.0 μg)
			6. Triphenylene	(0.021 μg)

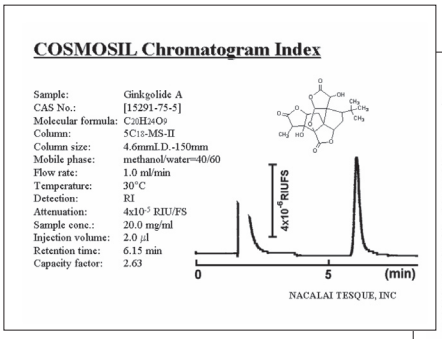
Column selection based on application data

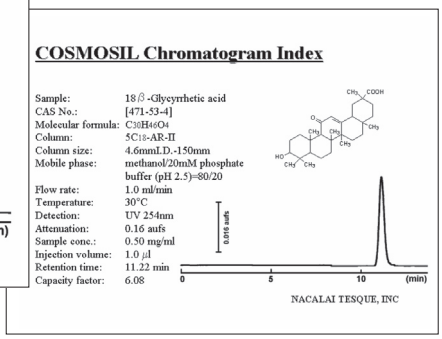
We prepare following application data to help you select separation conditions.

● COSMOSIL Chromatogram Index

More than 5,700 single compound elution profiles with full chromatographic condition description are available. They are not only an incredible help for chromatographers, but also can be used as references in choosing conditions for similar compounds. These data are available at our web site: http://www.nacalai.co.jp/cosmosil/

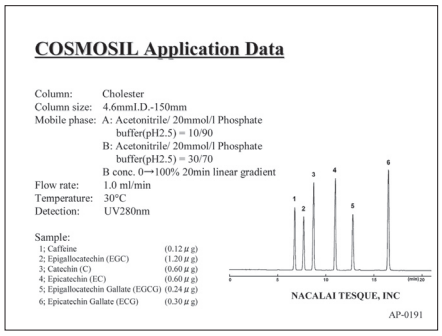


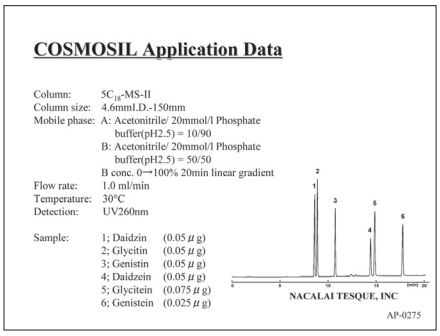




● COSMOSIL Application Data

COSMOSIL Application Data is now available on our website. The online version includes more than 1,000 application data using COSMOSIL columns. The online data are searchable by name of sample and column. If you have any questions regarding the application data or separations of compounds not listed here, please feel free to e-mail us at info.intl@nacalai.co.jp.



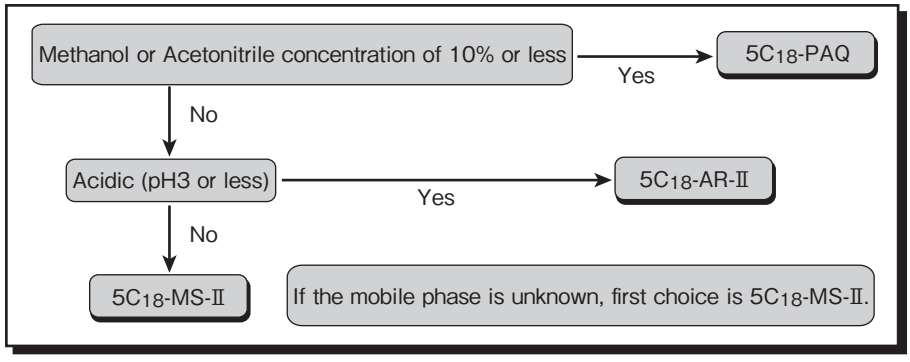


● Application data of substances in Japanese Pharmacopoeia, 15th version (222 data)

We prepare data of drugs using three kinds of C_{18} columns that are specified in HPLC analysis in Application Data of Substances in Japanese Pharmacopoeia, 15th version. The data are available at our web site.
http://www.nacalai.co.jp/en/cosmosil/TheJP15.htm, or type "Cosmosil Japanese Pharmacopoeia" at a search site.

Columns selection by mobile phase

- If a mobile phase is determined, use the following chart to select an appropriate COSMOSIL column.
- Refer to application data above for choosing a mobile phase of new analysis.
- Adjustment of pH is required for dissociative compounds.
- Generally acidic mobile phase is suitable for acidic compounds, and neutral mobile phase is suitable for basic compounds.
- If you are not sure about the mobile phase, try C_{18}-MS-Ⅱ first.



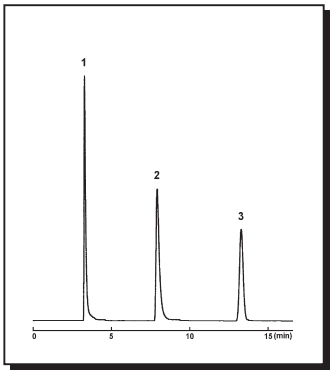
C_{18}-MS-Ⅱ

The COSMOSIL 5C_{18}-MS-Ⅱ is a well-balanced column with better basic performance such as sharper peaks of basic compounds and chelating compounds, and high theoretical plate number. It is the most popular HPLC column because we produce very consistent products and minimize variation from lot to lot. Furthermore we provide abundant application data with the column, so it will help you to choose an analysis condition of your sample.

Analysis of basic compounds

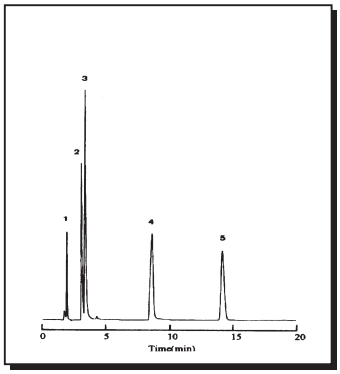
The COSMOSIL 5C_{18}-MS-Ⅱ column, taking advantage of a new end-capping treatment, can replace the original COSMOSIL C_{18} (ODS) column. A new end-capping treatment with polar groups for "shield effect" has significantly improved peak shape for basic compounds.

・Procainamide



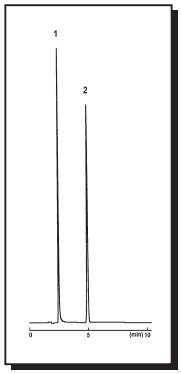
Column size	4.6 mm I.D. × 150 mm
Mobile phase	methanol : 20 mmol/l phosphate buffer (pH7) = 20 : 80
Flow rate	1.0 ml/min
Temperature	30℃
Detection	UV 254 nm
Sample	1. Procainamide (0.38 μg) 2. *N*-Acetylprocainamide (0.25 μg) 3. Benzyl Alcohol (5.63 μg)

・Benzylamine



Column size	4.6 mm I.D. × 150 mm
Mobile phase	methanol : 20 mmol/l phosphate Buffer (pH7) = 20 : 80
Flow rate	1.0 ml/min
Temperature	30℃
Detection	UV 254 nm 0.16AUFS
Sample	1. Uracil 2. Benzylamine 3. *N*-Methylbenzylamine 4. *N,N*-Dimethylbenzylamine 5. Benzyl Alcohol

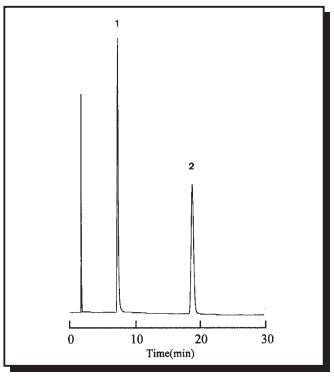
・Pyridine



Column size	4.6 mm I.D. × 150 mm
Mobile phase	acetonitrile : water = 30 : 70
Flow rate	1.0 ml/min
Temperature	30℃
Detection	UV 254 nm
Sample	1. Pyridine (0.4 μg) 2. Phenol (1.7 μg)

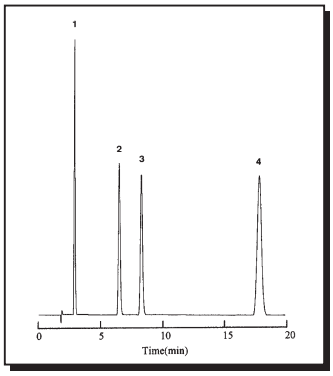
Application Data

・Cholinergic blocker



Column size	4.6 mm I.D. × 150 mm
Mobile phase	methanol : 20 mmol/l phosphate buffer (pH7) = 30 : 70
Flow rate	1.0 ml/min
Temperature	30℃
Detection	UV 210 nm 0.2AUFS
Sample	1. Astropine Sulfate 2. Scopolamine Hydrobromide

・Antipyretic analgesic



Column size	4.6 mm I.D. × 150 mm
Mobile phase	methanol : 20 mmol/l phosphate buffer (pH7) = 30 : 70
Flow rate	1.0 ml/min
Temperature	30℃
Detection	UV 254 nm 0.16AUFS
Sample	1. Acetoaminophen 2. Antipyrine 3. *4*-Aminoantipurine 4. Phenacetin

Stability under alkaline condition

The durability under extremely high pH is greatly improved in the new COSMOSIL 5C$_{18}$-MS-Ⅱ compared with old COSMOSIL 5C$_{18}$-MS. Although it is possible to use COSMOSIL under alkaline condition for a limited time, some deterioration may occur after long exposure. We recommend pH 7.5 and below for long-term usage. Between the measurements, methanol : 20 mM/l Tris buffer (pH 10.0) = 50 : 50 mobile phase was run at 1.0 ml/min flow rate, no significant deterioration could be observed.

Decrease of k'in %

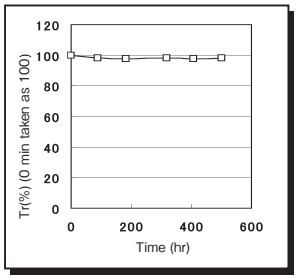


Figure 5/A, Retention factor for Naphthalene

Column size	4.6 mm I.D. × 150 mm
Mobile phase	methanol : water = 70 : 30
Flow rate	1.0 ml/min
Temperature	30℃
Detection	UV 254 nm
Sample	Baphthalene

Decrease of N in %

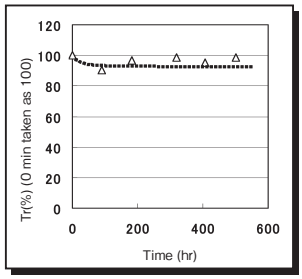


Figure 5/B, Theoretical plate numbers for Pyridine

Column size	4.6 mm I.D. × 150 mm
Mobile phase	acetonitrile : water = 30 : 70
Flow rate	1.0 ml/min
Temperature	30℃
Detection	UV 254 nm
Sample	Pyridine

Validation

The strict quality control system of Nacalai Tesque supports the customers with an individual "Inspection Report" which accompanies each and every COSMOSIL and COSMOGEL Packed Column (except guard columns) and an additional "Certificate of Analysis" for the COSMOSIL 5C$_{18}$-MS-Ⅱ (4.6 mm I.D. × 150 mm and 4.6 mm I.D. × 250 mm). For more information, please refer to page 12.

Ordering information

● Analytical column (Particle size : 5 μm)

COSMOSIL 5C$_{18}$-MS-Ⅱ Packed Column

Column size I.D. × length (mm)	Product number
1.0 × 50	02824-31
1.0 × 150	02896-01
2.0 × 30	05876-71
2.0 × 50	04355-21
2.0 × 100	05597-31
2.0 × 150	38025-91
2.0 × 250	05761-61
3.0 × 100	05458-51
3.0 × 150	34245-31
3.0 × 250	34254-11
4.6 × 30	34341-61
4.6 × 50	38017-01

Column size I.D. × length (mm)	Product number
4.6 × 100	38018-91
4.6 × 150	38019-81
4.6 × 250	38020-41
6.0 × 150	38021-31
6.0 × 250	38022-21
10 × 50	05789-21
10 × 150	34355-91
10 × 250	38023-11
20 × 150	05091-41
20 × 250	38024-01
28 × 250	05760-71

COSMOSIL 5C$_{18}$-MS-Ⅱ Guard Column

Column size I.D. × length (mm)	Product number
4.6 × 10	38014-31
4.6 × 10 Cartridge	38015-89
10 × 20	38016-11
20 × 20	05790-81
20 × 50	34371-71
28 × 50	34347-01

● Preparative column (Particle size : 15 μm)

COSMOSIL 15C$_{18}$-MS-Ⅱ Packed Column

Column size I.D. × length (mm)	Product number
28 × 250	34525-61
50 × 250	05886-41
50 × 500	34531-71

COSMOSIL 15C$_{18}$-MS-Ⅱ Guard Column

Column size I.D. × length (mm)	Product number
28 × 50	05885-51
50 × 50	34527-41

● Fast LC column (Particle size : 3 μm)

COSMOSIL 3C$_{18}$-MS-Ⅱ Packed Column

Column size I.D. × length (mm)	Product number
2.0 × 50	05514-01
4.6 × 10	38065-71
4.6 × 50	38066-61
4.6 × 100	38067-51

For more information on 15C$_{18}$-MS-Ⅱ , please see page 35.
For flow rate and device of semi-micro columns, or preparative columns, please see page 99.

C_{18}-AR-Ⅱ

The COSMOSIL 5C_{18}-AR-Ⅱ packed column features a polymeric type of C_{18} reversed phase material. The column employs an ultra-pure silica gel low in metal impurities and has near-perfect end-capping. In addition, it has stronger acid resistance than the COSMOSIL 5C_{18}-AR. The COMSOSIL 5C_{18}-AR-Ⅱ column is especially effective for the separation of chelating compounds as well as both acidic and basic compounds.

Acidic resistance

The acidic resistance of COSMOSIL 5C_{18}-AR-Ⅱ is much improved compared with commercially available monomeric type octadecyl stationary phases. It retains high performance even in case of acidic mobile phases commonly used to separate acidic compounds and peptides.

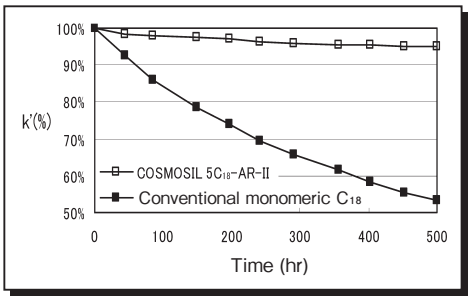
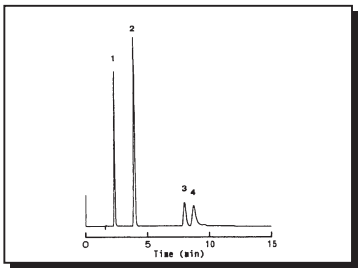


Figure. Decomposition test in 0.1% Trifluoroacetic Acid solution at 60℃ .
Capacity factor (k') = Naphthalene, Mobile phase: 70% methanol

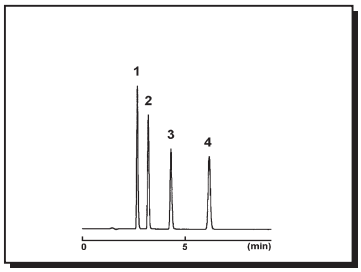
Application Data

・Organic Acid



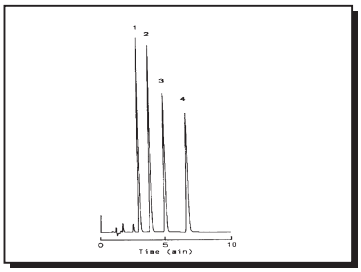
Column size	4.6 mm I.D. x 150 mm
Mobile phase	methanol : 20 mmol/l phosphoric acid = 20 : 80
Flow rate	1.0 ml/min
Temperature	30℃
Detection	UV 254 nm 0.5AUFS
Sample	1. Gallic acid (0.63 μg) 2. Protocatechuic Acid (0.63 μg) 3. Gentisic Acid (0.63 μg) 4. Phthalic Acid (0.63 μg)

・Paraben



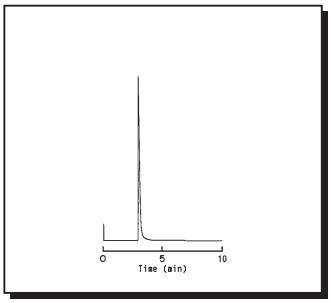
Column size	4.6 mm I.D. × 150 mm
Mobile phase	acetonitrile : water = 50 : 50
Flow rate	1.0 ml/min
Temperature	30℃
Detection	UV 254nm
Sample	1. Methyl *p*-Hydroxybenzoate (0.25 μg) 2. Ethyl *p*-Hydroxybenzoate (0.025 μg) 3. *n*-Propyl *p*-Hydroxybenzoate (0.025 μg) 4. *n*-Butyl *p*-Hydroxybenzoate (0.025 μg)

・Salicylic Acid



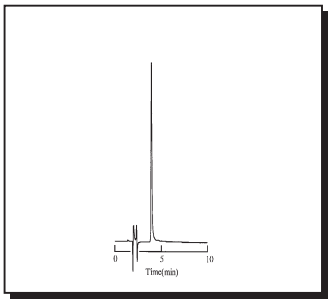
Column size	4.6 mm I.D. × 150 mm
Mobile phase	acetonitrile : water = 70 : 30
Flow rate	1.0 ml/min
Temperature	30℃
Detection	UV 254 nm 0.2 AUFS
Sample	1. Methyl Salicylate (2.3 μg) 2. Ethyl Salicylate (2.6 μg) 3. Propyl Salicylate (2.3 μg) 4. Butyl Salicylate (2.6 μg)

・Hinokitiol



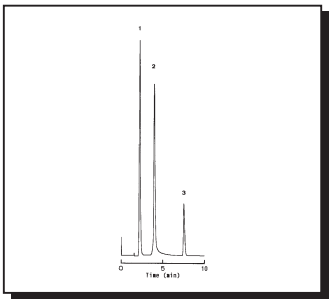
Column size	4.6 mm I.D. × 150 mm
Mobile phase	methanol : 1 mmol/l EDTA 20mmol/l phosphoric acid = 70 : 30
Flow rate	1.0 ml/min
Temperature	30℃
Detection	UV 254 nm 0.5 AUFS
Sample	Honokitiol (1 μg)

・Oxine-copper



Column size	4.6 mm I.D. × 150 mm
Mobile phase	acetonitrile : 20mmol phosphoric asid = 5 : 95
Flow rate	1.0 ml/min
Temperature	30℃
Detection	UV 240nm 0.2AUFS
Sample	Oxine-copper

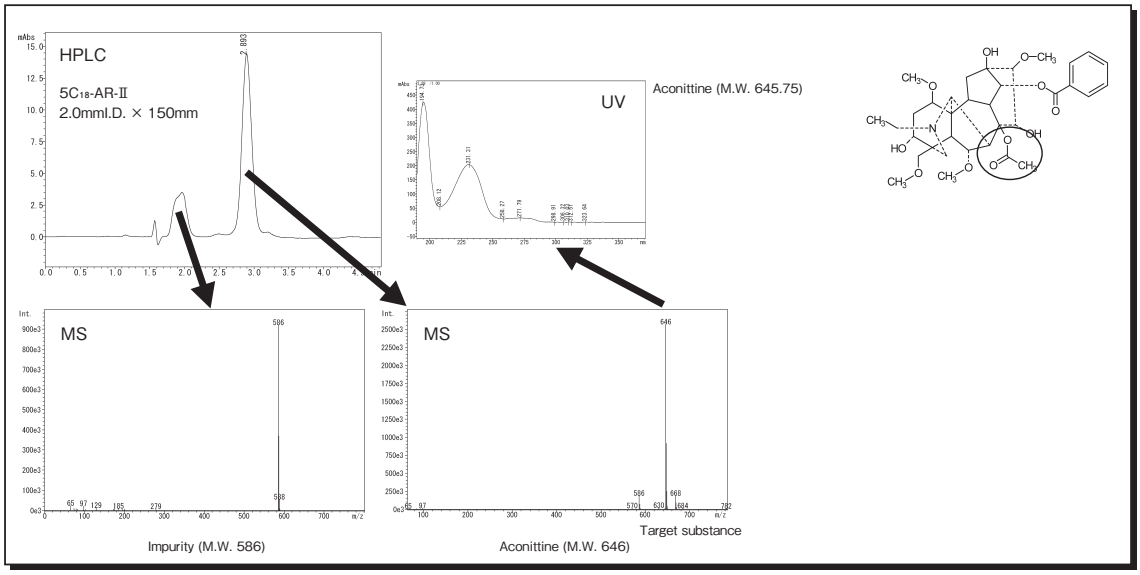
・Acetylacetone



Column size	4.6 mm I.D. × 150 mm
Mobile phase	acetonitrile : 20 mmol/l phosphate buffer (pH7) = 60 : 40
Flow rate	1.0 ml/min
Temperature	30℃
Detection	UV 240 nm 0.2AUFS
Sample	1. Acetylacetone (0.76 μg) 2. *8*-Quinolinol (0.70 μg) 3. Benzene (14.65 μg)

LC-MS Application data

Identification of herbal medicine constituents by LC-MS



Validation

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Ordering information

● Analytical column (Particle size : 5 μm)

COSMOSIL 5C18-AR-Ⅱ Packed Column

Column size I.D. × length (mm)	Product number
1.0 × 50	02955-21
1.0 × 150	02951-61
2.0 × 30	05098-71
2.0 × 50	34400-81
2.0 × 100	34469-11
2.0 × 150	37992-51
2.0 × 250	05272-71
3.0 × 100	05791-71
3.0 × 150	38028-61
3.0 × 250	38029-51
4.6 × 30	05877-61
4.6 × 50	38142-51
4.6 × 100	38143-41
4.6 × 150	38144-31
4.6 × 250	38145-21
6.0 × 150	38146-11
6.0 × 250	38147-01
10 × 50	05369-21
10 × 150	34350-41
10 × 250	38149-81
20 × 150	34316-01
20 × 250	38150-41
28 × 250	34362-91

COSMOSIL 5C18-AR-Ⅱ Guard Column

Column size I.D. × length (mm)	Product number
4.6 × 10	38141-61
4.6 × 10 Cartridge	38008-89
10 × 20	38148-91
20 × 20	34458-51
20 × 50	34479-81
28 × 50	34363-81

● Preparative column (Particle size : 15 μm)

COSMOSIL 15C18-AR-Ⅱ Packed Column

Column size I.D. × length (mm)	Product number
28 × 250	37978-51
50 × 250	38058-71
50 × 500	05884-61

COSMOSIL 15C18-AR-Ⅱ Guard Column

Column size I.D. × length (mm)	Product number
28 × 50	38030-11
50 × 50	38057-81

● Fast LC column (Particle size : 3 μm)

COSMOSIL 3C18-AR-Ⅱ Packed Column

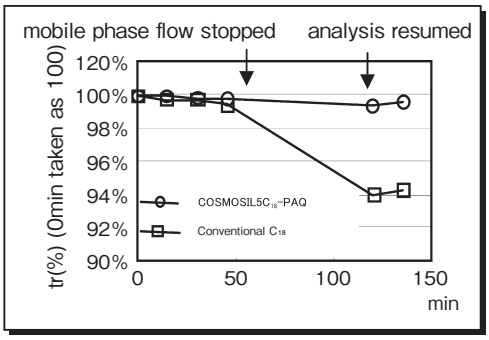
Column size I.D. × length (mm)	Product number
2.0 × 50	05478-91
4.6 × 10	38068-41
4.6 × 50	38069-31
4.6 × 100	38070-91

For more information on 15C18-AR-Ⅱ, please see page 35.
For flow rate and device of semi-micro columns, or preparative columns, please see page 99.

C_{18}-PAQ

The COSMOSIL 5C_{18}-PAQ is a new member of our C_{18} column family which already includes the monomeric type MS-Ⅱ and the polymeric type AR-Ⅱ. This new column maintains stable retention time even in 100% aqueous mobile phases. The new polymeric linking style gives this column a strong acidic resistance so that it is compatible with mobile phases of acidic pH that can permanently damage conventional octadecyl stationary phases. This type is especially good for separation of hydrophilic compounds.

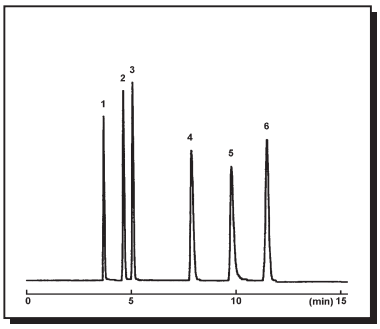
Stable Performance under 100% aqueous conditions



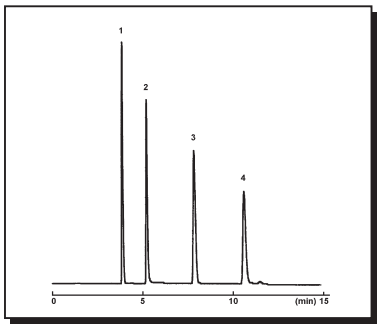
The figure shows the change of retention time for thymine with 100% aqueous mobile phase (20 mmol/l phosphate buffer pH7). The sample was analyzed 4 times. Flow of mobile phase was then stopped for 1 hour. The sample was analyzed under the same condition again after 1 hour. The conventional C_{18} column showed change of retention time, but COSMOSIL 5C_{18}-PAQ maintained stable retention time.

Application data

・Organic acid

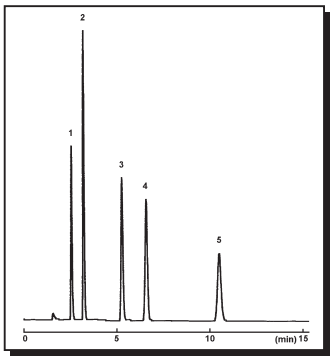


Column size	4.6 mm I.D. × 250 mm			
Mobile phase	20 mmol/l phosphoric acid			
Flow rate	1.0 ml/min			
Temperature	30℃			
Detection	UV 210 nm 0.16AUFS			
Sample	1. Glycolic Acid	(6.3 μg)	4. Citric Acid	(6.3 μg)
	2. Malic Acid	(6.3 μg)	5. Maleic Acid	(0.063 μg)
	3. Lactic Acid	(13 μg)	6. Fumaric Acid	(0.031 μg)



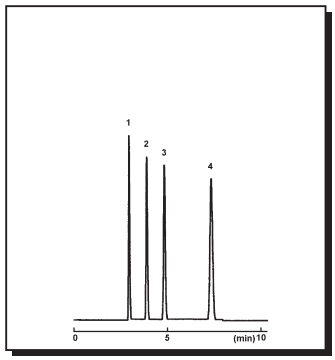
Column size	4.6 mm I.D. × 250 mm			
Mobile phase	20 mmol/l phosphoric acid			
Flow rate	1.0 ml/min			
Temperature	30℃			
Detection	UV 210 nm 0.16AUFS			
Sample	1. Tartaric Acid	(4.0 μg)		
	2. Acetic Acid	(13 μg)		
	3. Succinic Acid	(13 μg)		
	4. Propionic Acid	(13 μg)		

・Nucleic-acid base



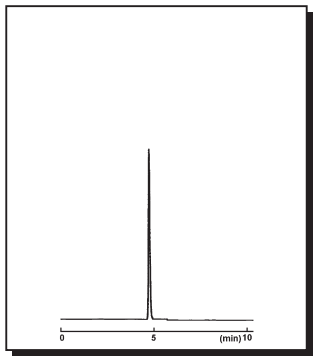
Column size	4.6 mm I.D. × 150 mm			
Mobile phase	20 mmol/l phosphate buffer (pH7)			
Flow rate	1.0 ml/min			
Temperature	30℃			
Detection	UV 254 nm 0.16AUFS			
Sample	1. Cytosine	(0.1 μg)		
	2. Uracil	(0.1 μg)		
	3. Guanine	(0.1 μg)		
	4. Thymine	(0.2 μg)		
	5. Adenine	(0.05 μg)		

・dNTP



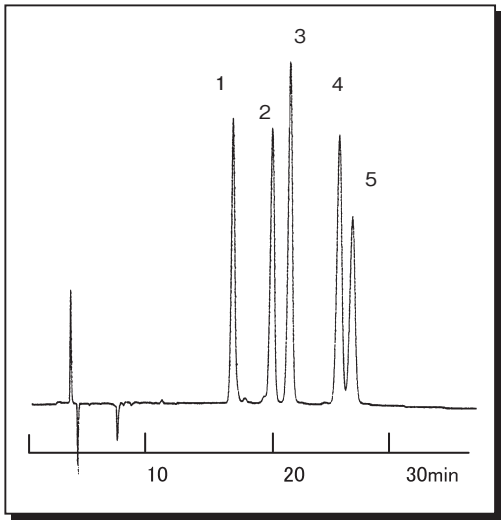
Column size	4.6 mm I.D. × 250 mm
Mobile phase	20 mmol/l phosphate buffer (pH7)
Flow rate	1.0 ml/min
Temperature	30℃
Detection	UV 254 nm 0.16AUFS
Sample	1. dCTP
	2. dTTP
	3. dGTP
	4. dATP
	1 mmol/ each
Injection vol.	0.5 μl

・Ascorbic acid



Column size	4.6 mm I.D. × 250 mm
Mobile phase	20 mmol/l Phosphoric Acid
Flow rate	1.0 ml/min
Temperature	30℃
Detection	UV 245 nm 0.16AUFS
Sample	Ascorbic Acid (Vitamin C) (0.1 μg)

・*2*-Phenylethyl glycoside



1 *2*-Phenylethyl-β-melibioside

2 *2*-Phenylethyl-β-gentiobioside

3 *2*-Phenylethyl-β-lactoside

4 *2*-Phenylethyl-β-cellobioside

5 *2*-Phenylethyl-β-maltoside

Column size	4.6 mm I.D. × 150 mm
Mobile phase	acetonitrile : methanol : water = 8 : 4 : 88
Flow rate	1.0 ml/min
Temperature	30℃
Detection	UV 210 nm 0.064AUFS
Pressure	10.5 MPa

Data courtesy of Dr. B. Shimizu, Dr. K. Sakata, Institute for Chemical Research, Kyoto University

Ordering information

● Analytical column (Particle size : 5 μm)

COSMOSIL 5C18-PAQ Packed Column

Column size I.D. × length (mm)	Product number
1.0 × 50	05792-61
1.0 × 150	05793-51
2.0 × 30	05878-51
2.0 × 50	05794-41
2.0 × 100	05470-71
2.0 × 150	34449-71
2.0 × 250	05795-31
3.0 × 100	05796-21
3.0 × 150	05797-11
3.0 × 250	05798-01
4.6 × 30	05879-41
4.6 × 50	34451-21

Column size I.D. × length (mm)	Product number
4.6 × 100	05799-91
4.6 × 150	02486-71
4.6 × 250	02485-81
6.0 × 150	34419-61
6.0 × 250	05800-41
10 × 50	05801-31
10 × 150	34466-41
10 × 250	34376-21
20 × 150	34476-11
20 × 250	34373-51
28 × 250	34456-71

COSMOSIL 5C18-PAQ Guard Column

Column size I.D. × length (mm)	Product number
4.6 × 10	02484-91
10 × 20	34457-61
20 × 20	05803-11
20 × 50	05804-01
28 × 50	34455-81

● Preparative column (Particle size : 15 μm)

COSMOSIL 15C18-PAQ Packed Column

Column size I.D. × length (mm)	Product number
28 × 250	05888-21
50 × 250	05890-71
50 × 500	05891-61

COSMOSIL 15C18-PAQ Guard Column

Column size I.D. × length (mm)	Product number
28 × 50	05887-31
50 × 50	05889-11

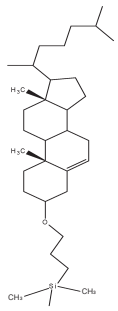
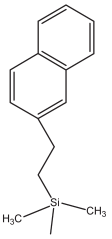
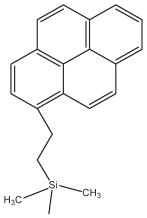
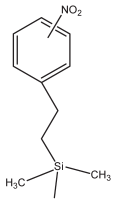
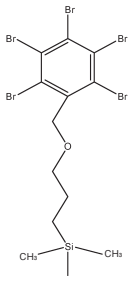
For more information on 15C18-PAQ, please see page 35.
For flow rate and device of semi-micro columns, or preparative columns, please see page 99.

3. Reversed Phase Chromatography - Specialty columns

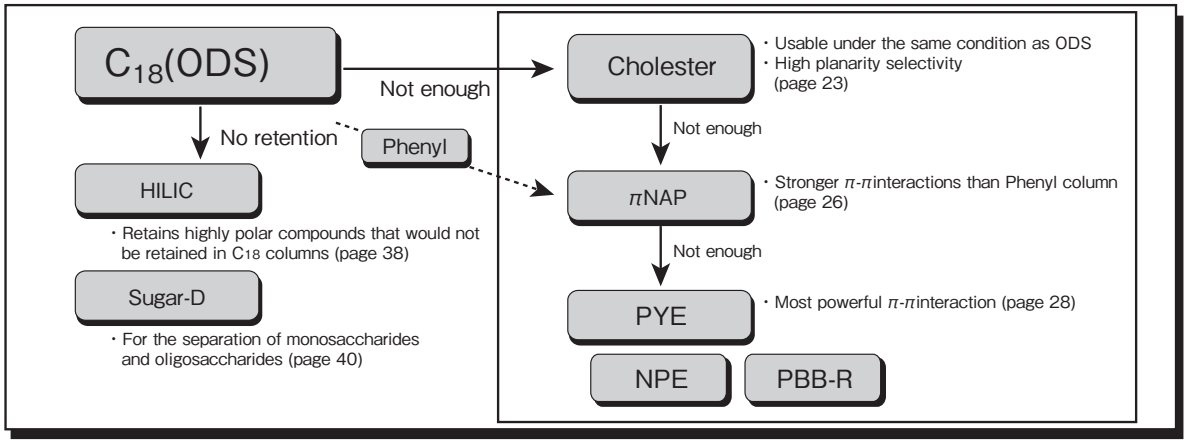
Introduction

Reversed phase HPLC columns have been widely used because of their superior resolution, high theoretical plate number and ease of use. Since hydrophobic interaction is the dominant separation mechanism in reversed phase chromatography, conventional stationary phases such as C_{18} and C_8 do not offer optimum selectivity for compounds with similar hydrophobicity. COSMOSIL offers a broad selection of columns with unique stationary phases for separation of these difficult analytes. These columns offer improved separation of structurally similar compounds that are difficult to analyze with a C_{18} type column.

Material characteristics

Packing material	Cholester	πNAP	PYE	NPE	PBB-R
Silica gel	high purity porous spherical silica				
Average particle size	5μm				
Average pore size	approx. 120 Å				
Specific surface area	approx. 300 m²/g				
Stationary phase	 Cholesteryl group	 Naphtylethyl proup	 Pyrenylethyl group	 Nitrophenylethyl group	 Pentabromobezyl group
Bonded type	monomeric type				
Main interaction	hydrohobic interaction molecular shape selectivity	hydrohobic interaction π-π interaction	hydrohobic interaction π-π interaction dispersion force charge-tansfer interaction	hydrohobic interaction π-π interaction dipole-Dipole interaction	hydrohobic interaction dispersion force
End-capping treatment	near-perfect treatment				
Carbon content	approx 20%	approx 11%	approx 18%	approx 9%	approx 8%
Feature	Usable under condition the same as C_{18} High molecular sharp selectivity	Stronger π-π interaction than Phenyl column	Strongest π-π interaction	Dipole-Dipole interaction	Dispersion force interaction

Column selection guide



Cholester

COSMOSIL Cholester is a reversed phase HPLC column with cholesteryl groups bonded silica packing material, which provides equivalent hydrophobicity like traditional alkyl group bonded silica packing materials (C_{18}, C_{30}). However, Cholester offers strong molecular shape selectivity for hydrophobic compounds to yield unique and reproducible separation patterns following the same analytical conditions used with other ODS columns.

Hydrophobic interaction

Figure 1 shows the comparison of hydrophobic interactions with competitive C_{18} columns. Cholester provides the same hydrophobicity as alkyl group bonded types (C_{18}, C_{30}). It is not necessary to change the analytical conditions when replacing C_{18} or C_{30} columns with Cholester.

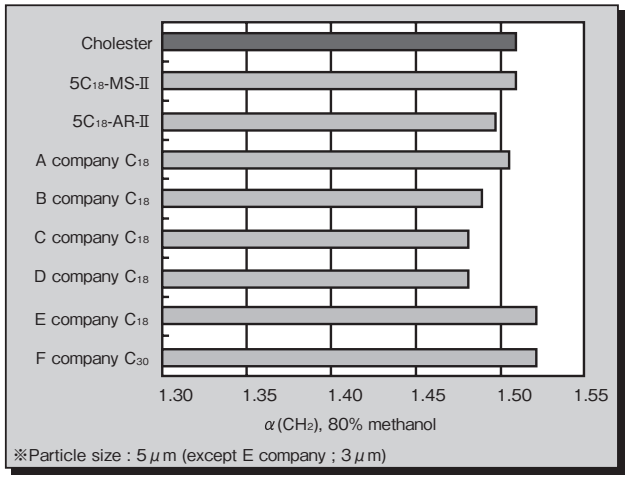
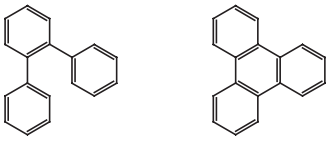


Figure1. Comparison of hydrophobic interactions

Molecular shape selectivity

The stationary phase of Cholester has very rigid structures and can distinguish different molecular shapes. Cholester offers improved separation for structurally similar compounds that are difficult to analyze with alkyl group bonded materials (C_{18} and C_{30}). As in the following example Cholester retains planar Triphenylene longer than stereoscopic *o*-Terphenyl.



o-Terphenyl　　Triphenylene

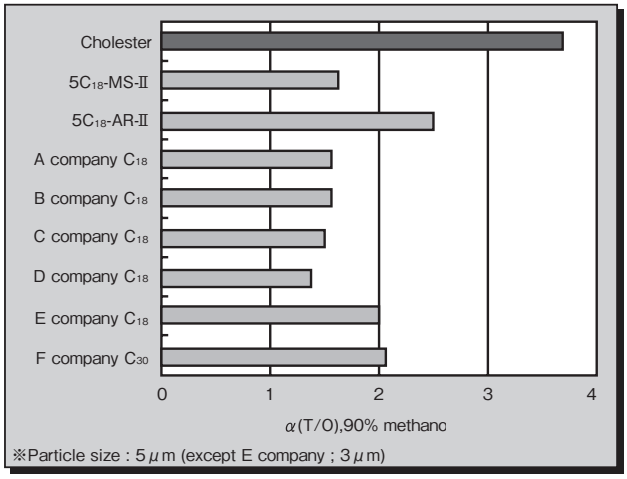
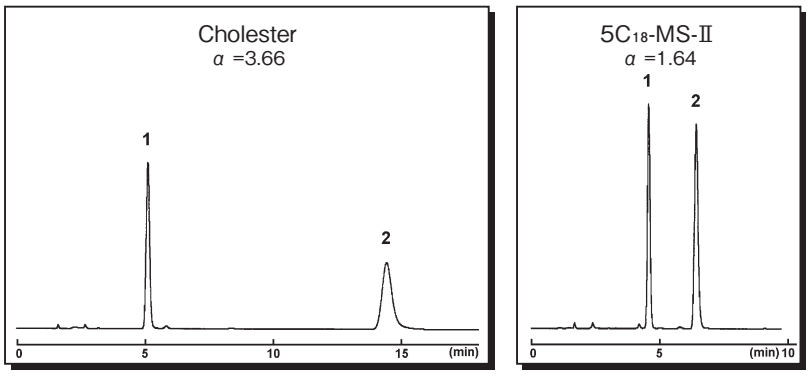


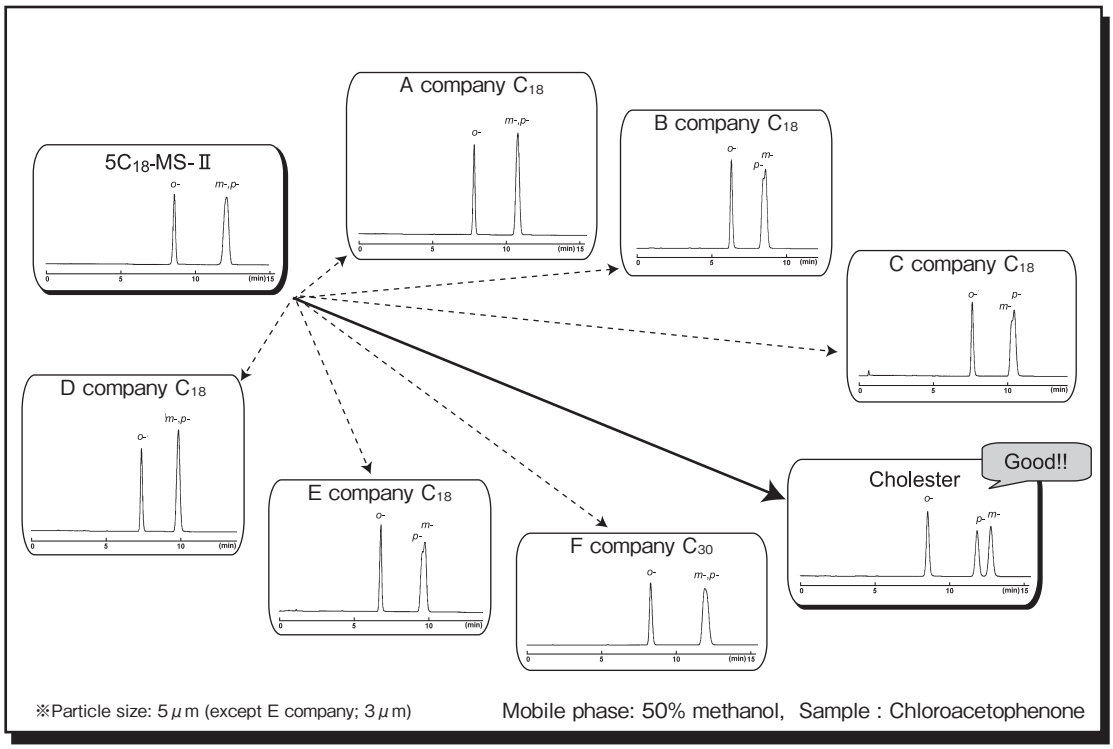
Figure 2. Comparison of molecular sharp selectivity



Column size	4.6 mm I.D. × 150 mm
Mobile phase	methanol : water = 90 : 10
Flow rate	1.0 ml/min
Temperature	30℃
Detection	UV 254 nm
Sample	1. *o*-Terphenyl (0.10μg)
	2. Triphenylene (0.01μg)

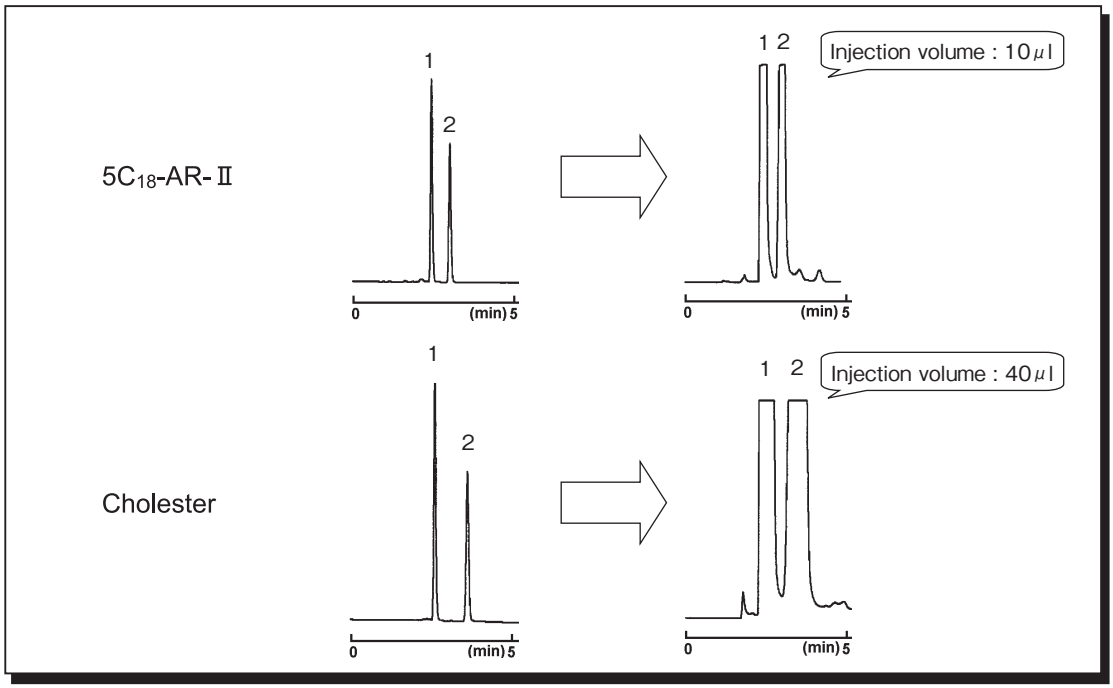
Improvement in separation

COSMOSIL Cholester provides enhanced selectivity over traditional C_{18} columns and offers greater performance in separating isomers or other closely related compounds. COSMOSIL Cholester is ideal for method development and serves as an excellent alternative to traditional C_{18} columns. The figure below shows analytical data of chloroacetophenone isomers. These isomers are difficult to separate with C_{18} and C_{30}, but they are well resolved by COSMOSIL Cholester.



Efficiency of preparative separation

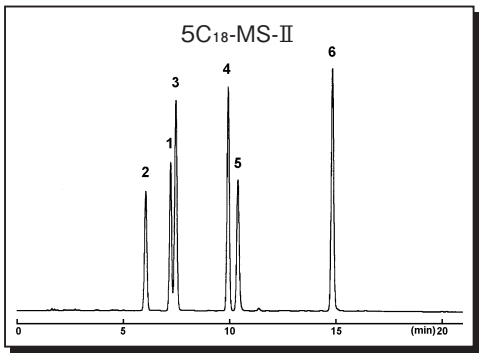
The figure below shows the comparison of efficiency of preparative separation with a C_{18} column. Both columns show good separation. However, sample loading capacity for preparative separations can be affected by a slight difference in separation ability. COSMOSIL Cholester can load 4 times of sample volume compared with C_{18} columns.

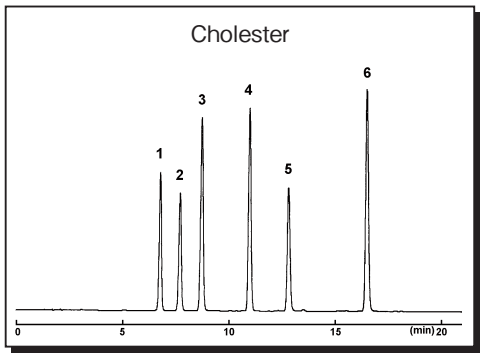


Column size	4.6 mm I.D. × 150 mm	Detection	UV 254 nm
Mobile phase	100% methanol	Sample	1. Bibenzyl
Flow rate	1.0 ml/min		2. Anthracene
Temperature	30℃		

Application Data

・Catechin

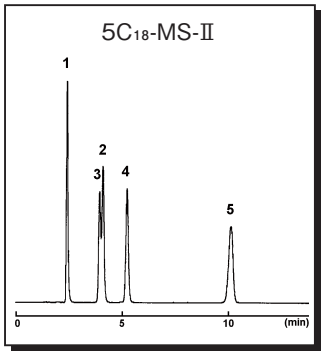


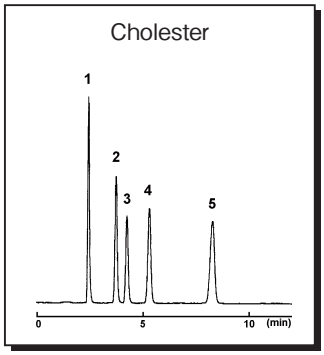


Column size	4.6 mm I.D. × 150 mm
Mobile phase	A : acetonitrile : 20 mmol/l phosphate buffer (pH2.5) = 10 : 90
	B : acetonitrile : 20 mmol/l phosphate buffer (pH2.5) = 30 : 70
	B conc. 0 → 100% 20 min linear gradient
Flow rate	1.0 ml/min
Temperature	30℃
Detection	UV 280 nm

Sample	1. Caffeine	(0.12 μg)
	2. Epigallocatechin (EGC)	(1.20 μg)
	3. Catechin (C)	(0.60 μg)
	4. Epicatechin (EC)	(0.60 μg)
	5. Epigallocatechin gallate (EGCG)	(0.24 μg)
	6. Epicatechin gallate (ECG)	(0.30 μg)

・Saikosaponin





Column size	4.6 mm I.D. × 150 mm
Flow rate	acetonitrile : water = 45 : 55
Mobile phase	1.0 ml/min
Temperature	30℃
Detection	ELSD (Gain = 6, Atten = 8)

Sample	1. Saikosaponin c	(1.5 μg)
	2. Saikosaponin a	(1.5 μg)
	3. Saikosaponin b_2	(1.5 μg)
	4. Saikosaponin b_1	(1.5 μg)
	5. Saikosaponin d	(1.5 μg)

Ordering information

COSMOSIL Cholester Packed Column

Column size I.D. × length (mm)	Product number
1.0 × 150	05968-71
1.0 × 250	05969-61
2.0 × 30	08565-51
2.0 × 150	05971-11
2.0 × 250	05972-01
3.0 × 150	05973-91
3.0 × 250	05974-81
4.6 × 50	06359-21
4.6 × 100	06591-61

Column size I.D. × length (mm)	Product number
4.6 × 150	05976-61
4.6 × 250	05977-51
10 × 150	08011-91
10 × 250	05979-31
20 × 150	06088-71
20 × 250	05982-71
28 × 250	05985-41

COSMOSIL Cholester Guard Column

Column size I.D. × length (mm)	Product number
4.6 × 10	05975-71
10 × 20	05978-41
20 × 20	05980-91
20 × 50	05981-81
28 × 50	05983-61

π NAP

COSMOSIL π NAP is a reversed phase HPLC column with naphtylethyl group bonded silica packing material. The naphthylethyl group is composed of two fused aromatic rings and forms strong π-π interactions with unsaturated compounds. This column offers improved separation of compounds such as positional isomers that are difficult to analyze with alkyl group bonded materials.

Comparison of π-π interactions

COSMOSIL π NAP shows stronger π-π interactions than phenyl columns.

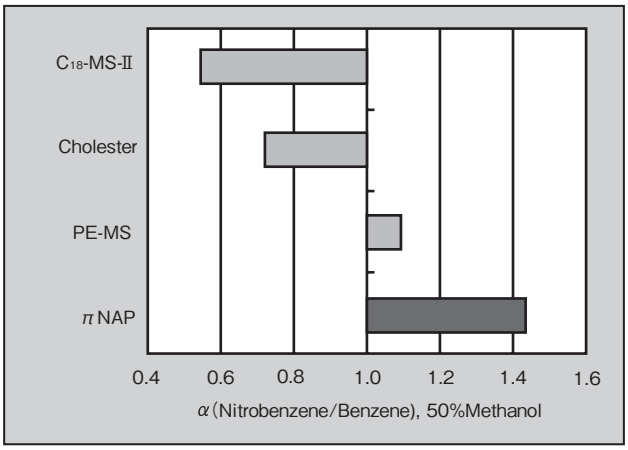
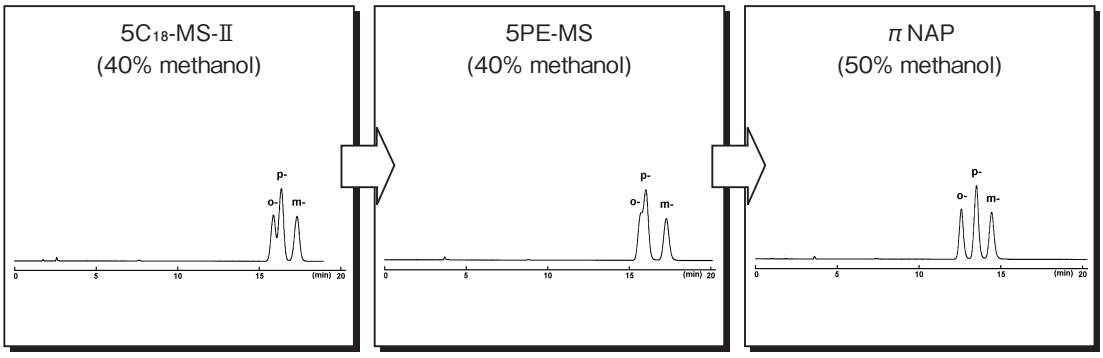


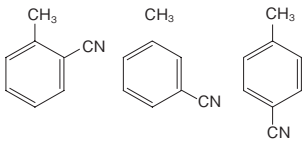
Figure. Comparison of π-π interactions

Analytical data

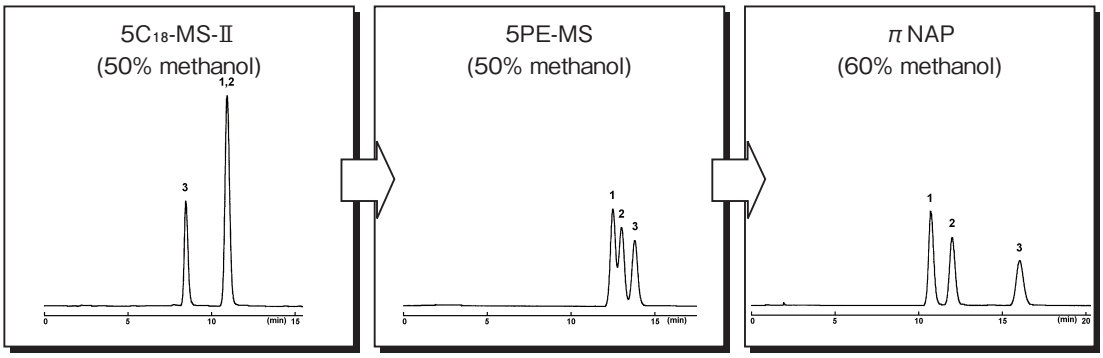
・Tolunitrile



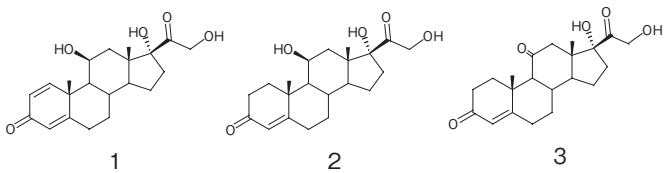
Column size	4.6 mm I.D. × 150 mm	
Flow rate	1.0 ml/min	
Temperature	30℃	
Detection	UV 254 nm	
Sample	*o*-Tolunitrile	(2.0 μg)
	m-Tolunitrile	(2.0 μg)
	p-Tolunitrile	(1.0 μg)



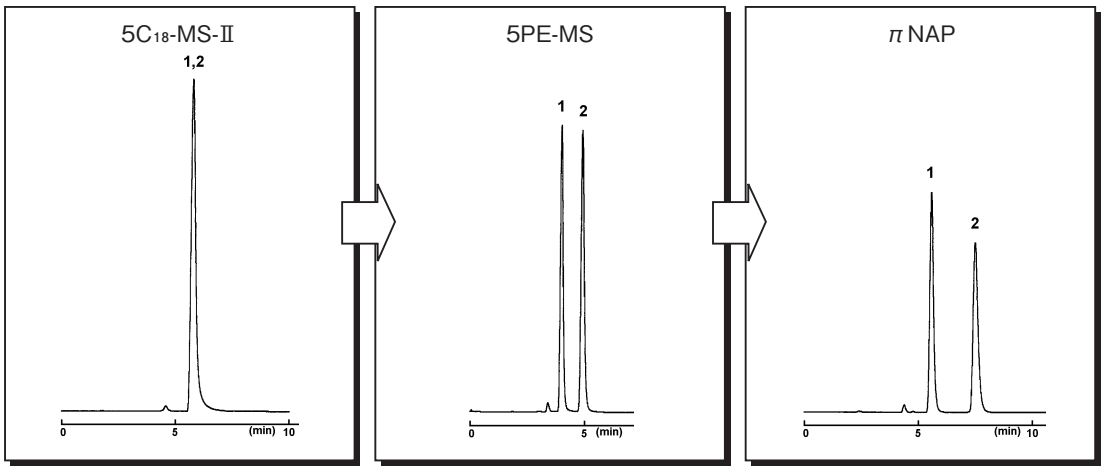
・Adrenal cortical hormone



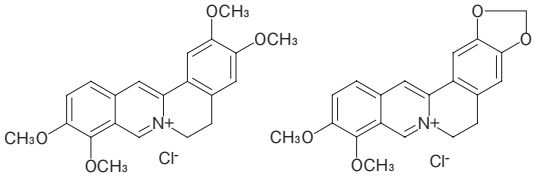
Column size	4.6 mm I.D. × 150 mm	
Flow rate	1.0 ml/min	
Temperature	30℃	
Detection	UV 254 nm	
Sample	1. Prednisolone	(0.33 μg)
	2. Hydrocortisone	(0.33 μg)
	3. Cortisone	(0.33 μg)



・Berberine

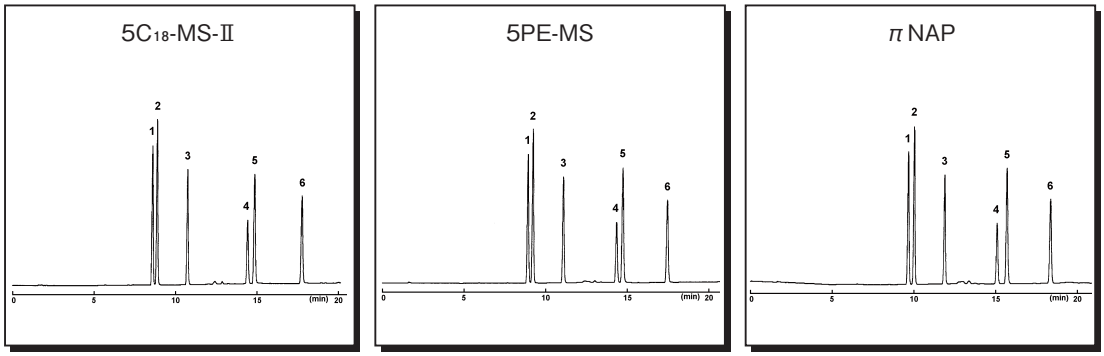


Column size	4.6 mm I.D. × 150 mm	
Mobile phase	methanol : 20 mmol/l phosphate buffer (pH2.5) =	
	$5C_{18}$-MS-Ⅱ	40 : 60
	5PE-MS	60 : 80
	πNAP	80 : 20
Flow rate	1.0 ml/min	
Temperature	30℃	
Detection	UV 254 nm	
Sample	1. Palmatine choride	(0.2 μg)
	2. Berberine Hydrochoride	(0.2 μg)



1 2

・Isoflavone



Column size	4.6 mm I.D. × 150 mm	
Mobile phase	A : 10% acetonitrile / 20mmol/l phosphate buffer (pH 2.5)	
	B : 50% acetonitrile / 20mmol/l phosphate buffer (pH 2.5)	
	B Conc. 0 → 100% 20min linear gradient	
Flow rate	1.0 ml/min	
Temperature	30℃	
Detection	UV 260 nm	
Sample	1. Daidzin	(0.05 μg)
	2. Glycitin	(0.05 μg)
	3. Genistin	(0.05 μg)
	4. Daidzein	(0.05 μg)
	5. Glycitein	(0.075 μg)
	6. Genistein	(0.025 μg)

Ordering information

COSMOSIL πNAP Packed Column

Column size I.D. × length (mm)	Product number
1.0 × 150	08076-61
1.0 × 250	08077-51
2.0 × 30	08566-41
2.0 × 50	08567-31
2.0 × 150	08078-41
2.0 × 250	08079-31
3.0 × 150	08080-91
3.0 × 250	08081-81
4.6 × 50	08083-61
4.6 × 100	08084-51

Column size I.D. × length (mm)	Product number
4.6 × 150	08085-41
4.6 × 250	08086-31
10 × 150	08088-11
10 × 250	08089-01
20 × 150	08092-41
20 × 250	08093-31
28 × 250	08095-11

COSMOSIL πNAP Guard Column

Column size I.D. × length (mm)	Product number
4.6 × 10	08082-71
10 × 20	08087-21
20 × 20	08090-61
20 × 50	08091-51
28 × 50	08094-21

PYE

COSMOSIL PYE column is a reversed phase column with 2-(1-pyrenyl) ethyl groups bonded silica packing material. This column utilizes π-π interactions originating from the planar pyrene ring structure to separate structural isomers.

Comparison of π-π interactions with πNAP

COSMOSIL PYE provides much stronger π-π interactions than πNAP.

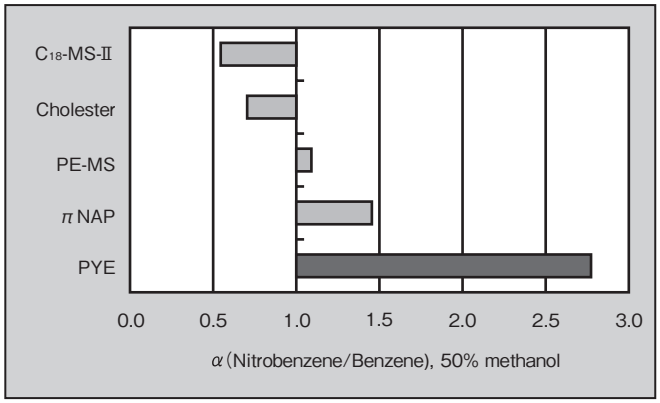
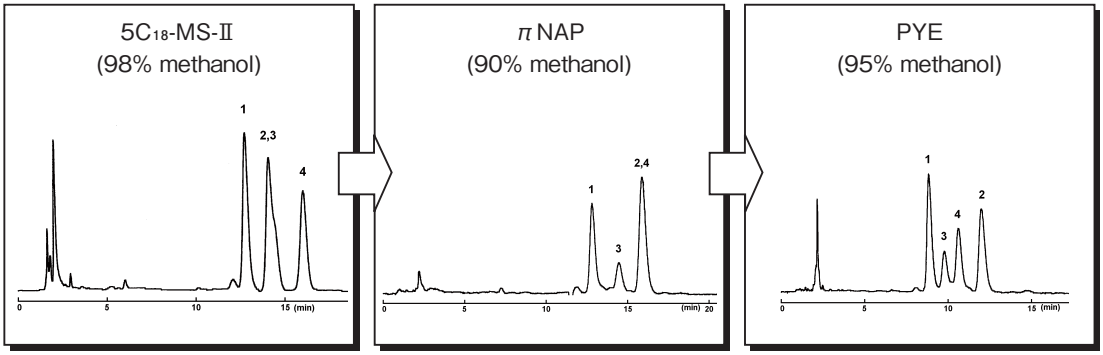


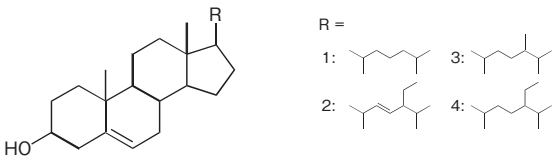
Figure. Comparison of π-π interactions

Analytical data

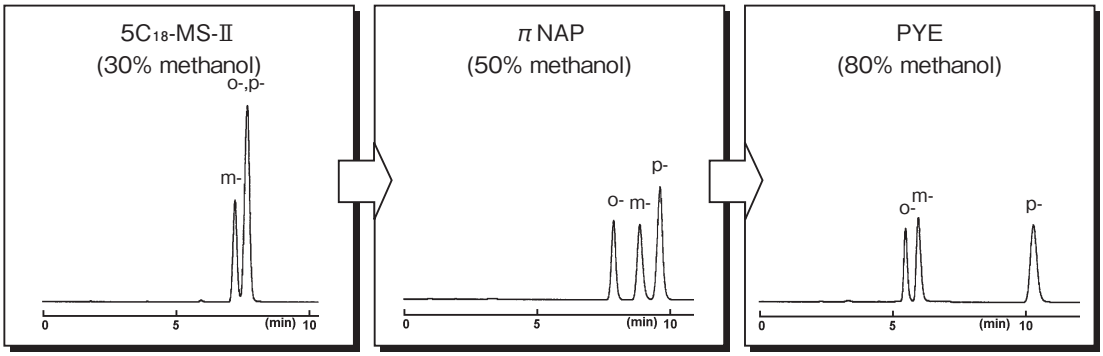
・Sterol



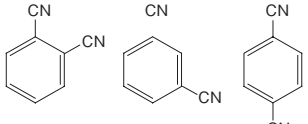
Column size	4.6 mm I.D. × 150 mm
Flow rate	1.0 ml/min
Temperature	30℃
Detection	UV 210 nm
SampleSample	1. Cholesterol
	2. Stigmasteroll
	3. Campesterol
	4. Sitosterol



・Phthalonitrile

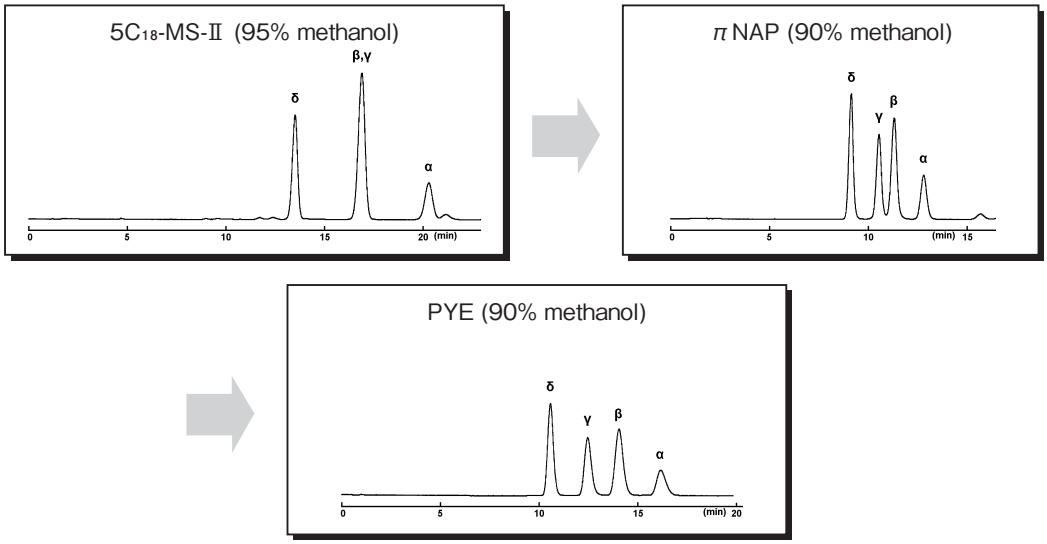


Column size	4.6 mm I.D. × 150 mm	
Flow rate	1.0 ml/min	
Temperature	30℃	
Detection	UV 254 nm	
Sample	*o*-Phthalonitrile (Phthalonitrile)	(0.3 μg)
	m-Phthalonitrile (Isophthalonitrile)	(3.0 μg)
	p-Phthalonitrile (Terephthalonitrile)	(0.15 μg)

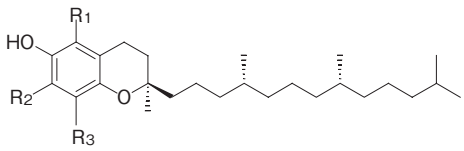


\- *m*- *p*-

・Tocopherol



Column size	4.6 mm I.D. × 150 mm
Flow rate	1.0 ml/min
Temperature	30℃
Detection	UV 295 nm
Sample	α-Tocopherol (1.0 μg)
	β-Tocopherol (1.0 μg)
	γ-Tocopherol (1.0 μg)
	δ-Tocopherol (1.0 μg)



	α	β	γ	δ
R_1	Me	Me	H	H
R_2	Me	H	Me	H
R_3	Me	Me	Me	Me

Ordering information

COSMOSIL 5PYE Packed Column

Column size I.D. × length (mm)	Product number
1.0 × 150	02851-71
2.0 × 150	38042-61
2.0 × 250	34450-31
4.6 × 150	37837-91
4.6 × 250	37989-11
10 × 250	37996-11
20 × 250	38044-41

COSMOSIL 5PYE Guard Column

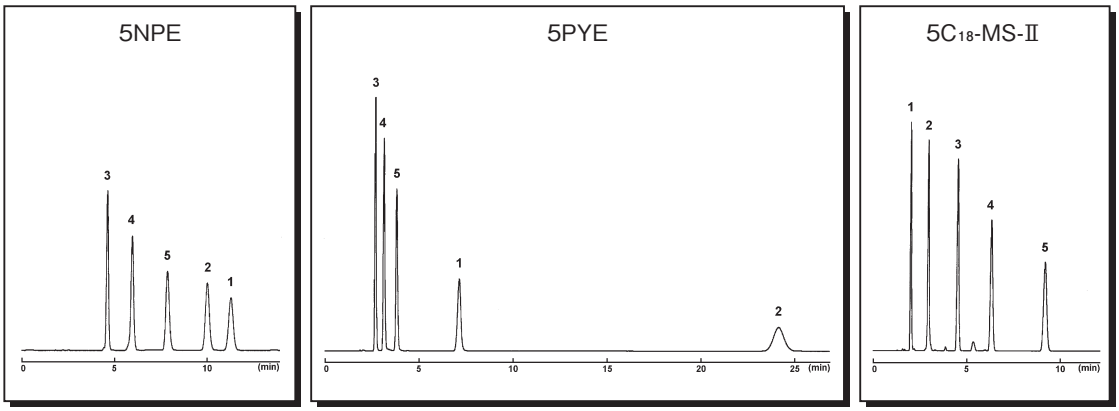
Column size I.D. × length (mm)	Product number
4.6 × 10	37903-11
10 × 20	38041-71
20 × 20	05867-91
20 × 50	34475-21

NPE

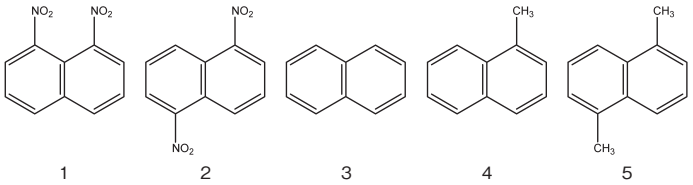
COSMOSIL NPE column is a reversed phase column with nitrophenylethyl groups bonded silica packing material. This column provides unique retention characteristics, slightly different from the COSMOSIL PYE column, utilizing both dipole-dipole and π-π interactions.

Selectivity for dipole

Selectivity for dipole is evaluated based on the separation of *1,5*-dinitronaphthalene and *1,8*-dinitronaphthalene. Dinitronaphthalenes (peak 1 and 2) were strongly retained on PYE and NPE because of π-π interaction compared with dimethylnaphthalenes. However, there is a slight difference between these two columns. While *1,5*-dinitronaphthalene (peak 2) was preferentially retained on PYE, *1,8*-dinitronaphthalene (peak 1) was retained longer on NPE. The results with NPE indicate the presence of strong dipole-dipole interaction. The two nitro group dipoles in *1,8*-dinitronaphthalene are aligned for a much greater dipolar coupling with the bonded nitrophenyl group in NPE than *1,5*-dinitronaphthalene.



Column size	4.6 mm I.D. × 150 mm		Sample	1. *1,8*-Dinitronaphthalene	(0.21 μg)
Mobile phase	5NPE	methanol : water = 70 : 30		2. *1,5*-Dinitronaphthalene	(0.11 μg)
	5PYE	methanol : water = 90 : 10		3. Naphthalene	(0.25 μg)
	$5C_{18}$-MS-Ⅱ	methanol : water = 80 : 20		4. *1*-Methylnaphthalene	(0.35 μg)
Flow rate	1.0 ml/min			5. *1,5*-Dimethylnaphthalene	(0.42 μg)
Temperature	30℃				
Detection	UV 254 nm				



Attention

1. Methanol is recommended as a mobile phase for COSMOSIL NPE column. Acetonitrile is not recommended because it has many π electrons and interferes π-π interactions between a sample and the stationary phase.
2. The stationary phase of COSMOSIL NPE, nitrophenyl group, has a large UV absorption. When the stationary phase detaches from silica gel and elutes, even a slight quantity can be detected and causes baseline noise. In such a case, wash the column with tetrahydrofuran. Detachment of a small amount of the stationary phase does not deteriorate a column's separation ability.
3. COSMOSIL NPE column is not suitable for gradient analysis.

Ordering information

COSMOSIL 5NPE Packed Column

Column size I.D. × length (mm)	Product number
1.0 × 150	05897-01
2.0 × 150	34328-51
2.0 × 250	34379-91
4.6 × 150	37902-21
4.6 × 250	37990-71
10 × 250	05469-11
20 × 250	38046-21

COSMOSIL 5NPE Guard Column

Column size I.D. × length (mm)	Product number
4.6 × 10	37904-01
10 × 20	38045-31
20 × 20	05868-81
20 × 50	05869-71

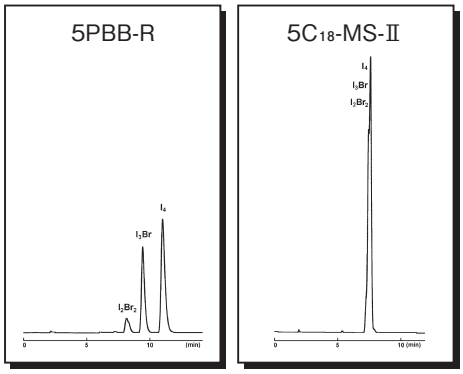
PBB-R

COSMOSIL PBB is a reversed phase column with pentabromobenzyl groups bonded silica packing material. This column provides unique selectivity for structurally similar compounds utilizing the dispersion force interaction. The dispersion force interaction of COSMOSIL PBB makes it useful for separation of structural isomers differing only by a double bond.

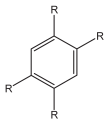
Application data

・Halogen exchange reaction products

COSMOSIL PBB-R strongly retains iodine atom which has a large dispersion force, than bromine atom. So it can separate halogen exchange reaction products that are difficult to analyze with C_{18} column.



Column size	4.6 mm I.D. × 150 mm	
Mobile phase	5PBB-R	methanol : water = 100 : 0
	$5C_{18}$-MS-Ⅱ	methanol : water = 90 : 10
Flow rate	1.0 ml/min	
Temperature	30℃	
Detection	UV 254 nm	
Sample	Halogen exchange reaction products	

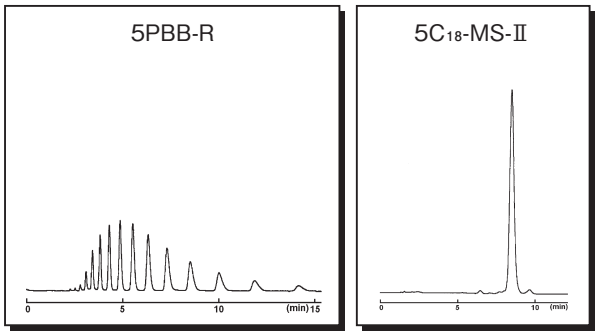


R=I or Br

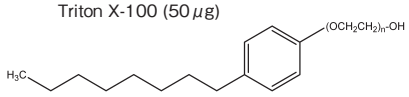
Sample courtesy of Dr. H. Yamamoto, RIKEN

・Surfactant agent

C_{18} column can not separate Triton X-100 mixturem, because (-OCH_2CH_2-) group has little hydrophobicity. However, COSMOSIL PBB-R can separate them because it distinguishes difference in the dispersion force, which depends on its molecular weight.



Column size	4.6 mm I.D. × 150 mm	
Mobile phase	5PBB-R	methanol : water = 100 : 0
	$5C_{18}$-MS-Ⅱ	methanol : water = 80 : 20
Flow rate	1.0 ml/min	
Temperature	30℃	
Detection	UV 254 nm	
Sample	Triton X-100 (50 μg)	



Ordering information

COSMOSIL 5PBB-R Packed Column

Column size I.D. × length (mm)	Product number
1.0 × 150	05899-81
2.0 × 150	05900-31
2.0 × 250	05904-91
4.6 × 150	05697-21
4.6 × 250	05698-11
10 × 250	05699-01
20 × 250	05700-51

COSMOSIL 5PBB-R Guard Column

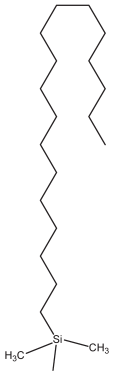
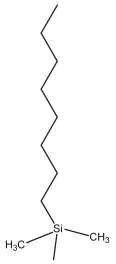
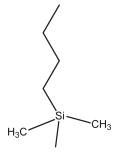
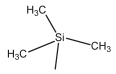
Column size I.D. × length (mm)	Product number
4.6 × 10	05704-11
10 × 20	05721-81
20 × 20	05911-91
20 × 50	05722-71

4. Reversed Phase Chromatography - Alkyl chains columns

C_{22}-AR-Ⅱ・C_8-MS・C_4-MS・TMS-MS

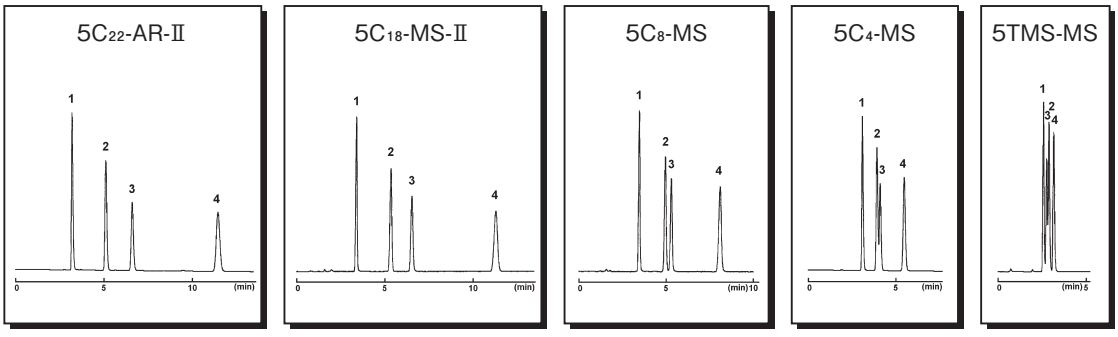
COSMOSIL alkyl type columns also include stationary phases with packing materials of C_{22} (Dococyl group), C_8 (Octadecyl group), C_4 (Butyl group) and TMS (Trimethyl group). The order of retention force by hydrophobicity of each packing material is C_{22}=C_{18}>C_8>C_4>TMS. The columns having lower hydrophobicity than C_{18} or C_{22} are effective for separation of high hydrophobic compounds and compounds with big difference in hydrophobic. Hydrophobicity of C_{22} is about the same as C_{18}. However, stereoselectivity of C_{22} is higher than C_{18}, and so C_{22} may provide better separation.

Material characteristics

Packing material	C_{22}-AR-Ⅱ	C_{18}-MS-Ⅱ	C_8-MS	C_4-MS	TMS-MS
Silica gel	high purity porous spherical silica				
Average particle size	5 μm				
Average pore size	approx. 120 Å				
Specific surface area	approx. 300 m²/g				
Stationary phase	 dococyl group	 octadecyl group	 octyl group	 butyl group	 trimethyl group
Bonding type	polymeric	monomeric			
Main interaction	hydrophobic interaction				
End capping treatment	near-perfect treatment				
Carbon content	approx. 19%	approx. 16%	approx. 10%	approx. 7%	approx. 5%

Effect of alkyl chain length on reversed phase

The shorter alkyl chain stationary phase shows shorter retention time for non-polar compounds such as benzene and toluene and longer retention for polar compounds such as acetophenone and benzoate.



Column size	4.6 mm I.D. × 150 mm	Sample	1. Acetophenone	(0.05 μg)
Mobile phase	methanol : water = 60 : 40		2. Methyl Benzoate	(0.5 μg)
Flow rate	1.0 ml/min		3. Benzene	(2.0 μg)
Temperature	30℃		4. Toluene	(2.0 μg)
Detection	UV 254 nm			

Ordering information

COSMOSIL 5C$_{22}$-AR-Ⅱ Packed Column

Column size I.D. × length (mm)	Product number
4.6 × 50	05848-41
4.6 × 100	05849-31
4.6 × 150	04598-51
4.6 × 250	04599-41
6.0 × 150	05850-91
6.0 × 250	05851-81
10 × 250	04969-91
20 × 250	05183-41

COSMOSIL 5C$_{22}$-AR-Ⅱ Guard Column

Column size I.D. × length (mm)	Product number
4.6 × 10	04881-21
10 × 20	05554-81

COSMOSIL 5C$_{8}$-MS Packed Column

Column size I.D. × length (mm)	Product number
4.6 × 50	38153-11
4.6 × 100	38154-01
4.6 × 150	38155-91
4.6 × 250	38156-81
6.0 × 150	38157-71
6.0 × 250	38158-61
10 × 250	38159-51
20 × 250	38160-11

COSMOSIL 5C$_{8}$-MS Guard Column

Column size I.D. × length (mm)	Product number
4.6 × 10	38151-31
10 × 20	38152-21

COSMOSIL 5C$_{4}$-MS Packed Column

Column size I.D. × length (mm)	Product number
4.6 × 50	38163-81
4.6 × 100	38164-71
4.6 × 150	38165-61
4.6 × 250	38166-51
6.0 × 150	38167-41
6.0 × 250	38168-31
10 × 250	38169-21
20 × 250	38170-81

COSMOSIL 5C$_{4}$-MS Guard Column

Column size I.D. × length (mm)	Product number
4.6 × 10	38161-01
10 × 20	38162-91

COSMOSIL 5TMS-MS Packed Column

Column size I.D. × length (mm)	Product number
4.6 × 50	38173-51
4.6 × 100	38174-41
4.6 × 150	38175-31
4.6 × 250	38176-21
6.0 × 150	38177-11
6.0 × 250	38178-01
10 × 250	38179-91
20 × 250	38180-51

COSMOSIL 5TMS-MS Guard Column

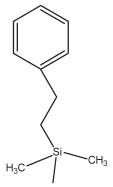
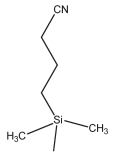
Column size I.D. × length (mm)	Product number
4.6 × 10	38171-71
10 × 20	38172-61

5. Reversed Phase Chromatography – Phenyl type, Cyano type

5PE-MS・5CN-MS

The COSMOSIL PE-MS columns (phenylethyl group) and the COSMOSIL CN-MS columns (cyanopropyl group) provide a secondary separation mode (π-π interaction). These columns are recommended when the other alkyl chain stationary phases do not offer optimum selectivity for structurally similar compounds.

Material characteristics

Packing material	PE-MS	CN-MS
Silica gel	high purity porous spherical silica	
Average particle size	approx. 5 μm	
Average pore size	approx. 120 Å	
Specific surface area	approx. 300 m²/g	
Stationary phase	 phenylethyl group	 cyanopropyl group
Bonding type	monomeric	
Main interaction	hydrophobic interaction π-π interaction	
End capping treatment	near-perfect treatment	
Carbon content	approx. 10%	approx. 7%

Ordering information

COSMOSIL 5PE-MS Packed Column

Column size I.D. × length (mm)	Product number
4.6 × 50	38183-21
4.6 × 100	38184-11
4.6 × 150	38185-01
4.6 × 250	38186-91

Column size I.D. × length (mm)	Product number
6.0 × 150	38187-81
6.0 × 250	38188-71
10 × 250	38189-61
20 × 250	38190-21

COSMOSIL 5PE-MS Guard Column

Column size I.D. × length (mm)	Product number
4.6 × 10	38181-41
10 × 20	38182-31

COSMOSIL 5CN-MS Packed Column

Column size I.D. × length (mm)	Product number
4.6 × 50	38233-61
4.6 × 100	38234-51
4.6 × 150	38235-41
4.6 × 250	38236-31

Column size I.D. × length (mm)	Product number
6.0 × 150	38237-21
6.0 × 250	38238-11
10 × 250	38239-01
20 × 250	38240-61

COSMOSIL 5CN-MS Guard Column

Column size I.D. × length (mm)	Product number
4.6 × 10	38231-81
10 × 20	38232-71

6. Silica Based Preparative Columns

COSMOSIL series is available in 10 mm I.D. and 20 mm I.D. for semi-preparative applications and 28 mm I.D. and 50 mm I.D. for preparative scales.
For column sizes and packing materials not listed below, contact either your local distributor or the manufacturer directly.

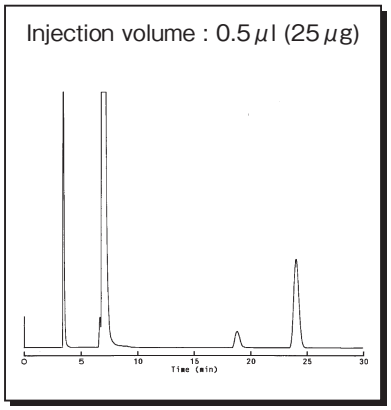
Material characteristics

Packing material	15C$_{18}$-MS-Ⅱ	15C$_{18}$-AR-Ⅱ	15C$_{18}$-PAQ
Silica gel	high purity porous spherical silica		
Average particle size	15 μm		
Average pore size	approx. 120 Å		
Specific surface area	approx. 300 m^2/g		
Stationary phase	octadecyl group (please refer to page 14)		
Bonding type	monomeric	polymeric	
Main interaction	hydrophobic interaction		
End capping treatment	near-perfect treatment		
Carbon content	approx. 16%	approx. 17%	approx. 11%
pH range	2 ~ 10※	1.5 ~ 7.5※	2 ~ 7.5
Feature	This phase is recommended for most of applications but particularly effective for basic organic compounds.	This phase is recommended for the separations requiring acidic mobile phase conditions. It also shows superior molecular shape selectivity to monomeric type C$_{18}$ columns.	This phase is designed to offer superior retention of polar compounds and excellent reproducibility in highly aqueous mobile phases, even in 100% aqueous.

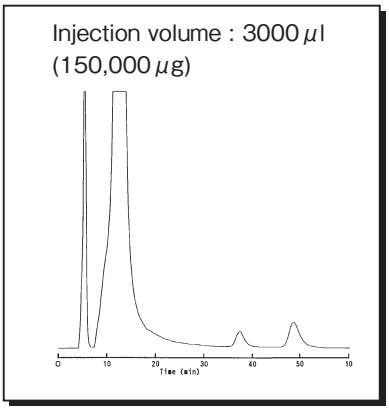
※ Optimum pH range of columns based on silica gel is between 2 and 7.5.

Preparative separation using 50 mm I.D. column

Carbazol is extracted from anthracene oil (coal tar) and required high purity because it is often used for analytical applications. Following is the preparative separation of carbazol using a 50 mm I.D. COSMOSIL 15C$_{18}$-AR-Ⅱ .



Column COSMOSIL 5C$_{18}$-AR-Ⅱ
4.6 mm I.D. × 150 mm
Flow rate 0.5 ml/min
Temperature room temperature
Detection UV 254 nm



Column COSMOSIL 15C$_{18}$-AR-Ⅱ
50 mm I.D. × 250 mm
Flow rate 60 ml/min
Temperature room temperature
Detection UV 254 nm

Please refer to TECHNICAL NOTE 8, Inner diameter of column (scale down and scale up) at page 99.

Ordering information

Please refer to page 17 for 15C$_{18}$-MS-Ⅱ , page 19 for 15C$_{18}$-AR-Ⅱ and page 21 for 15C$_{18}$-PAQ.

7. Normal Phase Chromatography column

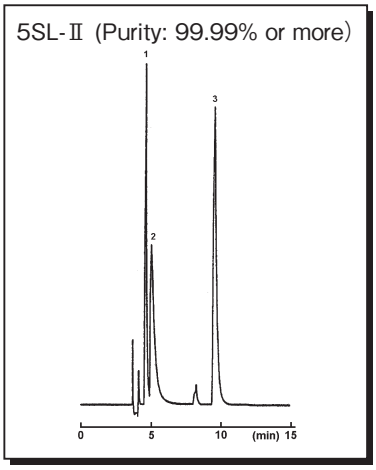
SL-Ⅱ

Ultra-pure silica gel of more than 99.99% purity is used for the COSMOSIL SL-Ⅱ packed column series. This column provides improved separation and reproducibility for compounds with carbonyl or phenol hydroxyl groups, which are often problematic to separate using conventional silica gel columns.

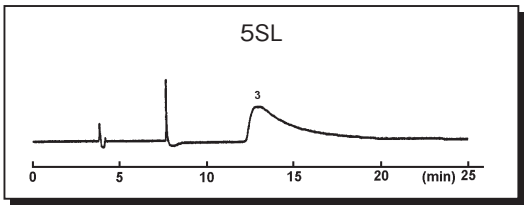
Material characteristics

Packing material	SL-Ⅱ
Silica gel	high purity porous spherical silica
Average particle size	3・5・15μm
Average pore size	approx. 120 Å
Specific surface area	approx. 300 m²/g
Feature	・High purity Silica Gel (>99.99%) with specially treatment ・Suitable for preparative separation

Analysis of acids and amide without ionic additives

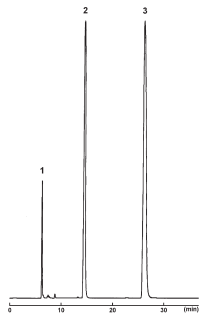
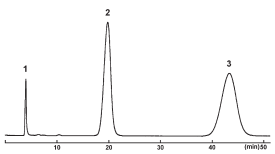
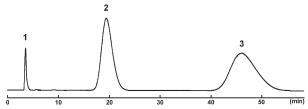


Column size 4.6 mm I.D. × 250 mm
Mobile phase ethanol : hexane = 10 : 90
Flow rate 1 ml/min
Temperature 30℃
Detection UV 254 nm, 0.5AUFS
Sample 1. Benzoic Acid (4.0μg)
2. Salicylic Acid (6.0μg)
3. Saliculamide (4.0μg)



Comparison with medium-pressure columns

COSMOSIL SL-Ⅱ offers sharper peak compared with packing materials for medium-pressure liquid chromatography and open chromatography.

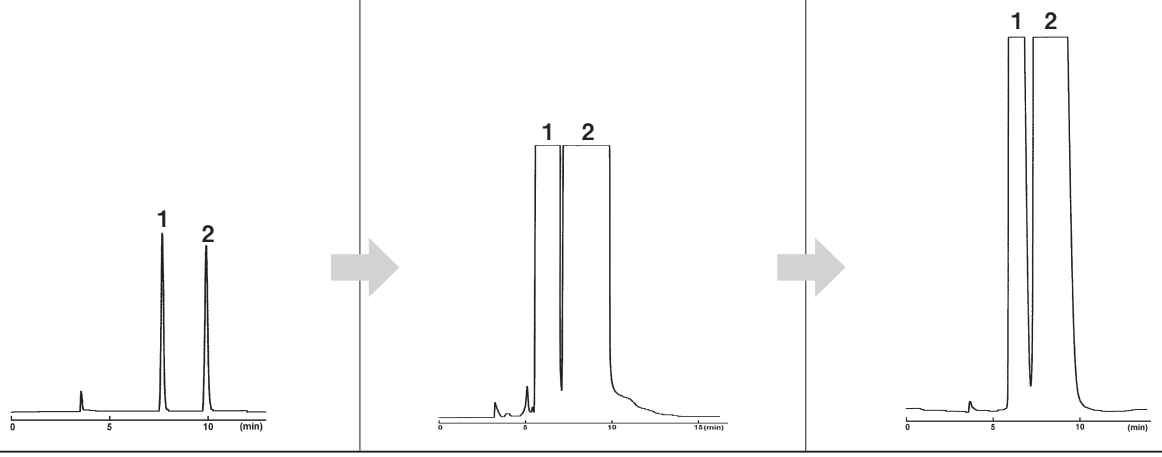
Column	5SL-Ⅱ	A company cartridge (30μm silica gel)	B company cartridge (60μm silica gel)
Column size	20 mm I.D. × 250 mm	26 mm I.D. × 104 mm	26 mm I.D. × 104 mm
Pressure	1.3MPa	0.25MPa	0.15MPa
Chromatogram			

Mobile phase ethanol : hexane = 5 : 95
Flow rate 10 ml/min
Temperature room temperature
Detection UV 254 nm

Sample 1. *p*-Xylene (8.3 mg)
2. Cinnamyl Alcohol (1.7 mg)
3. *p*-Nitrobenzyl Alcohol (4.2 mg)

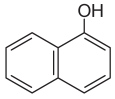
Scaling up from analytical to preparative separation

Step 1	Step 2	Step 3
Mobile phase is optimized by using 4.6 mm I.D. × 250 mm column	Maximum load capacity is optimized by using 4.6 mm I.D. × 250 mm column	Preparative separation by using 20 mm I.D. × 250 mm column
As mobile phase, highly soluble solvent for sample is suitable.	Maximum injection volume 56 μl (28 mg)	Maximum injection volume 1,050 μl (525 mg) (19 times higher than 4.6 mm I.D.)

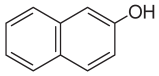


Mobile phase ethyl acetate : hexane = 10 : 90
Flow rate 4.6 mm I.D. : 1 ml/min
20 mm I.D. : 18.9 ml/min
Temperature room temperature
Detection UV 254 nm

Sample 1. *1*-Naphthol
2. *2*-Naphthol



1



2

Please refer to TECHNICAL NOTE 8, Inner diameter of column (scale down and scale up) at page 99.

Ordering information

● Analytical column (Particle size : 5 μm)

COSMOSIL 5SL-Ⅱ Packed Column

Column size I.D. × length (mm)	Product number
4.6 × 50	37999-81
4.6 × 100	38000-01
4.6 × 150	38001-91
4.6 × 250	38002-81

Column size I.D. × length (mm)	Product number
6.0 × 150	38003-71
6.0 × 250	38004-61
10 × 250	38005-51
20 × 250	38006-41
28 × 250	34358-61

COSMOSIL 5SL-Ⅱ Guard Column

Column size I.D. × length (mm)	Product number
4.6 × 10	37997-01
10 × 20	37998-91
20 × 20	05874-91
20 × 50	05875-81
28 × 50	34359-51

● Preparative column (Particle size : 15 μm)

COSMOSIL 15SL-Ⅱ Packed Column

Column size I.D. × length (mm)	Product number
28 × 250	05893-41
50 × 250	05895-21
50 × 500	05896-11

COSMOSIL 15SL-Ⅱ Guard Column

Column size I.D. × length (mm)	Product number
28 × 50	05892-51
50 × 50	05894-31

● Fast LC column (Particle size : 3 μm)

COSMOSIL 3SL-Ⅱ Packed Column

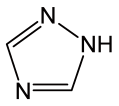
Column size I.D. × length (mm)	Product number
4.6 × 10	38059-61
4.6 × 50	38060-21
4.6 × 100	38061-11

8. Hydrophilic Interaction Chromatography

HILIC

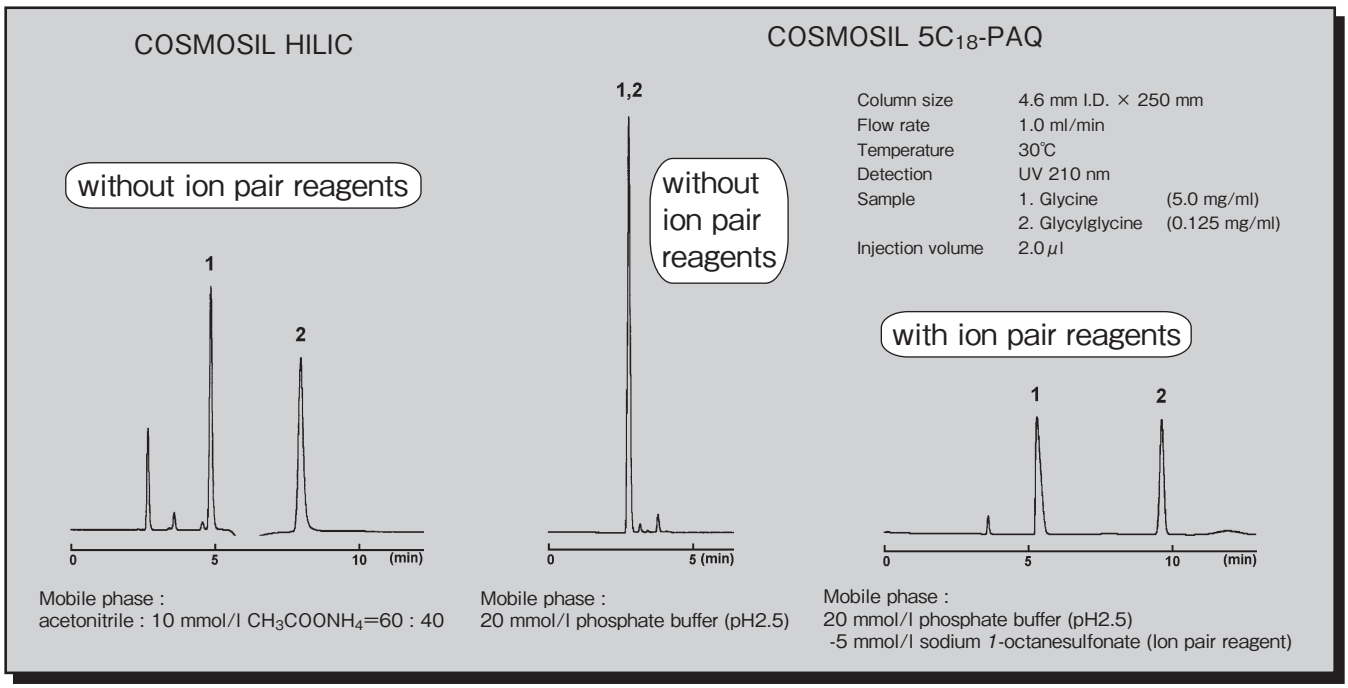
COSMOSIL HILIC is a new column for hydrophilic interaction chromatography with Triazole bonded silica packing material. The hydrophilic interaction chromatography is a variation of normal phase chromatography where a polar stationary phase is used with a mobile phase which contains a high concentration of water miscible organic solvent and a low concentration of aqueous eluent. The main retention mechanism is the partitioning of the polar analytes between the polar stationary and the non-polar mobile phase. As it is also called "aqueous normal phase", the elution order is similar to that of normal phase and the sample elution is in the order of increasing hydrophilicity. Without using ion-pair reagent COSMOSIL HILIC retains highly polar analytes that would not be retained in reversed phase chromatography. It also shows a weak anion-exchange mechanism with the positively charged stationary phase, thus acidic compound is strongly retained.

Material characteristics

Packing material	HILIC
Silica gel	high purity porous spherical silica
Average particle size	5 μm
Average pore size	approx. 120 Å
Specific surface area	approx. 300 m^2/g
Stationary phase	 Triazol
Interactions	hydrophilic interaction, anion exchange
Object substance	hydrophilic compound, acidic compound
Feature	Suitable for non-retaining by C_{18}

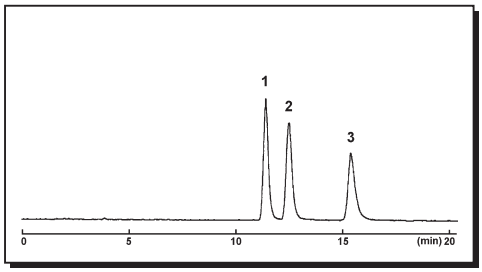
Comparison with C_{18}

COSMOSIL HILIC can separate glycine and glycylglycine without ion-pair reagent. Although C_{18} column can separate them with ion-pair reagents, there are some disadvantages such as column equilibration, preparation of mobile phase and column deterioration.



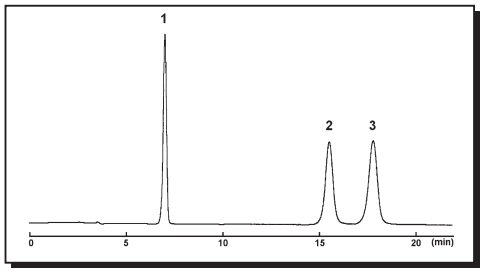
Application data

・BCAA(amino acid branched-chain)



Column	COSMOSIL HILIC
Column size	4.6 mm I.D. × 250 mm
Mobile phase	acetonitrile : 10 mmol/l CH_3COONH_4 = 85 : 15
Flow rate	1.0 ml/min
Temperature	30℃
Detection	ELSD(Gain = 6, Atten = 8)
Sample	1. Leucine (1.0 mg/ml)
	2. Isoleucine (1.0 mg/ml)
	3. Valine (1.0 mg/ml)
Injection vol.	3.0 μl

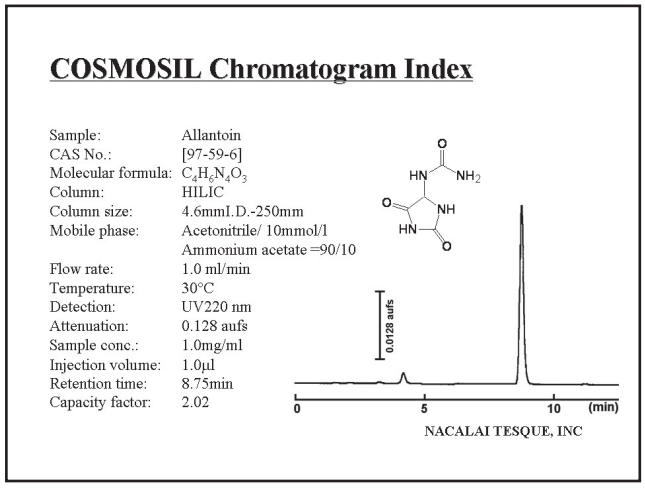
・Ascorbic Acid



Column	COSMOSIL HILIC
Column size	4.6 mm I.D. × 250 mm
Mobile phase	acetonitrile : 100 mmol/l CH_3COONH_4 = 80 : 20
Flow rate	1.0 ml/min
Temperature	30℃
Detection	UV 254 nm
Sample	1. Sorbic Acid (0.05 mg/ml)
	2. Isoascorbic Acid (0.3 mg/ml)
	3. Ascorbic Acid (0.3 mg/ml)
Injection vol.	1.0 μl

Optimizing analytical conditions

COMSOSIL HILIC Chromatogram Index, which includes 154 chromatograms using COSMOSIL HILIC, is now available online at http://www.nacalai.co.jp/en/cosmosil/. This index is useful for optimizing analytical conditions for hydrophilic interaction chromatography.



Ordering information

COSMOSIL HILIC Packed Column

Column size I.D. × length (mm)	Product number
1.0 × 150	07869-11
1.0 × 250	07870-71
2.0 × 30	08568-21
2.0 × 50	07052-91
2.0 × 100	08569-11
2.0 × 150	07054-71
2.0 × 250	07489-91

Column size I.D. × length (mm)	Product number
3.0 × 150	07871-61
3.0 × 250	07872-51
4.6 × 150	07056-51
4.6 × 250	07057-41
10 × 250	07059-21
20 × 250	07060-81
28 × 250	07875-21

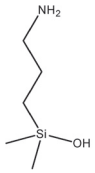
COSMOSIL HILIC Guard Column

Column size I.D. × length (mm)	Product number
4.6 × 10	07055-61
10 × 20	07058-31
20 × 20	07854-91
20 × 50	07873-41
28 × 50	07874-31

9. Saccharide Analysis

Saccharides are not retained on standard C_{18} columns because of the low hydrophobicity of compounds. COSMOSIL Sugar-D and NH_2-MS are specifically designed for separation of saccharides. COSMOSIL C_{18}-PAQ is recommended for hydrophobic glycosides or saccharide derivatives.

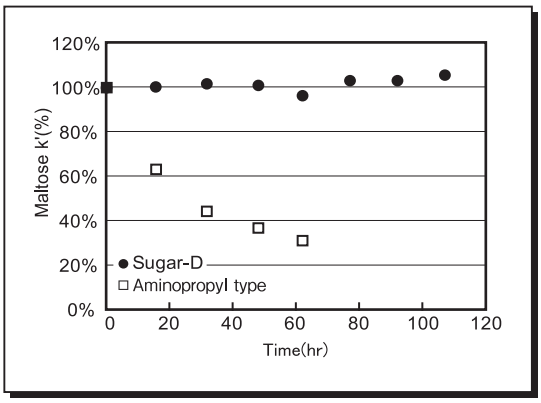
Material characteristics

Packing material	Sugar-D	NH_2-MS
Silica gel	high purity porous spherical silica	
Average particle size	5 μm	
Average pore size	—	approx. 120 Å
Specific surface area	—	approx. 300 m²/g
Stationary phase	— secondary/tertiary amine	 aminopropyl
Bonding type	—	polymeric
Object substance	monosaccharide, oligosaccharide	
End capping treatment	—	near-perfect treatment
Carbon content	—	approx. 4%
Feature	・High durability ・Good quantitative analysis	・Different selectivity from Sugar-D

Sugar-D

Conventionally aminopropyl bonded stationary phases are used for liquid chromatographic analysis of mono- and oligosaccharides. General shortcomings of the conventional aminopropyl bonded phases are tailing and adsorption of certain saccharides and the general low durability (short active life) of these columns. These problems are addressed and solved by the novel COSMOSIL Sugar-D, resulting in better (sharper) separation and much improved durability.

Durability

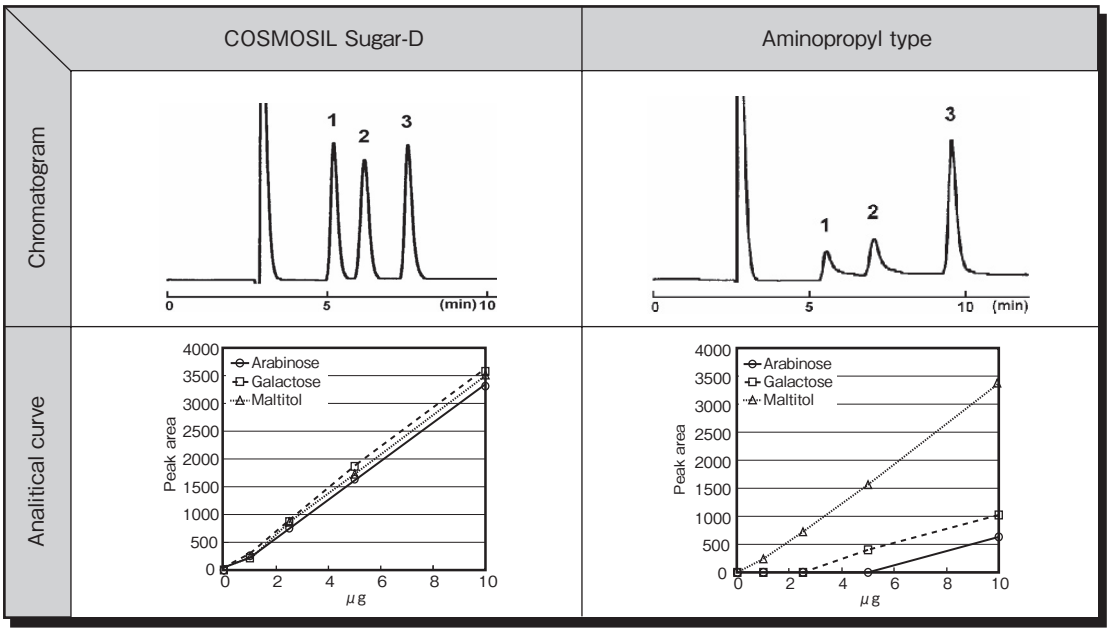


Decomposition condition

Solution	water
Flow rate	1.0 ml/min
Temperature	room temperature
Column	4.6 mm I.D. × 250 mm
Mobile phase	acetonitrile : water = 70 : 30
Flow rate	1.0 ml/min
Temperature	30℃
Detection	RI
Sample	Maltose

Adsorption characteristics

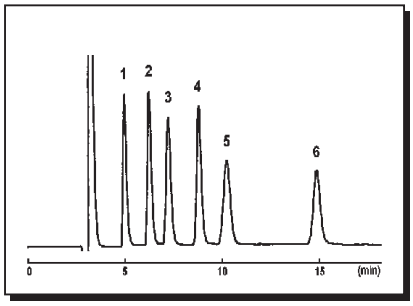
Certain types of saccharides such as arabinose or galactose are partially or temporarily adsorbed on conventional aminopropyl stationary phases causing tailing or no elution at all. COSMOSIL Sugar-D provides superior separation and high recovery for these saccharides.



Column size	4.6 mm I.D. × 250 mm	Sample	1. Arabinose
Mobile phase	acetonitrile : water = 70 : 30		2. Galactose
Flow rate	1.0 ml/min		3. Maltitol
Temperature	30℃		
Detection	RI		

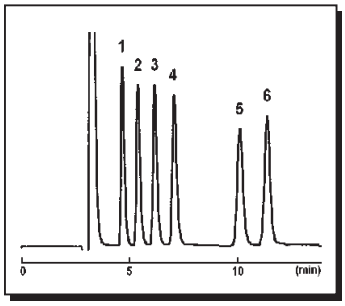
Application data

・Mono- and Oligosaccharides



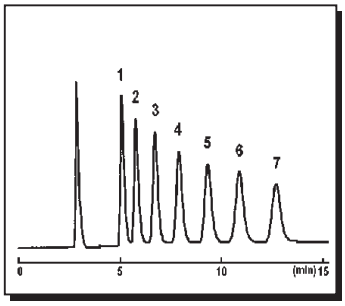
Column size	4.6 mm I.D. × 250 mm	
Mobile phase	acetonitrile : water = 75 : 25	
Flow rate	1.0 ml/min	
Temperature	30℃	
Detection	RI	
Sample	1. Rhamnose	(10 μg)
	2. Fructose	(10 μg)
	3. Glucose	(10 μg)
	4. Sucrose	(10 μg)
	5. Maltose	(10 μg)
	6. Raffinose	(10 μg)

・Polyols



Column size	4.6 mm I.D. × 250 mm	
Mobile phase	acetonitrile : water = 75 : 25	
Flow rate	1.0 ml/min	
Temperature	30℃	
Detection	RI	
Sample	1. Glycerol	(10 μg)
	2. *meso*-Erythritol	(10 μg)
	3. Xylitol	(10 μg)
	4. Glucitol	(10 μg)
	5. Maltitol	(10 μg)
	6. Inositol	(10 μg)

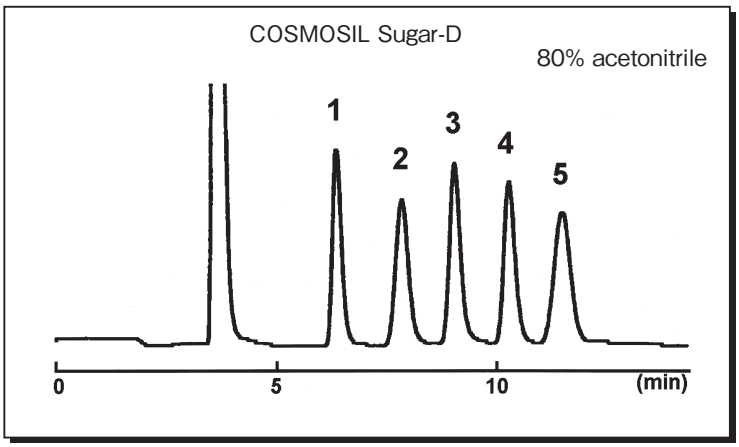
・Oligomaltoses



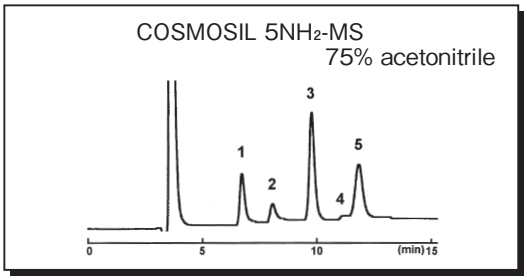
Column size	4.6 mm I.D. × 250 mm	
Mobile phase	acetonitrile : water = 65 : 35	
Flow rate	1.0 ml/min	
Temperature	30℃	
Detection	RI	
Sample	1. Glucose	(10 μg)
	2. Maltose	(10 μg)
	3. Maltotriose	(10 μg)
	4. Maltotetraose	(10 μg)
	5. Maltopentaose	(10 μg)
	6. Maltohexaose	(10 μg)
	7. Maltoheptaose	(10 μg)

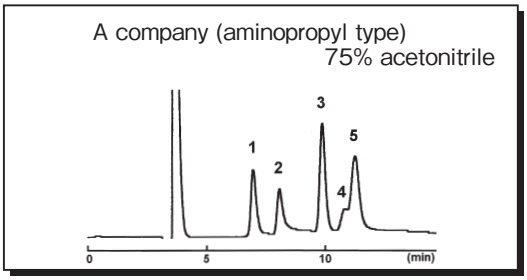
Comparison

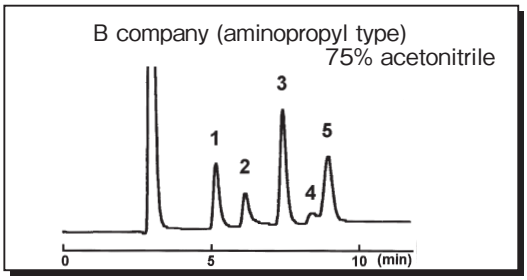
The separation and the adsorption of monosaccharides were compared using COSMOSIL Sugar-D and other companies' columns. Separation of aldoses, containing aldehyde group per molecule, is usually problematic with undesirable adsorption. COSMOSIL Sugar-D provides excellent separations for these saccharides.

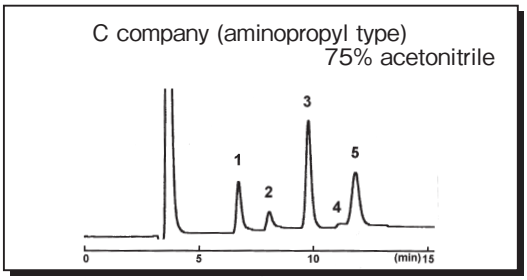


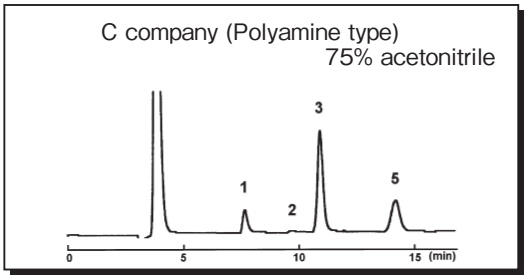
Column size	4.6 mm I.D. × 250 mm	
Mobile phase	acetonitrile : water	
Flow rate	1.0 ml/min	
Temperature	30℃	
Detection	RI	
Sample	1. Rhamnose	(10 μg)
	2. Xylose	(10 μg)
	3. Fructose	(10 μg)
	4. Mannose	(10 μg)
	5. Glucose	(10 μg)

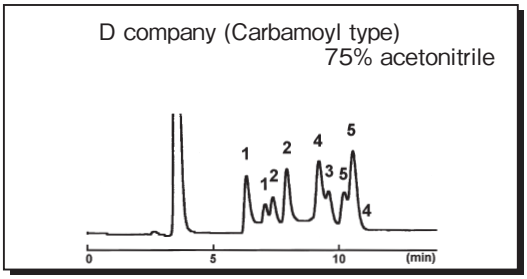


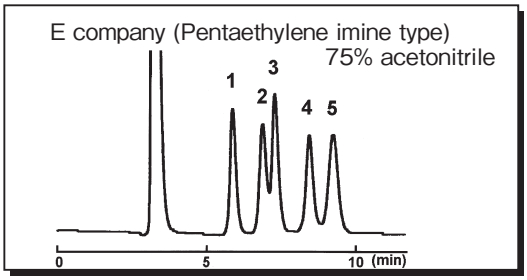


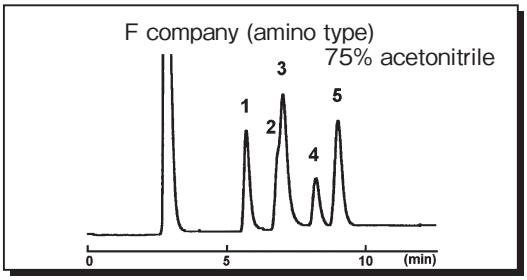






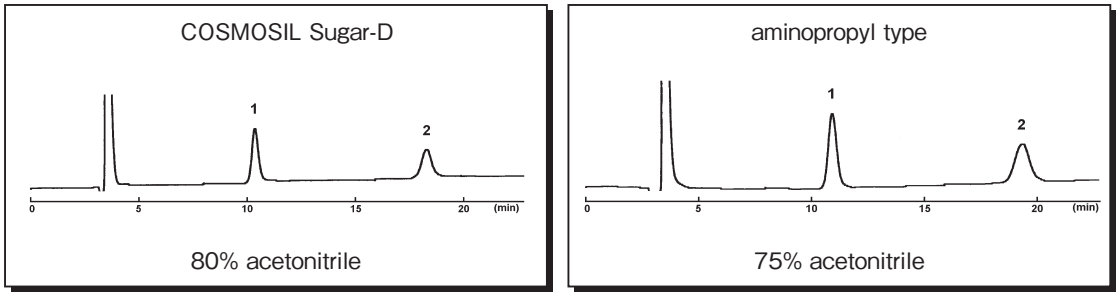






Retention

The conventional aminopropyl column is slightly more retentive than Sugar-D. The retention time of Sugar-D can be adjusted by increasing the concentration of acetonitrile in the mobile phase by 5%-10%.



Column size	4.6 mm I.D. × 250 mm	Detection	RI
Flow rate	1.0 ml/min	Sample	1. Glucose
Temperature	30℃		2. Maltose

Ordering information

COSMOSIL Sugar-D Packed Column

Column size I.D. × length (mm)	Product number
2.0 × 250	05689-31
3.0 × 150	05690-91
3.0 × 250	05691-81

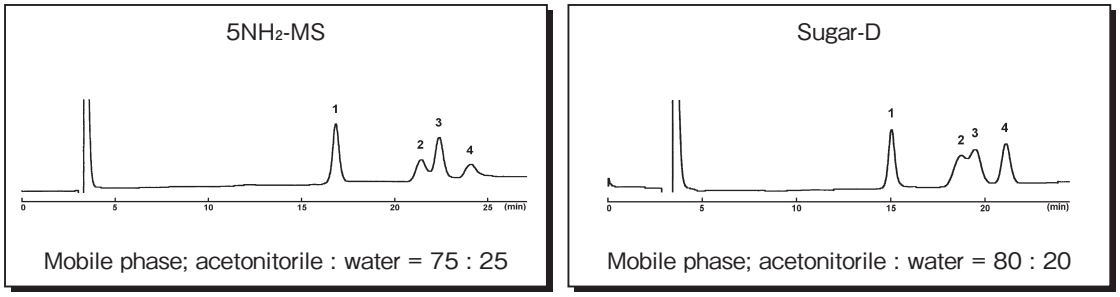
Column size I.D. × length (mm)	Product number
4.6 × 150	05395-71
4.6 × 250	05397-51
10 × 250	05692-71
20 × 250	05693-61

COSMOSIL Sugar-D Guard Column

Column size I.D. × length (mm)	Product number
4.6 × 10	05394-81
10 × 20	05696-31
20 × 50	05694-51

NH_2-MS

The COSMOSIL 5NH_2-MS is a column with aminopropyl group bonded silica packing material. It is recommended for separation of monosaccharides and oligosaccharides. While Sugar-D is our first choice for separation of saccharide, 5NH_2-MS can give better separation than Sugar-D in some cases depending on samples.



Column size	4.6 mm I.D. × 250 mm	Sample	1. Sucrose	(10 μg)
Flow rate	1.0 ml/min		2. Maltose	(10 μg)
Temperature	30℃		3. Lactose	(10 μg)
Detection	RI		4. Trehalose	(10 μg)

Ordering information

COSMOSIL 5NH_2-MS Packed Column

Column size I.D. × length (mm)	Product number
4.6 × 150	38245-11
4.6 × 250	38246-01

Column size I.D. × length (mm)	Product number
10 × 250	38249-71
20 × 250	38250-31

COSMOSIL 5NH_2-MS Guard Column

Column size I.D. × length (mm)	Product number
4.6 × 10	38241-51
10 × 20	38242-41
20 × 50	06093-91

10. Protein Separation Wide Pore Columns

Protein-R

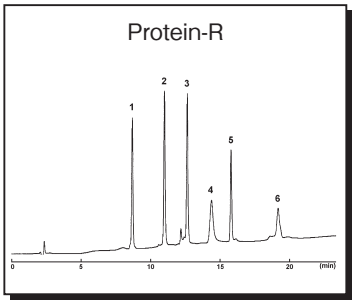
COSMOSIL Protein-R is a reversed phased HPLC column designed specifically for peptide and protein separations. This column provides improved separation, high recovery rate and acid-resistant, which are often problematic to separate using conventional C_{18}-300 Å (wide pore) and C_4-300 Å (wide pore).

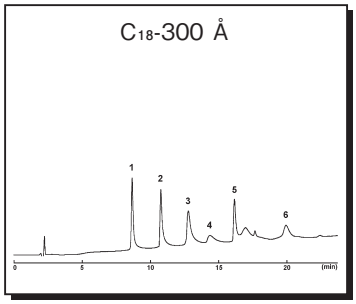
Material characteristics

Packing material	Protein-R
Silica gel	high purity porous spherical silica
Average particle size	5 μm
Average pore size	approx. 300 Å
Specific surface area	approx. 150 m²/g
Stationary phase	octadecyl group
Bonding type	polymeric
Main Interactions	hydrophobic interaction
End capping treatment	near-perfect treatment
Feature	・High recovery rate ・Acid-resistant

Comparison of separation

Protein-R shows sharper peaks for proteins than conventional C_{18} wide pore columns.

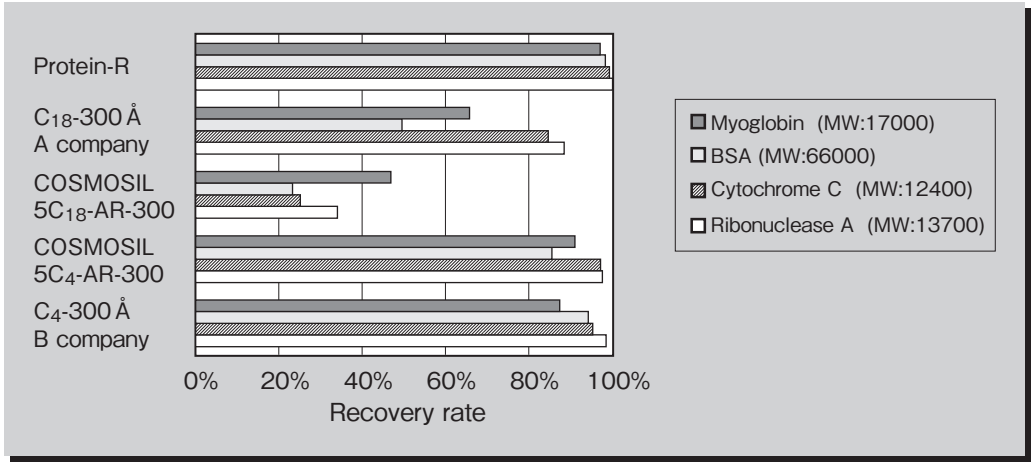




Column size	4.6 mm I.D. × 150 mm
Mobile phase	A : 0.05%TFA - 20% acetonitrile
	B : 0.05%TFA - 60% acetonitrile
	B conc. 0 → 100% 20 min linear gradient
Flow rate	1.0 ml/min
Temperature	30℃
Detection	UV 220 nm
Sample	1. Ribonuclease A (1.0 μg)
	2. Cytochrome C (1.0 μg)
	3. Lysozyme (1.0 μg)
	4. BSA (1.0 μg)
	5. Myoglobin (1.0 μg)
	6. Ovalbumin (1.0 μg)

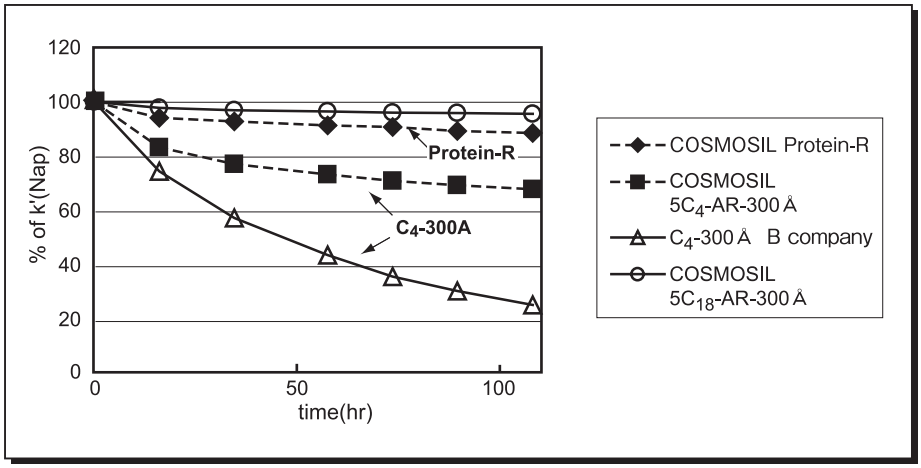
Recovery rate

The figure below shows recovery rates for proteins using different columns. Protein-R shows a higher recovery rate than C_4-300 and a much higher recovery rate than C_{18}-300.



Durability

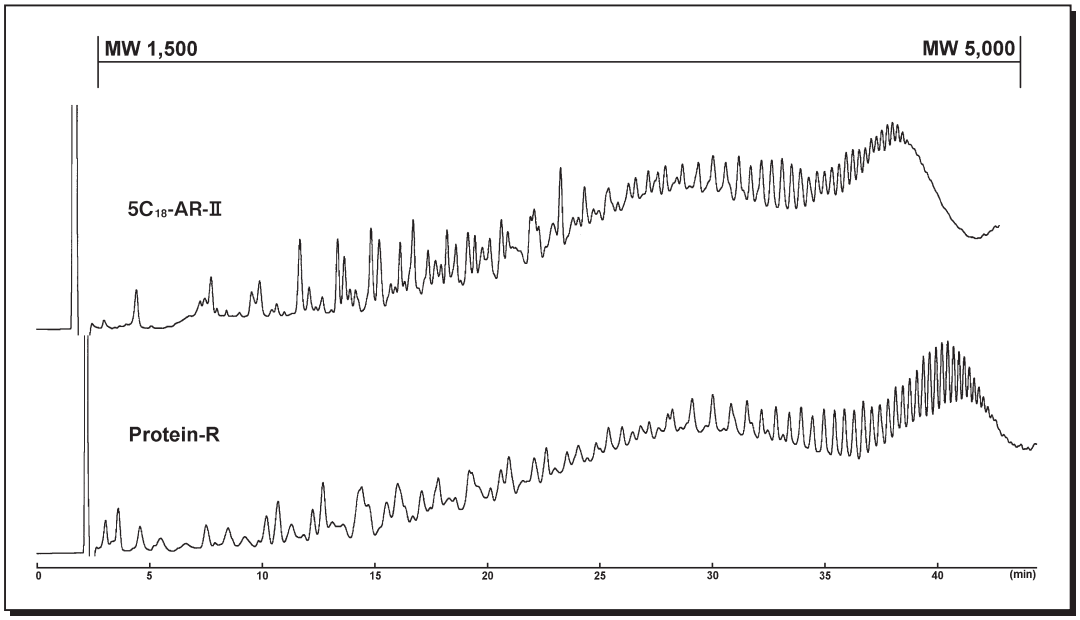
The figure below shows durability against acidic mobile phase of various columns. Protein-R shows a higher acid durability than C_4-300.



Degradation test with 0.1%-Trifluoroacetic Acid at 60℃
(k') : Napthalene in the mobile phase (methanol : water = 50 : 50)

Application of peptide separation

$5C_{18}$-MS-Ⅱ (pore size 120 Å) shows better separation of low molecular weight proteins, but Protein-R shows better separation of high-molecular weight proteins.



Ordering information

COSMOSIL Protein-R Packed Column

Column size I.D. × length (mm)	Product number
2.0 × 150	06514-71
4.6 × 50	06525-31
4.6 × 150	06526-21
4.6 × 250	06527-11

Column size I.D. × length (mm)	Product number
10 × 150	06529-91
10 × 250	06530-51
20 × 150	06531-41
20 × 250	06532-31

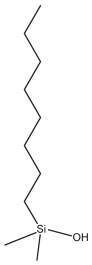
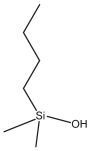
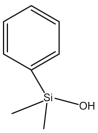
COSMOSIL Protein-R Guard Column

Column size I.D. × length (mm)	Product number
4.6 × 10	06518-31
10 × 20	06528-01

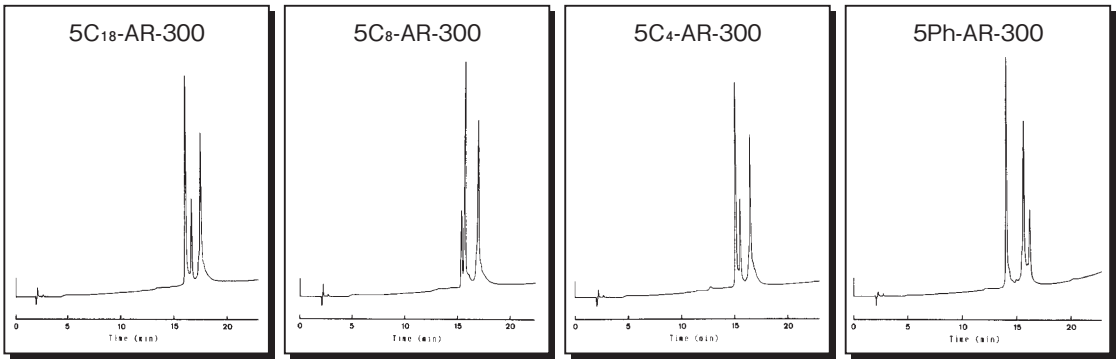
C_{18}-AR-300 · C_8-AR-300 · C_4-AR-300 · Ph-AR-300

COSMOSIL offers a variety of stationary phases with wide-pore silica gel material for separations of polypeptides and proteins.

Material characteristics

Packing material	5C_{18}-AR-300	5C_8-AR-300	5C_4-AR-300	5Ph-AR-300
Silica gel	high purity porous spherical silica			
Average particle size	5 μm			
Average pore size	approx. 300 Å			
Specific surface area	approx. 150 m²/g			
Stationary phase	 octadecyl group	 octyl group	 butyl group	 phenyl group
Bonding type	polymeric			
Main Interaction	hydrophobic interaction			hydrophobic interaction π-π interaction
End capping treatment	near-perfect treatment			
Carbon content	approx. 12%	approx. 7%	approx. 6%	approx. 7%

Comparison of separation



Column size 4.6 mm I.D. × 150 mm
Mobile phase A : 0.05%TFA - 20% acetonitrile
B : 0.05%TFA - 60% acetonitrile
B conc. 0 → 100% 20 min linear gradient
Flow rate 1 ml/min
Temperature 30℃
Detection UV 220 nm
Sample Hemoglobine, Bovine (10 μg)

Ordering information

COSMOSIL 5C$_{18}$-AR-300 Packed Column

Column size I.D. × length (mm)	Product number
4.6 × 50	37911-01
4.6 × 150	37913-81
4.6 × 250	37914-71

Column size I.D. × length (mm)	Product number
10 × 150	37917-41
10 × 250	37918-31
20 × 150	37919-21
20 × 250	37920-81

COSMOSIL 5C$_{18}$-AR-300 Guard Column

Column size I.D. × length (mm)	Product number
4.6 × 10	37910-11
10 × 20	37965-11

COSMOSIL 5C$_{8}$-AR-300 Packed Column

Column size I.D. × length (mm)	Product number
4.6 × 50	37951-81
4.6 × 150	37953-61
4.6 × 250	37954-51

Column size I.D. × length (mm)	Product number
10 × 150	34345-21
10 × 250	34247-11
20 × 150	05861-51
20 × 250	34364-71

COSMOSIL 5C$_{8}$-AR-300 Guard Column

Column size I.D. × length (mm)	Product number
4.6 × 10	37950-91
10 × 20	34464-61

COSMOSIL 5C$_{4}$-AR-300 Packed Column

Column size I.D. × length (mm)	Product number
4.6 × 50	37956-31
4.6 × 150	37958-11
4.6 × 250	37959-01

Column size I.D. × length (mm)	Product number
10 × 150	34249-91
10 × 250	38047-11
20 × 150	34477-01
20 × 250	38048-01

COSMOSIL 5C$_{4}$-AR-300 Guard Column

Column size I.D. × length (mm)	Product number
4.6 × 10	37955-41
10 × 20	05862-41

COSMOSIL 5Ph-AR-300 Packed Column

Column size I.D. × length (mm)	Product number
4.6 × 50	37961-51
4.6 × 150	37963-31
4.6 × 250	37964-21

Column size I.D. × length (mm)	Product number
10 × 150	05865-11
10 × 250	34267-51
20 × 150	05866-01
20 × 250	34468-21

COSMOSIL 5Ph-AR-300 Guard Column

Column size I.D. × length (mm)	Product number
4.6 × 10	37960-61
10 × 20	34268-41

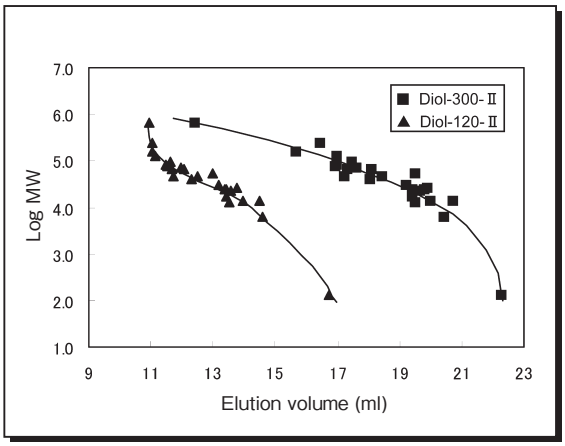
Gel filtration chromatography - Diol-120-Ⅱ・Diol-300-Ⅱ

COSMOSIL Diol-120-Ⅱ and Diol-300-Ⅱ gel filtration columns are ideal for the size-based separation of proteins and other water soluble polymers. The separation MW range is 5,000 – 700,000 daltons for proteins and 300 – 300,000 daltons for water soluble polymers when Diol-120-Ⅱ and Diol-300-Ⅱ are used in series.

Material characteristics

Packing material		5Diol-120-Ⅱ	5Diol-300-Ⅱ
Silica gel		high purity porous spherical silica	
Average particle size		5 μm	
Average pore size		approx. 120 Å	approx. 300 Å
Specific surface area		diol group	
Object substance		protein, water soluble polymer	
Flow rate		0.5-1.0 (ml/min)	
Selection of pore size	Protein	5,000-100,000 Da	10,000-700,000 Da
	Water soluble polymer	300-30,000 Da	500-300,000 Da

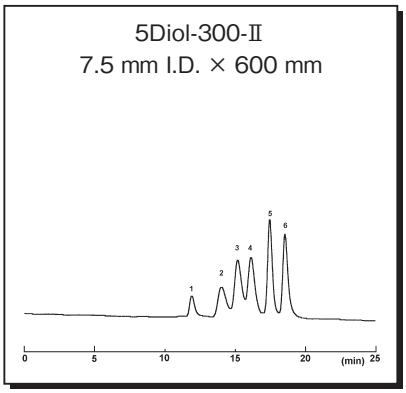
Calibration curve of proteins

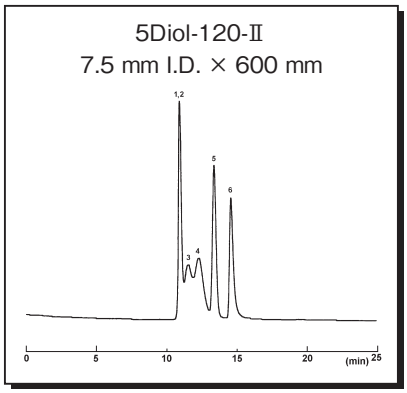


Column COSMOSIL 5Diol-Ⅱ 7.5 mm I.D. × 600 mm
Mobile phase 20 mmol/l phosphate buffer (pH 7.0) + 100 mmol/l Na_2SO_4
Flow rate 1.0 ml/min
Temperature 30℃

Sample	M. W.	Sample	M. W.
Thyroglobulin	660,000	Albumin (Ovalbumin)	45,000
Catalase	250,000	Peroxidase	40,000
Glucose Oxidase	160,000	Carbonic Anhydrase	30,000
Uricase	128,000	α-Chymotrypsinogen A	25,700
Choline Oxidase	95,000	α-Chymotrypsin	25,200
Transferrin	85,000	Trypsinogen	24,000
Conalbumin	77,500	Trypsin (bovine)	23,300
Malate Dehydrogenase	70,000	Myoglobin	17,000
α-Glucosidase	68,500	Lysozyme	14,300
Albumin (BSA)	66,000	Ribonuclease A	13,700
α-Amylase	52,500	Cytochrome C	12,400
Fetuin	48,000	Aprotinin	6,500
		Gly-Gly	132

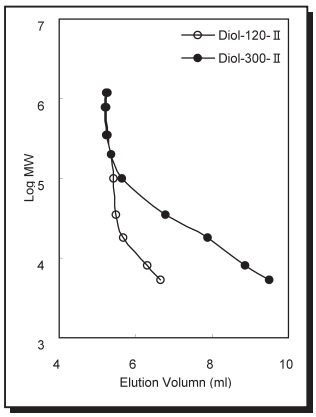
Application data

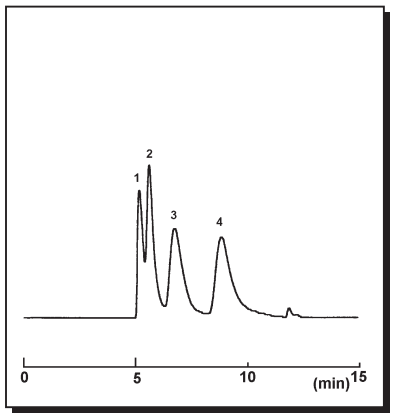




Column COSMOSIL 5Diol
Mobile phase 20 mmol/l phosphate buffer (pH 7.0)
+100 mmol/l Na_2SO_4
Flow rate 1.0 ml/min
Temperature room temperature
Detection UV 220 nm
Sample 1. Thyroglobulin
2. Glucose Oxidase
3. Conalbumin
4. Peroxidase
5. Myoglobin
6. Aprotinin

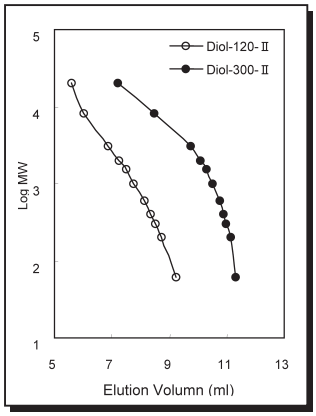
・Water soluble polystyrene

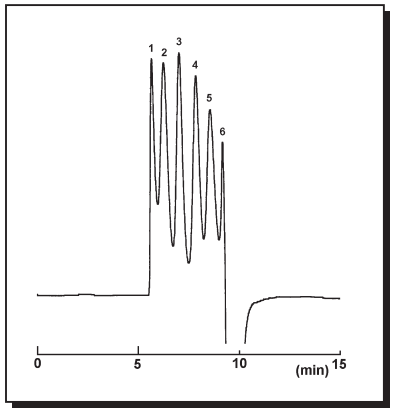




Column	COSMOSIL 5Diol-300-Ⅱ
	7.5 mm I.D. × 300 mm
Mobile phase	20 mmol/l phosphate buffer（pH 6.7）
Flow rate	1.0 ml/min
Temperature	30℃
Detection	UV 254 nm
Sample	Sodium Polystyrene Sulfonate
	1. MW 780,000
	2. MW 100,000
	3. MW 35,000
	4. MW 8,000

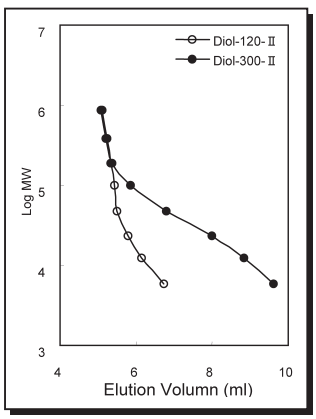
・Polyethylene glycol

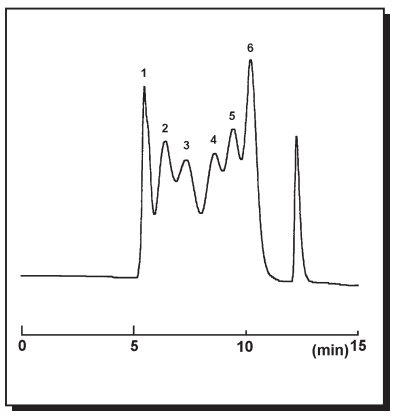




Column	COSMOSIL 5Diol-120-Ⅱ
	7.5 mm I.D. × 300 mm
Mobile phase	methanol : water = 20 : 80
Flow rate	1.0 ml/min
Temperature	30℃
Detection	RI
Sample	Polyethylene Glycol
	1. PEG 20,000
	2. PEG 6,000
	3. PEG 4,000
	4. PEG 1,000
	5. PEG 300

・Pullulan





Column	COSMOSIL 5Diol-300-Ⅱ
	7.5 mm I.D. × 300 mm
Mobile phase	water
Flow rate	1.0 ml/min
Temperature	30℃
Detection	UV 254 nm
Sample	Pullulan
	1. P-800
	2. P-100
	3. P-50
	4. P-20
	5. P-5

Ordering information

COSMOSIL 5Diol-120-Ⅱ Packed Column

Column size I.D. × length (mm)	Product number
7.5 × 300	38050-51
7.5 × 600	38051-41

COSMOSIL 5Diol-120-Ⅱ Guard Column

Column size I.D. × length (mm)	Product number
7.5 × 50	38049-91

COSMOSIL 5Diol-300-Ⅱ Packed Column

Column size I.D. × length (mm)	Product number
7.5 × 300	38053-21
7.5 × 600	38054-11

COSMOSIL 5Diol-300-Ⅱ Guard Column

Column size I.D. × length (mm)	Product number
7.5 × 50	38052-31

Ion-exchange Chromatography – DEAE・QA・CM・SP

The packing materials for COSMOGEL ion-exchange glass columns are based on hydrophilic polymethacrylate 10 μm particles with a 1000 Å pore size. COSMOGEL packed columns are available with DEAE, a weak anion exchanger; QA, a strong anion exchanger ; CM, a weak cation exchanger; and SP, a strong cation exchanger. The availability of four different ion exchangers provides chromatographers with the flexibility of column selection based on charge differences of samples.

Material characteristics

Packing material	DEAE	QA	CM	SP
Type	Diethylaminoethyl type weak anion exchange	Quaternary ammonium type strong anion exchange	Carboxymethyl type weak cation exchange	Sulfopropyl type strong cation exchange
Gel	totally porous spherical hydrophilic polymer			
Average particle size	10 μm			
Average pore size	approx. 1000 Å			
Functional group	$N^+H(C_2H_5)_2$	$N^+(CH_3)_3$	COO^-	SO_3^-
Counter ion	Cl^-	Cl^-	Na^+	Na^+
Capacity (meq/g)	0.6	0.4	0.3	0.4
50% Ionization pH	10.8	11.0	5.7	2.6
pH range	< 11	whole area	> 4	whole area
Flow rate (appropriate)	7.5 mm I.D. / 8.0 mm I.D. ; 0.5 ~ 1.0 ml/min		20 mm I.D. ; 4.0 ~ 6.0 ml/min	
Flow rate (maximum)	7.5 mm I.D. / 8.0 mm I.D. ; 1.5 ml/min		20 mm I.D. ; 8.0 ml/min	
Pressure (maximum)	1.5 MPa			
Temperature	10 ~ 50℃			

Selection of the mobile phase

Generally, anion-exchange columns are operated with the mobile phase pH at least one point higher than the isoelectric point (pI) of samples, while cation-exchange columns are operated with the mobile phase pH at least one point lower than the pI. The elution force of bivalent ions such as Bis-tris HCl is stronger than univalent ions such as Tris HCl.

Table. Buffer type and pH

Anion exchange (DEAE, QA)	pH	Cation exchange (CM, SP)
	4.0	Formic acid buffer
Piperazine buffer	5.0	Acetic acid buffer
Bis-Tris buffer	6.5	Phosphoric acid buffer
Tris buffer	8.0	HEPES buffer
Monoethanolamine buffer	9.5	

The initial mobile Phase (A) is 20-50 mmol/l of one of the above mentioned buffer solutions and the final mobile phase (B) is the mobile phase (A) with an addition of 20-600 mmol/l of salt.

Selection of salts

High concentration of salts, generally NaCl, is used in elution buffers. When stronger elution buffer is needed, $CaCl_2$ or $MgCl_2$ can be used for DEAE and QA columns, and Na_2SO_4 can be used for CM and SP columns.

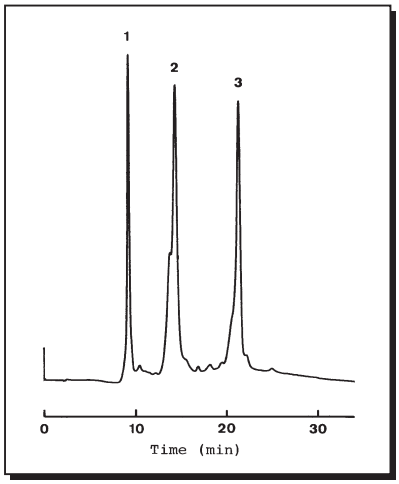
Selection of organic solvents

Water miscible solvents can be used when the elution is not strong enough. COSMOGEL columns can be used with up to 20% of water miscible solvents such as acetonitrile and *2*-propanol.

Application data

・Separation of proteins on anion exchange columns

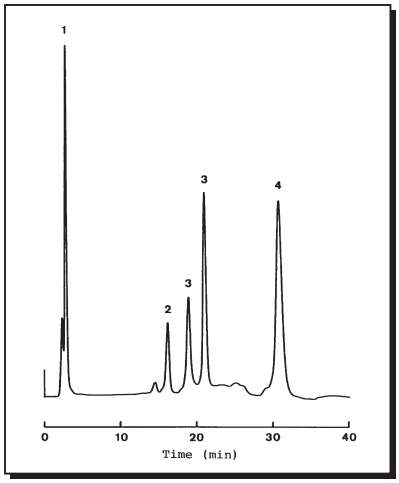
The higher the negative charge, the longer the sample is retained on an anion exchange column. As shown below, with a weak alkaline mobile phase, the lower the isoelectric point, the longer the sample is retained.



Column	COSMOGEL DEAE 7.5 mm I.D. × 75 mm
Mobile phase	A: 20 mmol/l Tris-HCl buffer (pH 8.2)
	B: 500 mmol/l NaCl in buffer A
	B 0%→ 100% /30min linear gradient
Flow rate	1.0 ml/min
Temperature	30℃
Detection	UV 280 nm, 0.16AUFS
Sample	1. Myoglobin (20 μg)
	2. Transferrin (50 μg)
	3. Trypsin Inhibitor (70 μg)

・Separation of proteins on cation exchange columns

The higher the positive charge, the longer the sample is retained on cation-exchange column. As shown below, with a weak acidic mobile phase, the higher the isoelectric point, the longer the sample is retained.

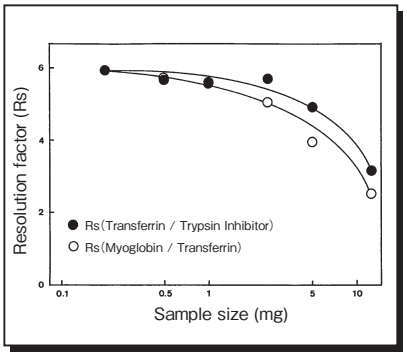


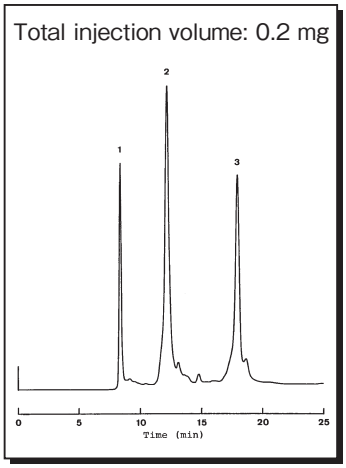
Column	COSMOGEL CM 7.5 mm I.D. × 75 mm
Mobile phase	A: 20 mmol/l phosphate buffer (pH 6.5)
	B: 500 mmol/l NaCl in buffer A
	B 0 → 100% /30 min linear gradient
Flow rate	1.0 ml/min
Temperature	30℃
Detection	UV 280 nm, 0.32AUFS
Sample	1. Myoglobin (50 μg)
	2. Ribonuclease A (50 μg)
	3. Cytochrome C (50 μg)
	4. Lysozyme (50 μg)

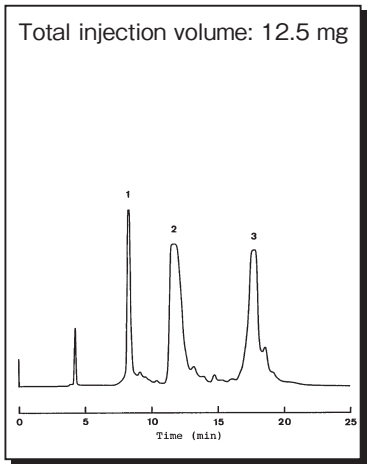
Sample loading capacity and resolution

Up to 1 mg of sample can be well separated on an 8 mm I.D. column.

If the resolution is high enough, 10 mg of sample can be separated.







Condition

Column	COSMOGEL DEAE GLASS Packed Column, 8.0 mm I.D. × 75 mm
Mobile phase	A: 20 mmol phosphate buffer (pH 8.2)
	B: 500 mmol NaCl in buffer A
	B 0 → 100%/30 min liner gradient
Flow rate	1.0 ml/min
Temperature	30℃
Detection	UV 280 nm
Sample	1. Myoglobin
	2. Transfferin
	3. Trypsin Inhibitor,Soybean

Ordering information

COSMOGEL Glass Packed Column

Product name	Column size I.D. × length (mm)	Product number
COSMOGEL DEAE Glass Packed Column	8.0 × 75	37845-81
COSMOGEL QA Glass Packed Column		37846-71
COSMOGEL CM Glass Packed Column		37844-91
COSMOGEL SP Glass Packed Column		37847-61

COSMOGEL Stainless Packed Column

Product name	Column size I.D. × length (mm)	Product number
COSMOGEL DEAE Stainless Packed Column	7.5 × 75	43371-91
COSMOGEL QA Stainless Packed Column		43373-71
COSMOGEL CM Stainless Packed Column		43375-51
COSMOGEL SP Stainless Packed Column		43377-31

Hydrophobic Interaction Chromatography - HIC

COSMOSIL 5HIC is designed for one step desalting and separation of proteins. Hydrophobic interaction chromatography (HIC) is an effective method for purification and separation of proteins (especially enzymes) based on differences in their surface hydrophobicity. Since this method does not use organic solvents like reversed phase chromatography, there is only a little loss in enzyme activity and the tertiary structure of proteins.

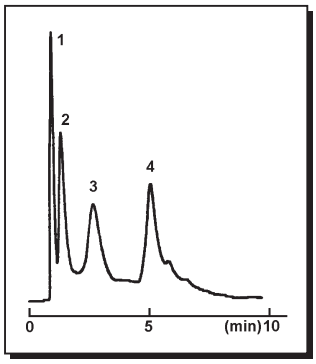
Material characteristics

Packing material	HIC
Silica gel	high purity porous spherical silica
Average particle size	5 μm
Average pore size	approx. 300 Å
Specific surface area	approx. 150 m^2/g
Main interaction	hydrophobic interaction

Application data

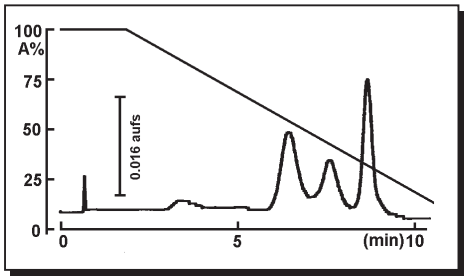
・Separation of protein standards

A buffer with high salt concentration, usually 1-2 mol/l of $(NH_4)_2SO_4$, is used as an initial mobile phase for adsorption of samples to a weakly hydrophobic stationary phase. The elution is done with a decreasing salt gradient.



Column size	4.6 mm I.D. × 50 mm	
Mobile phase	A: 20 mmol/l phosphate buffer + 100 mmol/l Na_2SO_4 + 1.5 mol/l $(NH_4)_2SO_4$ (pH6.0)	
	B: 20 mmol/l phosphate buffer + 100 mmol/l Na_2SO_4 (pH6.7)	
	B 0 → 100% /10 min linear gradient	
Flow rate	1.0 ml/min	
Temperature	30℃	
Detection	UV 220 nm	
Sample	1. Myoglobin	(0.5 mg/ml)
	2. β-Lactoglobulin	(1.0 mg/ml)
	3. Hemoglobin	(2.5 mg/ml)
	4. BSA	(1.0 mg/ml)
Injection vol.	mixture 2.0 μl	

・Separation of crude beta-Glucosidase



Column size	4.6 mm I.D. × 50 mm
Mobile phase	A: 20 mmol/l phosphate buffer + 100 mmol/l Na_2SO_4 + 2.0 mol/l $(NH_4)_2SO_4$ (pH6.0)
	B: 20 mmol/l phosphate buffer + 100 mmol/l Na_2SO_4 (pH6.7)
	B 0 → 100% /10 min linear gradient
Flow rate	1.0 ml/min
Temperature	30℃
Detection	UV 220 nm
Concentration	10.0 mg/ml
Injection vol.	1.5 μl

Ordering information

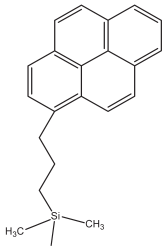
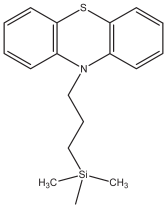
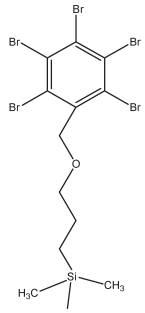
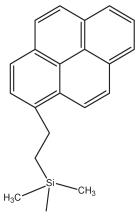
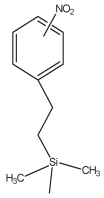
COSMOSIL 5HIC Packed Column

Column size I.D. × length (mm)	Product number
4.6 × 50	04263-21

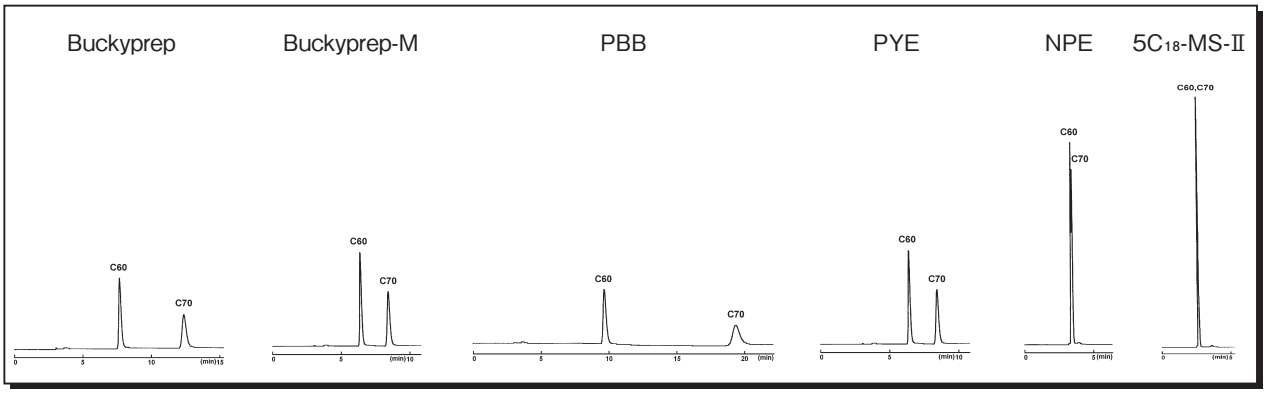
11. Special Column for Fullerenes

Separation of fullerenes, especially preparative-scale separation, on conventional HPLC columns is always problematic due to the low solubility and small recovery of fullerenes. COSMOSIL offers a variety of columns designed for preparative-scale separation of fullerenes including higher fullerenes, metallofullerenes and fullerene derivatives.

Material characteristics

Packing material	Buckyprep	Buckyprep-M	PBB	PYE	NPE
Silica gel	high purity porous spherical silica				
Average particle size	5 μm				
Average pore size	approx. 120 Å				
Specific surface area	approx. 300 m²/g				
Stationary phase	 pyrenylpropyl	 phenothiazinyl group	 pentabromobenzyl group	 pyrenylethyl group	 nitrophenylethyl group
Bonding type	monomeric				
End capping treatment	near-perfect	none	near-perfect	near-perfect	near-perfect
Carbon content	approx. 17%	approx. 13%	approx. 8%	approx. 18%	approx. 9%
Feature	Standard column for fullerenes separation.	Designed to separate metallofullerenes.	Designed for preparative separation of C_{60}, C_{70}.	Separation of fullerene and structural isomers.	Separation of fullerene derivatives

Comparison of retention in toluene



Column 4.6 mm I.D × 250 mm
Mobile phase toluene
Flow rate 1.0 ml/min
Temperature 30℃
Detection UV 312 nm
Sample 1. C_{60}
2. C_{70}

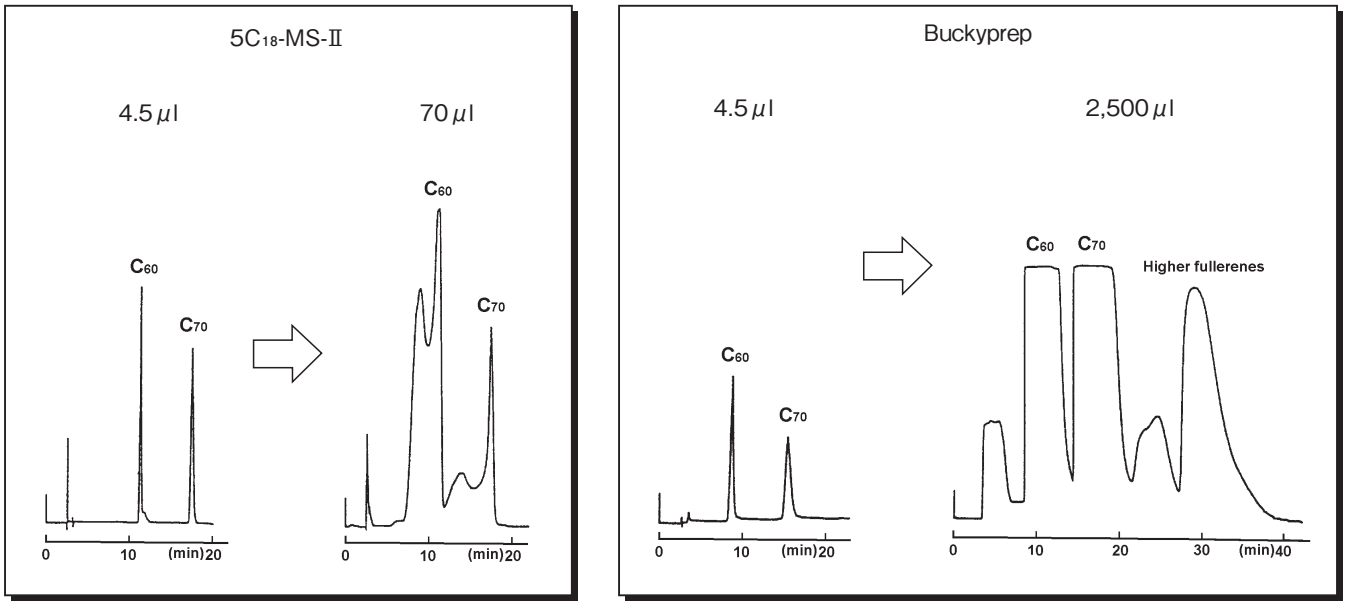
Solubility and boiling point of each solvent for C_{60}

Solvent	mg/ml	b.p. (℃)
Methanol	0.001	64.5
Acetonitrile	0.018	81.8
n-Hexane	0.046	68.7
Toluene	3.2	111
Chlorobenzene*	7.0	132
Carbon disulfide	12	46.3
o-Dichlorobenzene*	27	180
1,2,4-Trichlorobenzene	21.3	213

*: R.S.Ruoff, et al., J.Phy.Chem.,97,3379 (1993)

Toluene is the most commonly used solvent for fullerene separation.

Comparison with C_{18}



Column	4.6 mm I.D × 250 mm
Mobile phase	$5C_{18}$-MS-Ⅱ : toluene : acetonitrile = 55 : 45
	Buckyprep : toluene
Flow rate	1.0 ml/min
Temperature	30℃
Detection	UV 285 nm
Sample	Fullerene toluene extract (2.5 mg/ml)

Suggested solvents

Chlorobenzene	Stronger eluent than toluene. Recommended for higher fullerenes.
o-Dichlorobenzene	Stronger eluent than chlorobenzene.
1,2,4-Trichlorobenzene	Strongest eluent. It can be used as a washing solvent for higher fullerenes. To wash a column, inject 3 ml of *1,2,4*-trichlorobenzene to a 4.6 mm I.D. × 250 mm column and 50 ml to a 20 mm I.D. × 250 mm column after every operation.
n-Hexane	Weak eluent. Recommended for weakly retained fullerenes.
Acetonitrile	Weak eluent. Recommended for weakly retained fullerenes.

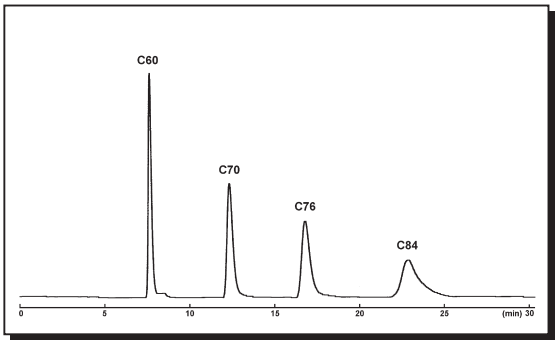
Note : Use them after filtration or distillation, if they are not for HPLC.

Buckyprep

COSMOSIL Buckyprep is a pyrenylpropyl group bonded silica based column specifically designed for fullerene separation. The unparalleled separation capabilities have enabled COSMOSIL Buckyprep to become the world benchmark of HPLC column for fullerene separation. COSMOSIL Buckyprep retains fullerenes very strongly with a mobile phase of 100% toluene and exceeds the injection volume of a standard C_{18} column by a factor of 35. Therefore, preparative-scale separation can be obtained with a 250 mm × 4.6 mm I.D. analytical column.

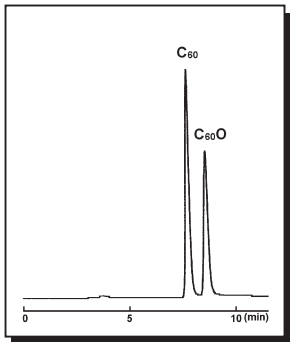
Application data

・Higher fullerene



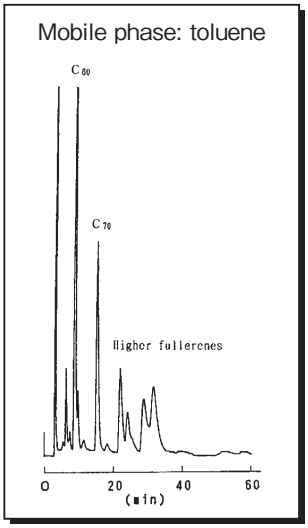
Column	4.6 mm I.D. × 250 mm
Mobile phase	toluene
Flow rate	1.0 ml/min
Temperature	30℃
Detection	UV 312 nm
Sample	C_{60}, C_{70}, C_{76}, C_{84}

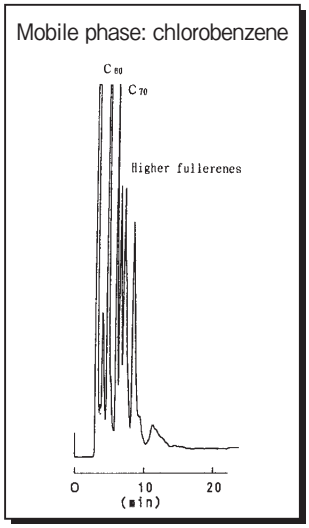
・Detivatized fullerene



Column	4.6 mm I.D. × 250 mm
Mobile phase	toluene
Flow rate	1.0 ml/min
Temperature	30℃
Detection	UV 312 nm
Sample	C_{60}, $C_{60}O$

・Separation of higher fullerenes on Buckyprep





Column	4.6 mm I.D. × 250 mm
Flow rate	1.0 ml/min
Temperature	30℃
Detection	UV 285 nm

Ordering information

COSMOSIL Buckyprep Packed Column

Column size I.D. × length (mm)	Product number
4.6 × 250	37977-61
10 × 250	37981-91
20 × 250	37982-81
28 × 250	34346-11

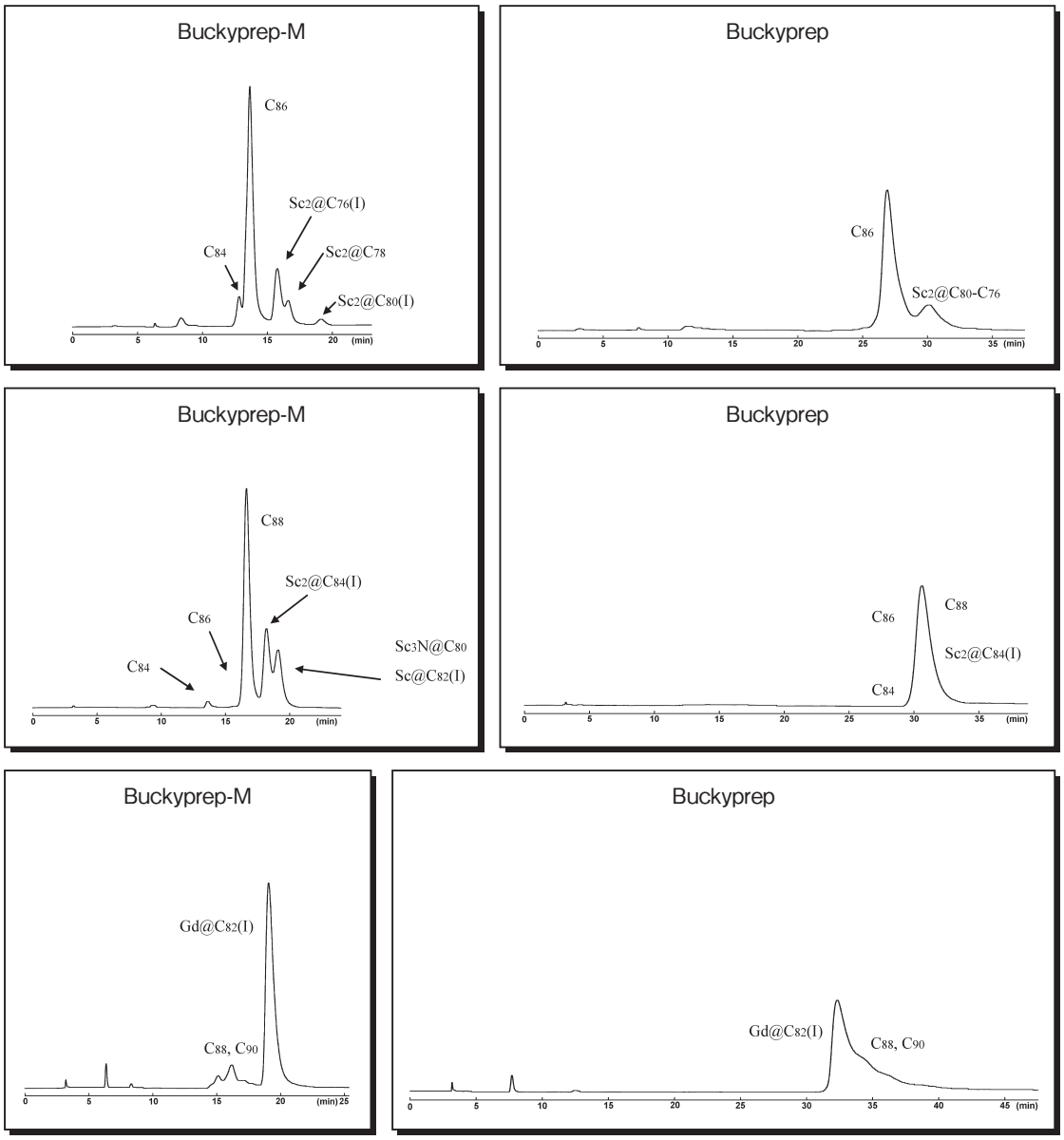
COSMOSIL Buckyprep Guard Column

Column size I.D. × length (mm)	Product number
4.6 × 10	37983-71
10 × 20	37984-61
20 × 50	34374-41
28 × 50	05871-21

Buckyprep-M

COSMOSIL Buckyprep-M is a phenothiazinyl group bonded silica based column specifically designed for metallofullerene separation. Metallofullerenes are retained more strongly than other fullerenes on this column. COSMOSIL Buckyprep-M is also effective for the separation of higher fullerenes and fullerene derivatives.

Application data



Column size	4.6 mm I.D × 150 mm
Mobile phase	toluene
Flow rate	1.0 ml/min
Temperature	30℃
Detection	UV 312 nm

Sample courtesy of Dr. H. Shinohara, Department of Chemistry, Nagoya University.

Ordering information

COSMOSIL Buckyprep-M Packed Column

Column size I.D. × length (mm)	Product number
4.6 × 250	04138-71
10 × 250	04141-11
20 × 250	04142-01
28 × 250	05873-01

COSMOSIL Buckyprep-M Guard Column

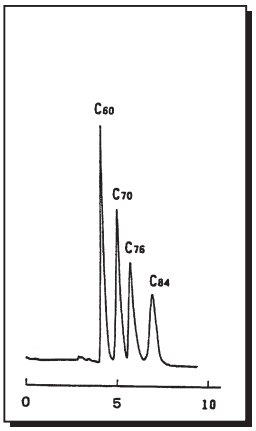
Column size I.D. × length (mm)	Product number
4.6 × 10	04139-61
10 × 20	04140-21
20 × 50	34474-31
28 × 50	05872-11

PBB

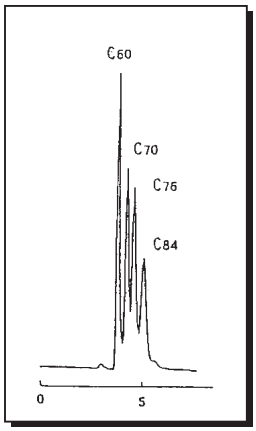
COSMOSIL PBB is a pentabromobenzyl group bonded silica based column useful for preparative-scale separation of fullerenes. It can be used with *o*-Dichlorobenzene, which has greater solubility for fullerenes than toluene. The loading capacity of COSMOSIL PBB for C_{60} and C_{70} can be three times greater than COSMOSIL Buckyprep.

Application data

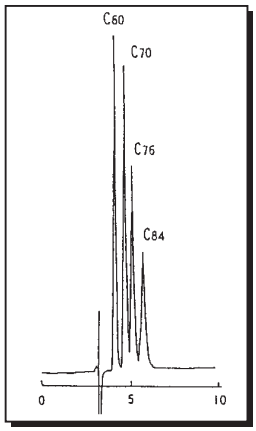
・Separation of higher fullerenes



Mobile phase *o*-Dichlorobenzene
Detection UV 310 nm



Mobile phase *1,2,4*-Trichlorobenzene
Detection UV 310 nm

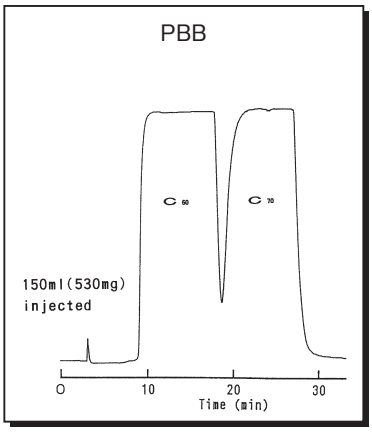


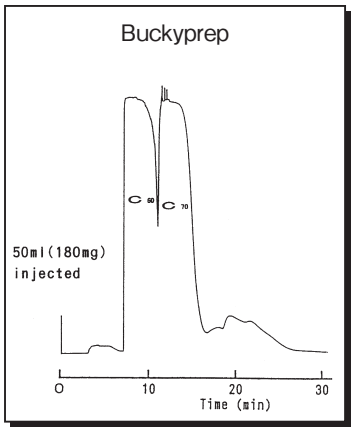
Mobile phase Carbon Disulfide
Detection UV 380 nm

Column size 4.6 mm I.D. × 250 nm
Flow rate 1.0 ml/min
Temperature 30℃
Sample C_{60},C_{70},C_{76},C_{84}

Preparative separation of fullerenes

The loading capacity of COSMOSIL PBB for C_{60} and C_{70} can be three times greater than COSMOSIL Buckyprep.





Column Size 20 mm I.D. × 250 mm
Mobile phase toluene
Flow rate 18 ml/min
Temperature room temperature
Detection UV 285 nm
Sample Crude fullerenes (3.5 mg/ml)

Ordering information

COSMOSIL 5PBB Packed Column

Column size I.D. × length (mm)	Product number
4.6 × 250	37980-01
10 × 250	37985-51
20 × 250	37986-41

COSMOSIL 5PBB Guard Column

Column size I.D. × length (mm)	Product number
4.6 × 10	37987-31
10 × 20	37988-21
20 × 50	34375-31

Ordering information

COSMOSIL 5PYE Packed Column

Column size I.D. × length (mm)	Product number
4.6 × 250	37989-11
10 × 250	37996-11
20 × 250	38044-41
28 × 250	34300-91

COSMOSIL 5PYE Guard Column

Column size I.D. × length (mm)	Product number
4.6 × 10	37903-11
10 × 20	38041-71
20 × 50	34475-21

COSMOSIL 5NPE Packed Column

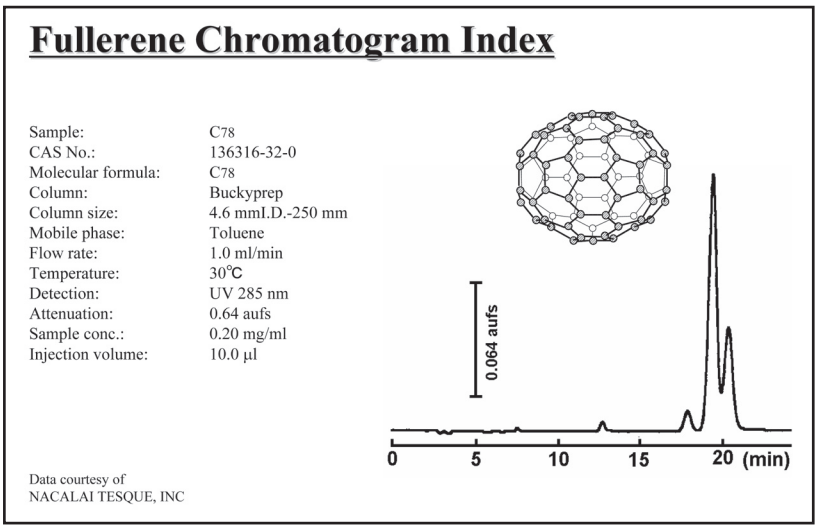
Column size I.D. × length (mm)	Product number
4.6 × 150	37902-21
4.6 × 250	37990-71
10 × 250	05469-11
20 × 250	38046-21

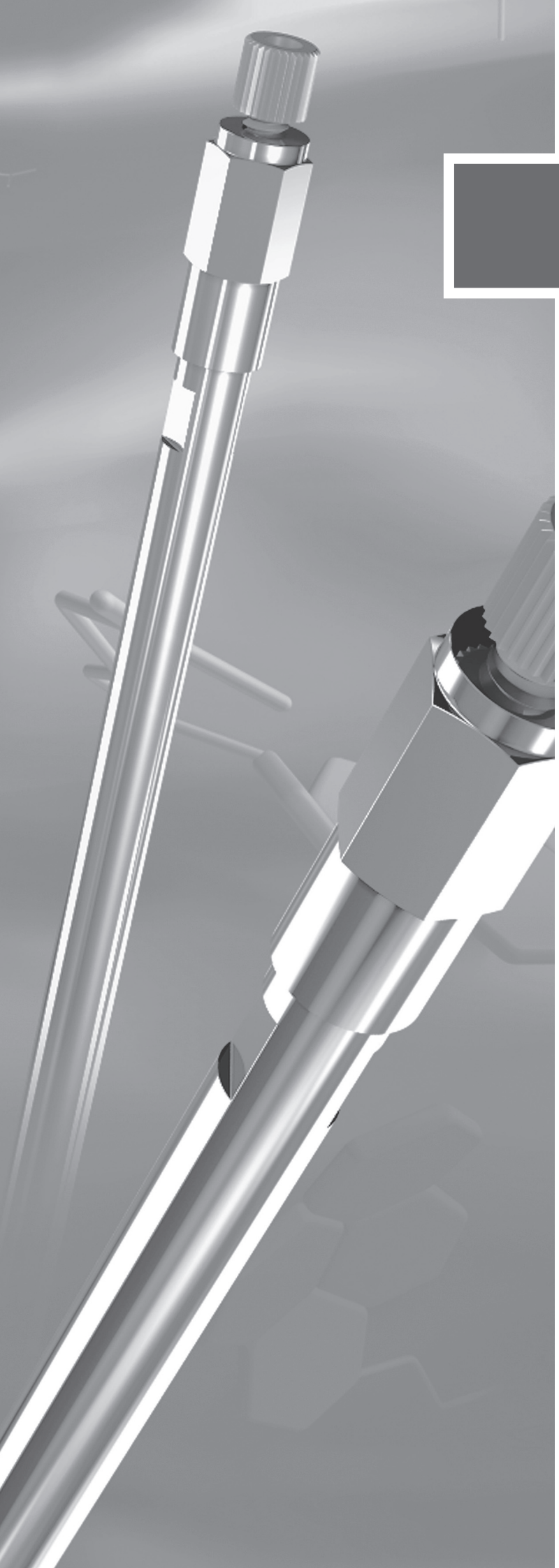
COSMOSIL 5NPE Guard Column

Column size I.D. × length (mm)	Product number
4.6 × 10	37904-01
10 × 20	38045-31
20 × 50	05869-71

Fullerene Chromatogram Index

Fullerene Chromatogram Index includes more than 100 chromatograms. If you are interested in this index, please feel free to e-mail us at info.intl@nacalai.co.jp.





II

LIQUID CHROMATOGRAPHY RELATED PRODUCTS

1. Packing Materials for Column Chromatography
2. Liquid Chromatography Related Products
3. Prefiltration Tools for Liquid Chromatography

1. Packing Materials for Column Chromatography

Introduction

Open column chromatography is an excellent and easy technique for large-scale preparation and purification at low cost. COSMOSIL offers both normal and reversed phase packing materials based on totally porous spherical silica, which provides higher separation, less pressure and higher reproducibility than irregular silica.

Packing materials for reversed phase preparative liquid chromatography

Material characteristic

	C_{18}-OPN	C_{18}-PREP
Silica gel	high purity porous spherical silica	
Average particle size	75, 140 μm	40, 75, 140 μm
Average pore size	approx. 120 Å	
Specific surface area	approx. 300 m^2/g	
Stationary phase	octadecyl group	
Carbon content	—	approx. 19%
End capping treatment	—	treated

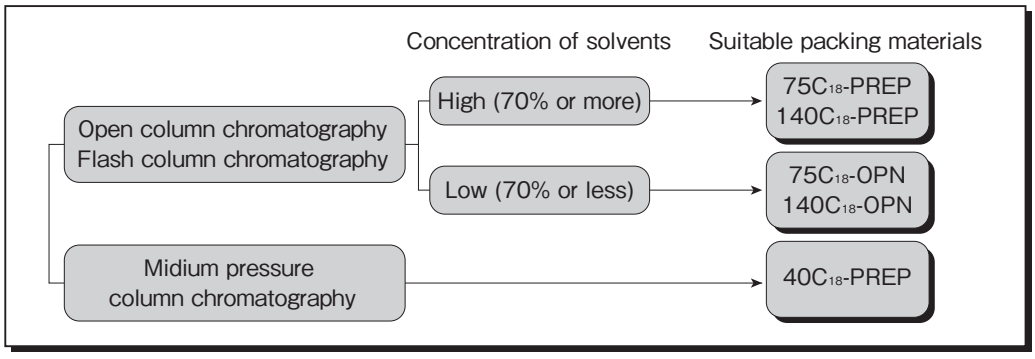
Useful range of C_{18}-OPN and C_{18}-PREP

	concentration of organic solvent	$75C_{18}$-OPN $140C_{18}$-OPN	$40C_{18}$-PREP	$75C_{18}$-PREP $140C_{18}$-PREP
Open column chromatography	70% or less	+++	-	-
	70% or more	++	+	+++
Middle pressure column chromatography		+	+++	++

+++ : very suitable, ++ ; suitable, +, applicable, - : not applicable

Selection guide

How to select C_{18} preparative packing materials.



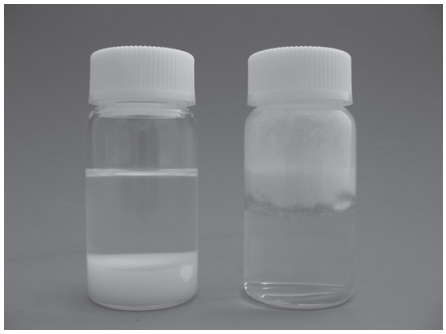


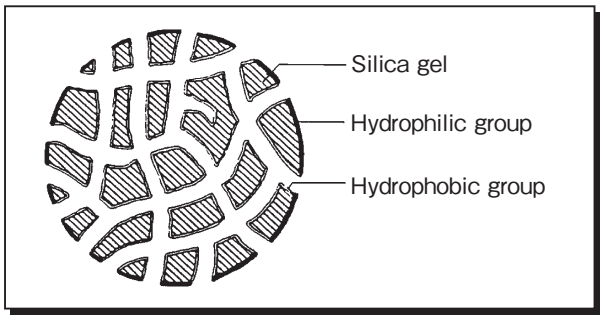
Figure. Packing material in water
Left : C_{18}-OPN provides good resolution
Right : C_{18}-PREP float up

C_{18}-OPN

Conventional reversed phase C_{18} packing materials are restricted to about 30-50% water in the mobile phase. The COSMOSIL C_{18}-OPN is a new "Water-Wet" C_{18} packing material developed for reversed phase open column chromatography. The C_{18}-OPN material can be used in 100% aqueous effluents.

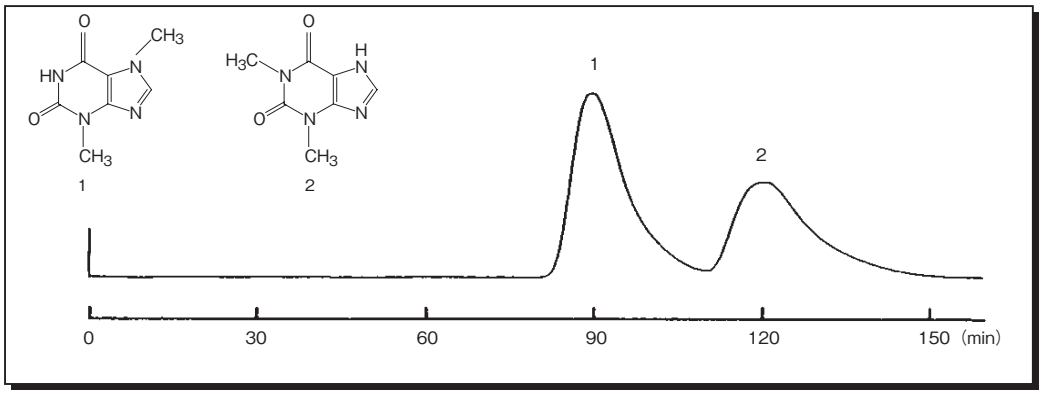
Characteristic

The external surface of the C_{18}-OPN gel is coated with hydrophilic group to increase wettability of the gel, and octadecyl group is bonded in the pore of the gel. This physical characteristic of the gel makes the reversed phase open column chromatography possible with 100 % water.



Separation of Theobromine and Theophylline

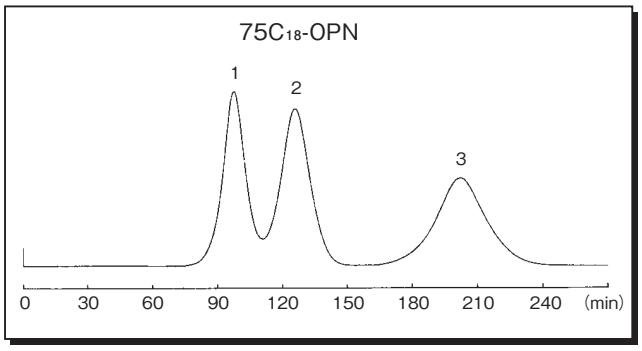
Figure shows the samples are clearly separated by reversed open column chromatography with 70% of water.

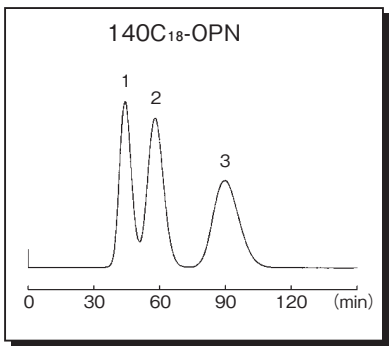


Packing material	COSMOSIL 75C_{18}-OPN	Sample	1. Theobromide (100 mg)
Column size	20 mm I.D. × 250 mm bed height		2. Theophylline (100 mg)
Mobile phase	methanol : water = 30 : 70		
Flow rate	0.2 ml/min		
Temperature	room temperature		
Detection	UV 254 nm		

Figure. Chromatogram of Theobromide and Theophylline separated on C_{18}-OPN

Influence of particle size





Column size	10 mm I.D. × 250 mm bed height
Mobile phase	methanol : water = 20 : 80
Temperature	room temperature
Detection	UV 254 nm
Sample	1. Theobromide (TB) 2. Theophylline (TP) 3. Caffeine (CF)

Table. Comparison between 75 μm and 140μm particle size silica

Particle (μm)	Flow rate (ml/min)	Theoretical plate number			Rs		Separation time(min)	Solvent consumption(ml)
		TB	TP	CF	TB/TP	TP/CF		
75	0.25	400	390	340	1	1.74	240	60
140	0.6	300	280	260	0.9	1.4	100	60

Flow rate

Since reversed phase chromatography generally employs high viscosity solvents such as water and methanol, the flow rate is lower than that of normal phase chromatography. The flow rate of reversed phase depends on the mobile phase composition. Figure indicates that the flow rate of the COSMOSIL 140C_{18}-OPN (140 μm in particle size) is about 2.5 times higher than that of the COSMOSIL 75C_{18}-OPN.

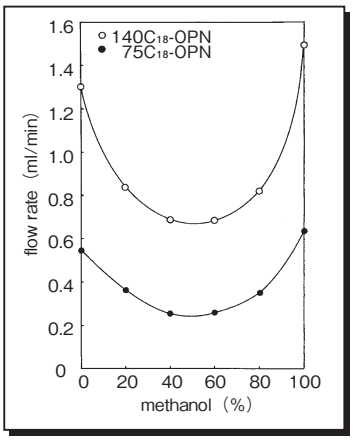
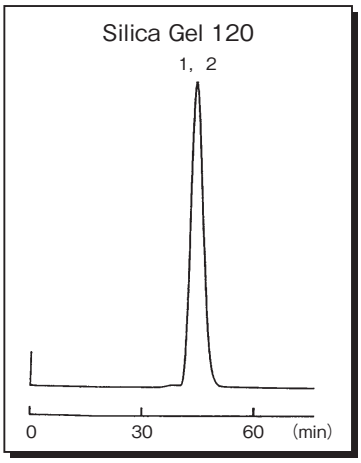


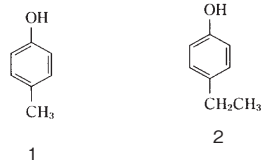
Figure.
Concentration of methanol against flow rate

Column 10 mm I.D. × 180 mm bed height
(gravitational liquid flow)

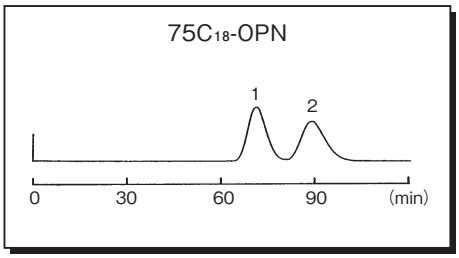
Separation of *p*-Cresol and *p*-Ethylphenol by normal and reversed phase mode

Since the structural difference between *p*-Cresol and *p*-Ethylphenol is only one methylene group, it is difficult to separate such samples under normal phase condition. On the other hand, the samples are clearly separated under reversed phase condition with COSMOSIL C_{18}-OPN packing material.





Packing material	Silica Gel 120 (150-325 mesh)
Column size	10 mm I.D. × 180 mm bed hight
Mobile phase	*n*-Hexane : ethyl acetate = 90 : 10
Flow rate	0.7 ml/min
Temperature	room temperature
Detection	UV 254 nm
Sample	1. *p*-Cresol 2. *p*-Ethylphenol



Packing material	COSMOSIL 75C_{18}-OPN
Column size	10 mm I.D. × 180 mm bed hight
Mobile phase	methanol : water = 60 : 40
Flow rate	0.2 ml/min
Temperature	room temperature
Detection	UV 254 nm
Sample	1. *p*-Cresol 2. *p*-Ethylphenol

Column size and required amount of packing material

Figure. Column size and required amount of C_{18}-OPN packing material

Column I.D.(mm)	Bed height(mm)	Amount of C_{18}-OPN(g)
10	150	4
	250	7
20	150	17
	250	28
30	150	38
	250	63

Reproducibility and washing methods

Wash the COSMOSIL C_{18}-OPN packing material with tetrahydrofuran, chloroform or other solvents to remove the impurities. This packing material has excellent reproducibility and can be used repeatedly.

"CAUTION"

Do not wash with basic solvents of pH 7 or more which will dissolve the silica gel or pH 2 or less which will cleave the C_{18} stationary phase. Dry the packing material at 50°C or less. See end of this chapter for packing method.

Ordering information

COSMOSIL C_{18}-OPN

Product name	Product number	PKG size
COSMOSIL 75C_{18}-OPN	37842-66	100 g
	37842-95	500 g
	37842-11	1 kg
COSMOSIL 140C_{18}-OPN	37878-16	100 g
	37878-45	500 g
	37878-61	1 kg

The large particle size C_{18} bulk materials are widely used for lab to process scale purifications. COSMOSIL offers three different particle sizes of C_{18} packing materials.

Particle size, flow rate and theoretical plate number

Because reversed phase chromatography employs effluents of high viscosity such as methanol and water, the flow rate is lower than that of normal phase chromatography, which uses effluents of low viscosity such as hexane and ethyl acetate.

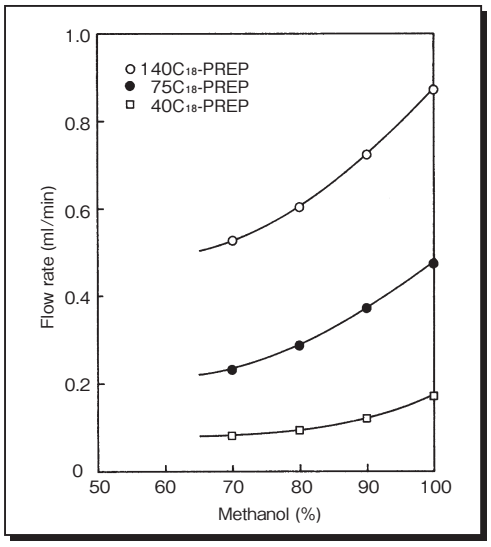


Figure 1. Concentration of methanol against flow rate
Column : 10 mm I.D. × 180 mm bed height (gravitational liquid flow)

Higher theoretical plate number can be obtained with lower flow rate.

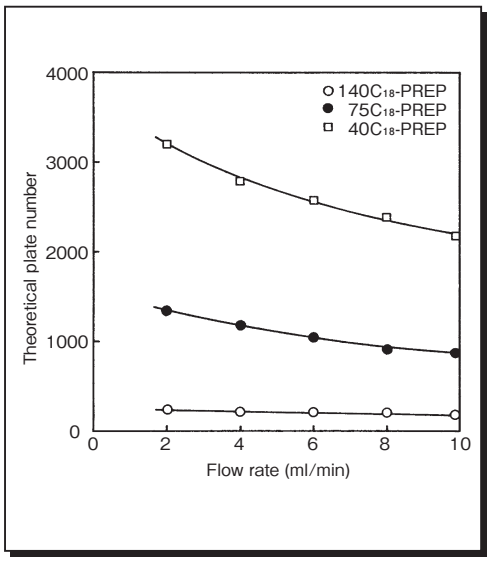
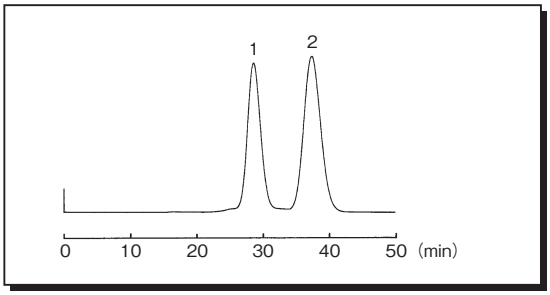


Figure 2. Flow rate against theoretical plate number
Column : 20 mm I.D. × 300 mm

Application Data

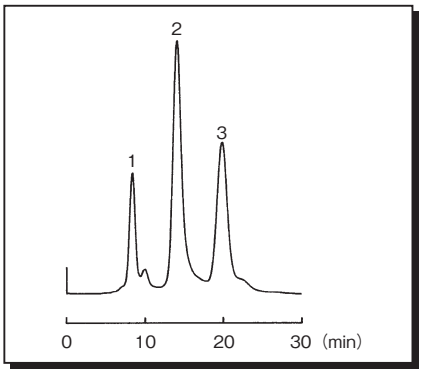
・Separation of Vitamin E



Packing material	COSMOSIL 40C_{18}-PREP
Column size	20 mm I.D. × 300 mm bed hight
Mobile phase	methanol
Flow rate	9.9 ml/min
Temperature	room temperature
Detection	UV 280 nm 0.2ALFS
Sample	1. *DL*-α-Tocopherol (5 mg)
	2. *DL*-α-Tocopherol Acetate (5 mg)

・Separation of crude drugs

Crude drugs that are strongly hydrophobic can be separated by the medium pressure chromatography (closed columns).



Packing material	COSMOSIL 40C_{18}-PREP
Column size	20 mm I.D. × 300 mm bed hight
Mobile phase	methanol : 0.05% TFA = 70 : 30
Flow rate	9.9 ml/min
Temperature	room temperature
Detection	UV 254 nm, 0.05ALFS
Sample	1. Baicalin (40 mg)
	2. Baicalein (120 mg)
	3. Wogonin (40 μg)

Column size and required amount of C_{18}-PREP packing material

Column I.D.(mm)	Bed height(mm)	Column volume(ml)	Amount of C_{18}-PREP(g)
8	300	15	9
	500	25	15
10	300	25	15
	500	40	25
20	300	95	55
	500	160	95
30	300	210	125
	500	350	220
50	300	560	350
	500	980	600

Packing method

Please refer to TECHNICAL NOTE 9 ; Packing instruction at page 101.

Ordering information

COSMOSIL C_{18}-PREP

Product name	Product number	PKG size
COSMOSIL 40C_{18}-PREP	37932-86	100 g
	37932-15	500 g
	37932-31	1 kg
COSMOSIL 75C_{18}-PREP	37933-76	100 g
	37933-05	500 g
	37933-21	1 kg
COSMOSIL 140C_{18}-PREP	37934-66	100 g
	37934-95	500 g
	37934-11	1 kg

Packing materials for normal phase preparative liquid chromatography

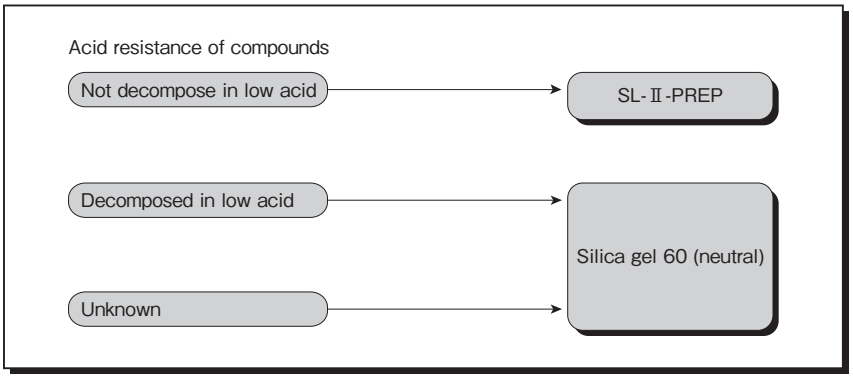
Material Characteristic

	SL-Ⅱ-PREP		Silica gel 60 neutral	
Silica gel	high purity porous spherical silica			
Average particle size	75 μm	140 μm	75 μm	140 μm
Particle size range	42 ~ 105 μm (150 ~ 325 mesh)	74 ~ 210 μm (70 ~ 230 mesh)	42 ~ 105 μm (150 ~ 325 mesh)	74 ~ 210 μm (70 ~ 230 mesh)
Average pore size	approx. 120 Å		approx. 60 Å	
Specific surface area	approx. 300 m²/g		approx. 500 m²/g	
Application	Open column chromatography/Flash column chromatography			

Different type of silica gel is also available, please see page 69.

Selection guide

Selection flow of packing materials for normal phase preparative chromatography.



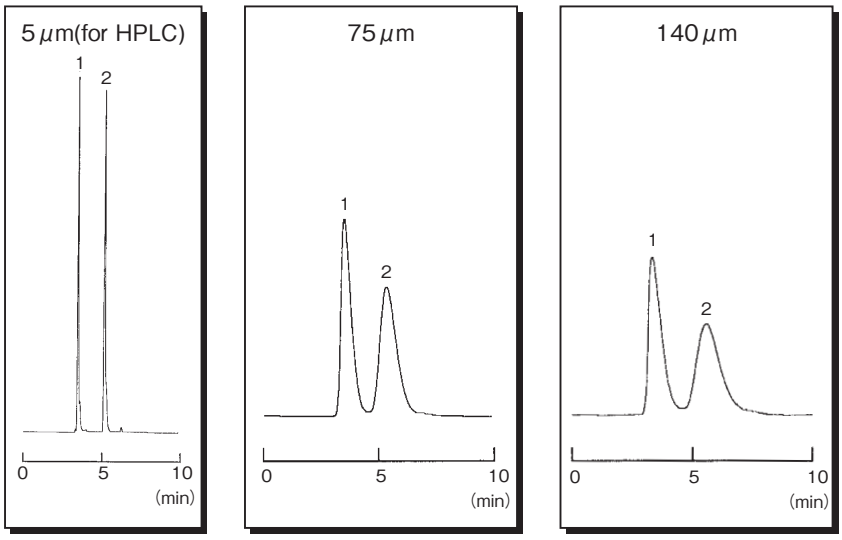
SL-Ⅱ-PREP

COSMOSIL SL-Ⅱ-PREP is ultra pure silica gel packing material more than 99.99% purity. COSMOSIL SL-Ⅱ-PREP provides improved separation and reproducibility for compounds with carbonyl or phenol hydroxyl groups, which are often problematic on conventional silica gel materials.

*All chromatograms shown below are obtained with silica gel packed into stainless steel columns.

Influence of the particle size on peak shape

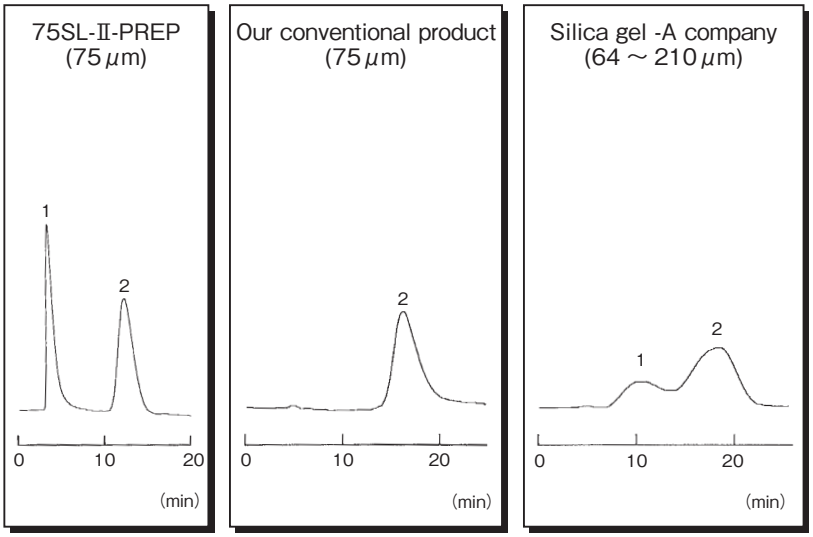
The SL-Ⅱ-PREP is available in five sizes : 3, 5, 15, 75 and 140 μm (particle diameter). The peak shapes depend on the particle size, however, the elution order remains the same.



Column size	10 mm I.D. × 250 mm
Mobile phase	hexane : ethanol = 95 : 5
Flow rate	5.0 ml/min
Temperature	30℃
Detection	UV 254 nm 0.16AUFS (5 μm 0.32AUFS)
Sample	1. Chrysazin 2. *p*-Cresol

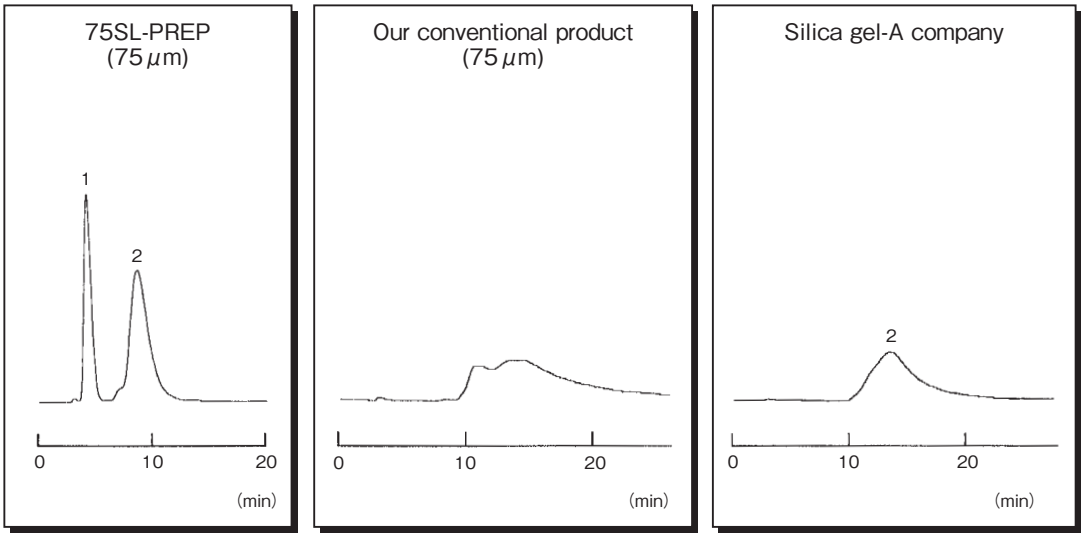
・Performance for chelating compounds

Highly purified silica gel of Cosmosil SL-Ⅱ-PREP enables separation of metal coordination compounds without adsorption.



Column size	10 mm I.D. × 250 mm
Mobile phase	hexane : ethanol = 95 : 5
Flow rate	5.0 ml/min
Temperature	30℃
Detection	UV 254 nm, 0.16AUFS
Sample	1. Quinizarin
	2. *p*-Nitrobenzyl Alcohol

・Performance for organic acid and amide



Column size	10 mm I.D. × 250 mm
Mobile phase	hexane : ethanol = 90 : 10
Flow rate	5.0 ml/min
Temperature	30℃
Detection	UV 254 nm, 0.16AUFS
Sample	1. Salicylic Acid
	2. Salicylamide

Packing method

Please refer to TECHNICAL NOTE 9 ; Packing instruction at page 101.

Ordering information

COSMOSIL SL-Ⅱ-PREP

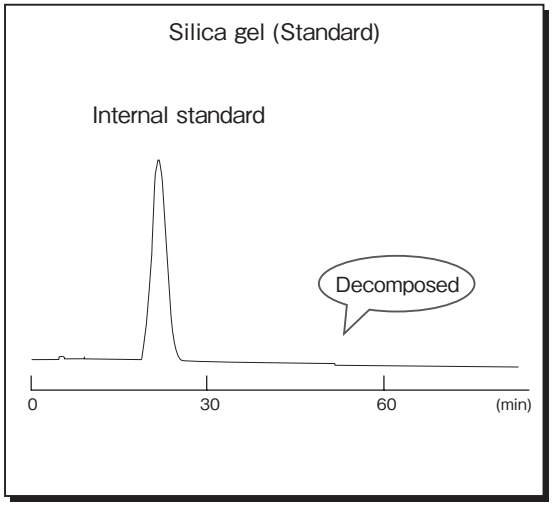
Product name	Product number	PKG size
COSMOSIL 75SL-Ⅱ-PREP	38012-64	100 g
	38012-35	500 g
	38012-51	1 kg
COSMOSIL 140SL-Ⅱ-PREP	38013-54	100 g
	38013-25	500 g
	38013-41	1 kg

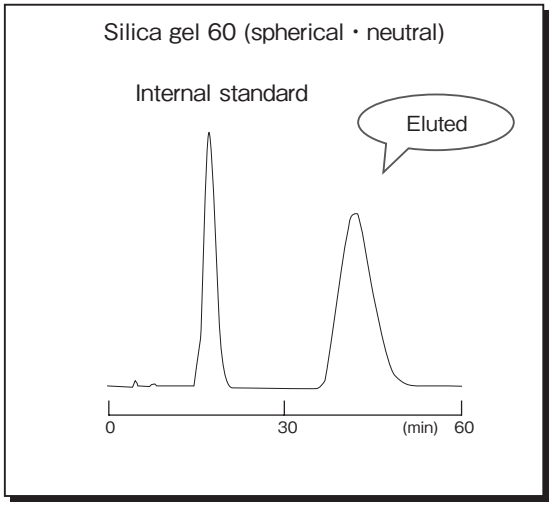
Silica gel (spherical・neutral)

Since conventional silica gels are weakly acidic, some pH sensitive compounds may be decomposed during the purification by column chromatography with the acidic silica gels. The pH of Silica gel 60 (spherical・neutral) is adjusted to nearly neutral for the separation of not only pH sensitive compounds but also new compounds that the physical properties are still unknown.
*All chromatograms shown below are obtained with silica gel packed into stainless and steel columns.

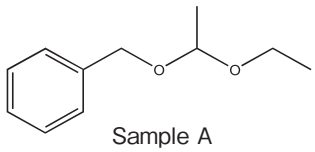
Comparison with conventional silica gel

・Purification of Acetal

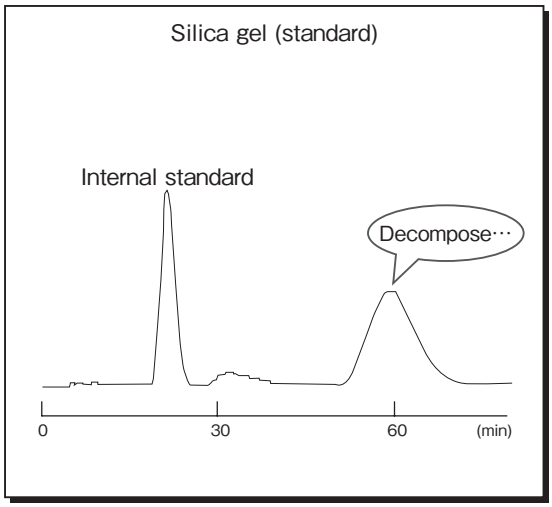


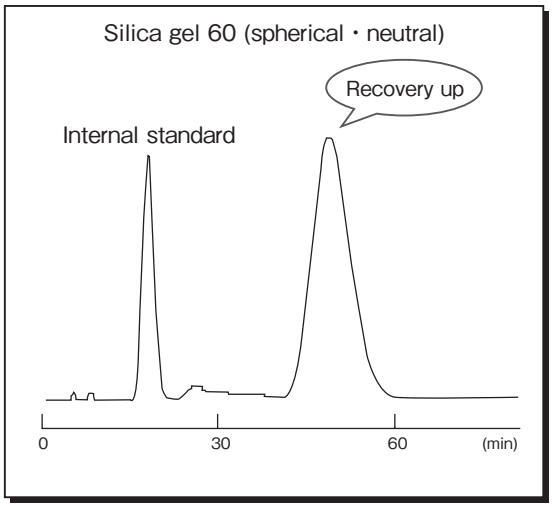


Column size	4.6 mm I.D. × 250 mm
Mobile phase	ethyl acetate : hexane = 1 : 99
Flow rate	1.0 ml/min
Temperature	30℃
Detection	UV 254 nm, 0.08AUFS
Sample	Sample A (100 mg/ml)
Internal standard	Methyl Benzoate (10 mg/ml)
Injection volume	3 μl

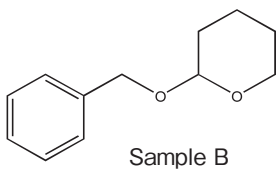


Sample A





Column size	4.6 mm I.D. × 250 mm
Mobile phase	ethyl acetate : hexane = 1 : 99
Flow rate	1.0 ml/min
Temperature	30℃
Detection	UV 254 nm, 0.08AUFS
Sample	Sample B (200 mg/ml)
Internal standard	Methyl Benzoate (10 mg/ml)
Injection volume :	3 μl



Sample B

Ordering information

Silica gel 60 (spherical, neutral)

Product name	Product number	PKG size
Silica gel 60, spherical, neutral 75 μm	30511-64	100 g
	30511-35	500 g
	30511-51	1 kg
	30511-06	5 kg
	30511-22	25 kg
Silica gel 60, spherical, neutral 140 μm	30518-94	100 g
	30518-65	500 g
	30518-81	1 kg
	30518-52	25 kg

Silica gel (for column chromatograph)

Ordering information

Product name	Particle size	Product number	PKG size
Silica gel (irregular)			
Silica gel 60	approx. 70-230 mesh	30724-55	500 g
		30724-71	1 kg
		30724-84	5 kg
		30724-42	25 kg
	approx. 230-400 mesh	30721-85	500 g
		30721-01	1 kg
		30721-14	5 kg
		30721-72	25 kg
	approx. 2-20 μm	30737-24	5 kg
Silica gel (spherical)			
Silica gel 60, spherical	approx. 70-230 mesh	30731-71	1 kg
		30731-42	25 kg
	approx. 150-325 mesh	30733-51	1 kg
		30733-22	25 kg
Silica gel 120, spherical	approx. 70-230 mesh	30734-41	1 kg
	approx. 150-325 mesh	30735-31	1 kg

Please refer to TECHNICAL NOTE 9 ; Packing instruction at page 101.

2. Liquid Chromatography Related Products

Ion pair reagents

The use of ion pair reagents as mobile phase additives extends the applicability of reversed phase HPLC. Ionic or highly polar compounds are difficult to analyze by reversed phase using only organic solvent and buffer solution because of the short retention time. Ion pair reagents are strong hydrophobic ions which form neutral ion pairs with oppositely charged samples molecules, making the efficient ODS columns amenable to separate ionic or highly polar samples.

Nacalai offers a broad range of ion pair reagents for pharmaceutical compounds and other highly polar materials.

When using ion pair regents, ample time should be allowed for establishing equilibrium and for cleaning the column.
When using ion pair regents with an alkyl chain of C_{10} or shorter, it typically takes 20 minutes for establishing equilibrium and 30 minutes for cleaning. It may take more than 1 hour to clean the column when using ion pair reagents with an alkyl chain longer than C_{10}. Therefore, it is highly recommended to prepare a column for exclusive use with ion pair reagents.

・General use of ion pair reagents in the mobile phase.

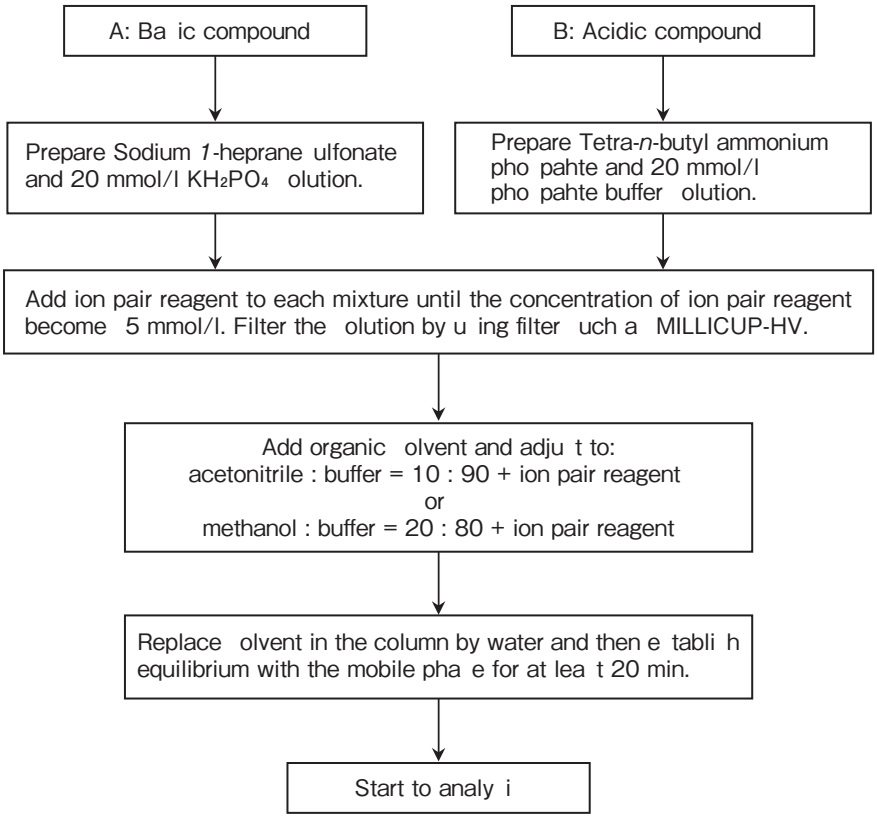


Table 1. Retention time and separation adjustment

Troubles	Solutions
1. Good peak separations but retention time is too long or too short.	1. Too long : increase organic solvent concentration by 10%. Too short : decrease organic solvent concentration by 10%.
2. Good retention time bur peaks are not sharply separated.	2. Use ion pair of longer alkyl chain or the same ion pair but double the concentration.
3. No difference in separations between using ion pair and not using, or no change appears trying solution No.2.	3. For basic compounds analysis, decrease pH value. For acidic compounds analysis, increase pH value.
4. Retention time of the interested compound is too long.	4. Use ion pair of shorter alkyl chain or same ion pair of half concentration.

Ordering information

・for Basic samples

Product name	Grade	Storage	Product number	PKG size
Sodium *1*-Butanesulfonate	SP	RT	31331-94	5 g
Sodium *1*-Butanesulfonate (0.5M solution)	SP	RT	31332-84	5×10 ml
Sodium *1*-Pentanesulfonate	SP	RT	31730-64	5 g
			31730-22	25 g
Sodium *1*-Hexanesulfonate	SP	RT	31529-24	5 g
			31529-82	25 g
Sodium *1*-Hexanesulfonate (0.5M solution)	SP	RT	31532-64	10 ml
			31532-06	5×10 ml
Sodium *1*-Heptanesulfonate	SP	RT	31528-34	5 g
			31528-92	25 g
Sodium *1*-Octanesulfonate	SP	RT	31729-04	5 g
			31729-62	25 g
Sodium *1*-Octanesulfonate (0.5M solution)	SP	RT	31733-34	10 ml
			31733-76	5×10 ml
Sodium *1*-Nonanesulfonate	SP	RT	31626-44	5 g
Sodium *1*-Decanesulfonate	SP	RT	31429-34	5 g
Sodium *1*-Undecanesulfonate	SP	RT	32030-04	5 g
Sodium *1*-Dodecanesulfonate	SP	RT	31426-64	5 g
Sodium Lauryl Sulfate	SP	RT	31623-32	25 g

・for Acid samples

Product name	Grade	Storage	Product number	PKG size
Tetra-*n*-butylammonium Bromide	SP	R	32824-72	25 g
Tetra-*n*-butylammonium Chloride	EP	R	32935-64	5 g
			32935-22	25 g
Tetra-*n*-butylammonium Hydrogensulfate	GR	RT	32924-62	25 g
Tetra-*n*-butylammonium Iodide	SP	R	32905-54	5 g
			32905-12	25 g
Tetra-*n*-butylammonium Perchlorate	SP	R	32906-44	5 g
			32906-02	25 g
Tetra-*n*-butylammonium Phosphate	SP	R	32929-54	5 g
Tetra-*n*-butylammonium Phosphate (0.5M solution)	SP	RT	32926-26	10 ml
			32926-84	5×10 ml

(storage) A : Cool and dark, RT : Room temprature, R : Refrigerator

Labeling reagents

Ordering information

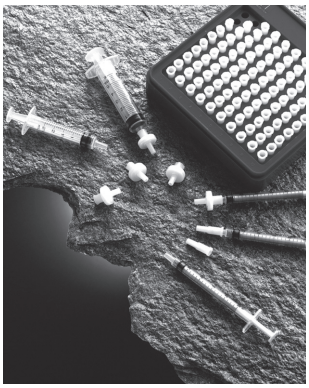
Product name	Grade	Storage	Product number	PKG size
Dansyl Chloride	SP	RT	10427-91	1 g
p-Bromophenacyl Bromide (PBPB)	GR	R	05802-92	25 g
3,5-Dinitrobenzoyl Chloride (DNBC)	SP	A	13530-44	5 g
N-(*9*-Acridinyl)maleimide (NAM)	SP	R	00842-64	50mg
NBD Chloride	SP	R	24113-61	1 g
o-Phthalaldehyde (OPA)	SP	R	27824-61	1 g
			27824-74	5 g
			27824-32	25 g

(storage) A : Cool and dark, RT : Room temprature, R : Refrigerator

3. Prefiltration Tool for Liquid Chromatography

Cosmonice filter

Injection of samples containing particulates (microparticles, precipitates, colloid substances) will clog HPLC columns, shorten injector life, and result in extensive maintenance on pumps. Cosmonice filters are used to remove particulates from samples and prolong the life of HPLC system components. There are two types of Cosmonice filters as stated below.



W series (Aqueous solution)

W series are installed with low adsorption hydrophilic durapore filter (polyvinylidenedifluoride, PVDF). W series can be used with both aqueous and organic solvents. They are best suited for prefiltration of protein and other biological samples.

S series (Organic solvents)

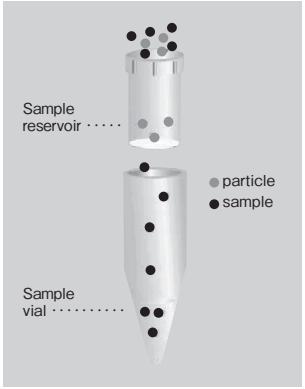
S series are installed Teflon filter (polytetrafluoroethylene, PTFE) with strong resistance to organic solvents, acids and alkalis. They are best suited for prefiltration of samples with aggressive organic solvents such as chloroform and tetrahydrofuran.

Ordering information

Product name	Pore size	Process volume	Hold-up volume	Connection	Product number	PKG size
Cosmonice Filter W (Aqueous) 4 mm	0.45 μm	less than 1 ml	< 10 μl	Inlet : luer-lock Outlet : luer-slip Connectable needles	06543-04	100 pkg
Cosmonice Filter W (Aqueous) 13 mm	0.45 μm	0.5-10 ml	< 30 μl		06544-94	100 pkg
Cosmonice Filter W (Aqueous) 25 mm	0.45 μm	3-50 ml	< 100 μl		06545-84	50 pkg
Cosmonice Filter W (Solvent) 4 mm	0.45 μm	less than 1 ml	< 10 μl		06541-24	100 pkg
Cosmonice Filter W (Solvent)) 13 mm	0.45 μm	0.5-10 ml	< 30 μl		06542-14	100 pkg

Cosmospin filter

Cosmospin filters are used to remove fine particles and precipitates from samples by centrifugation. They utilize Omnipore hydrophilic PTFE membrane filter, which has a wide range of chemical resistance. Cosmospin filters are the best choice for HPLC sample filtration. Two pore sizes, G (0.2 μm) and H (0.45 μm), are available.



- Add the sample into Sample reservoir
- Centrifuge after capping
- Recover the filtrate from Sample collection tube

Ordering information

Product name	Pore size	Maximum sample volume	Hold-up volume	Maximum centrifugal force	Rotor size (fixed-angle)	Filtration area	Color	Product number	PKG size
Cosmospin Filter G	0.2 μm	0.4 ml	5 μl	5,000×g	1.5 ml	0.2 cm²	brown	06549-44	100 pkg
Cosmospin Filter H	0.45 μm	0.4 ml	5 μl	5,000×g	1.5 ml	0.2 cm²	white	06540-34	100 pkg

Dimension : Diameter 10.6 mm × Length 45 mm Membrane : Omnipore Hydrophilic PTFE Sample reservoir and collection tube : Polypropylene

Solvent	Cosmonice W series	Cosmonice S series	Cosmospin	Solvent	Cosmonice W series	Cosmonice S series	Cosmospin
Acetic acid, glacial	+	+	+	Hydrogen peroxide (3%)	+	+	
Acetic acid, 5%	+	+	+	Hypo (photo)	+	+	+
Acetone	-	+	+	Isobutyl alcohol	+	+	+
Acetonitrile	+	+	+	Isopropyl acetate	+	+	+
Ammonia solution (conc.)	+	+		Isopropyl alcohol	+	+	+
Ammounium hydroxide (6N)	+	+	+	Kerosene	+	+	+
Amyl alcohol	+	+	+	Methyl alcohol	+	+	+
Benzene	+	+	-	Methyl ethyl ketone	-	+	+
Benzyl alcohol (1%)	+	+	-	Methyl isobutyl ketone	+	+	-
Boric acid	+		+	Nitric acid (6N)	+	+	
Butyl acetate		+		Nitrobenzene	+	+	-
Carbon tetrachloride	+	+	+	Ozone (10ppm in water)	-	+	-
CelloSolve (ethyl) solvent	+	+	+	Paraldehyde		+	
Chloroform	+	+	+	Pet base oils	+	+	+
Cyclohexanone	-	+	-	Pentane	+	+	-
Dichloromethane	+	+	-	Petroleum ether	+	+	
Dimethylacetamide	-	+	+	Phenol (5.0%)	+	+	-
Dimethylformamide	+	+	+	*2*-Propanol	+	+	+
Dioxane	+	+	+	Phosphate buffer solution	+		+
DMSO	-	+	-	Seawater	+	+	+
Ehtyl alcohol	+	+	+	Silicone oils	+	+	+
Ethers	+	+	+	Sodium hydroxide (conc.)	+	+	+
Ethyl acetate	+	+	+	Sulfuric acid (6N)		+	
Ethylene glycol	+	+	+	Toluene	+	+	-
Formaldehyde	+	+	+	THF	-	+	
Freon, TF or PCA solvent	+	+	+	Trichloroacetic acid	+	+	+
Gasoline	+	+	+	Trichloroethane	+	+	-
Glycerine (Glycerol)	+	+	+	Trichloroethylene	+	+	-
Hexane	+	+	-	TFA	+	+	-
Hydrochloride (6N)	+	+	+	Xylene	+	+	+
Hydrofluoric acid	-	+	-				

+ : Recommended, - : Not recommended, (blank) : No data

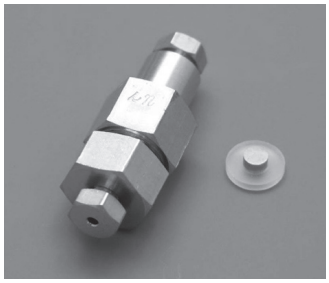
Ordering information

COSMOSIL Guard Cartridge Holder

Product name	Product number	PKG size
COSMOSIL Guard Cartridge Holder	38009-79	1PKG

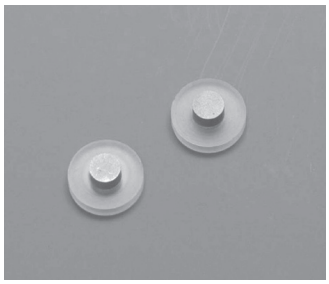
COSMOSIL Column Prefilter

Product name	Product number	PKG size
COSMOSIL Column Prefilter	39361-19	1PKG



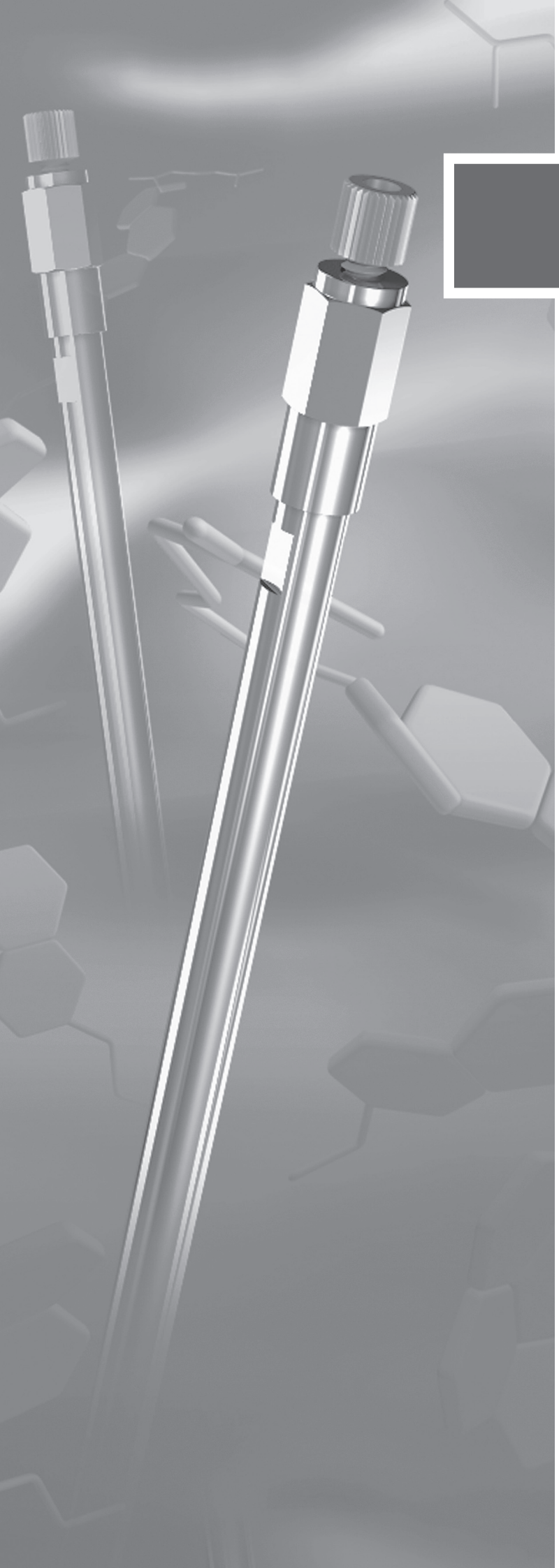
COSMOSIL Column Spare Filter for Prefilter

Product name	Product number	PKG size
COSMOSIL Column Spare Filter for Prefilter	39539-09	2PKG



COSMOSIL Column Connecting Tube

Product name	Product number	PKG size
COSMOSIL Column Connecting Tube	37843-69	1PKG



III

TECHNICAL NOTE

1. Selectivity of packing materials in reversed phase liquid chromatography
2. Preparation of mobile phase for HPLC
3. Sample pretreatment for HPLC
4. Baseline noise in gradient elution
5. Troubleshooting for increased pressure
6. Effect of guard column
7. Troubleshooting for normal phase chromatography
8. Inner diameter of column (scale down and scale up)
9. Packing instruction

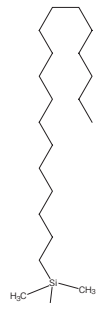
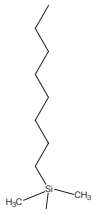
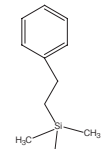
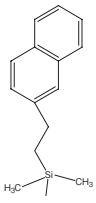
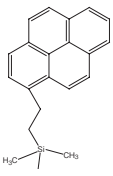
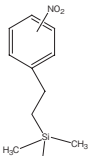
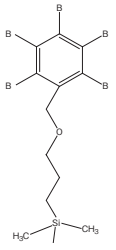
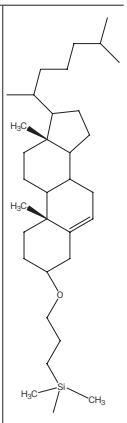
TECHNICAL NOTE

1. Selectivity of packing materials in reversed phase liquid chromatography

Reversed phase chromatography is the most commonly used method of HPLC, because of the high theoretical plate number, excellent separation characteristics, reproducibility, and ease of use. Columns packed with octadecyl group bonded type silica gel (C_{18}, ODS) are the most widely used reversed phase chromatography. However, C_{18} columns provide insufficient separation for compounds similar in hydrophobicity because the main separation mechanism of C_{18} column is based on hydrophobic interaction. It may improve separation of compounds with similar hydrophobicity by using longer columns, changing mobile phases or changing temperature. However, in many cases, it is probably most effective to use different packing materials which retain compounds base on a secondary interaction in addition to hydrophobic interaction.

At Nacalai, we offer a variety of COSMOSIL reversed phase packing materials. Summary of these packing materials and their respective retention mechanism are in Table 1. Retention of compounds in each stationary phase depends on summation of the interactions. Therefore, comprehension of each interaction leads to selection of an appropriate column.

Table1. Stationary phase and interaction of packing materials

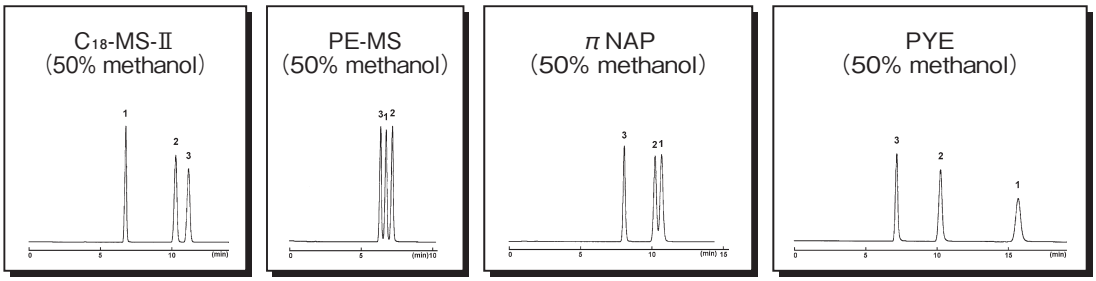
	C_{18}-MS-Ⅱ	C_{18}-AR-Ⅱ	C_8-MS	PE-MS	πNAP	PYE	NPE	PBB-R	Cholester
Silica gel	high purity porous spherical silica								
Average particle size	3・5・15μm		5μm						
Average pore size	approx. 120 Å								
Specific surface area	approx. 300m²/g								
Stationary phase									
	octadecyl	octadecyl	octyl	phenylethyl	naphthylethyl	pyrenylethyl	nitrophenylethyl	pentabromobenzyl	cholesteryl
Types	monomeric	polymeric	monomeric	monomeric	monomeric	monomeric	monomeric	monomeric	monomeric
Interaciton	hydrophobic interaction	hydrophobic interaction	hydrophobic interaction	hydrophobic interaction π-π interaction	hydrophobic interaction π-π interaction	hydrophobic Interaction π-π interaction dispersion Interaction shape selectivity	hydrophobic Interaction π-π interaction dipole-dipole interaction	hydrophobic interaction dispersion interaction	hydrophobic interaction shape selectivity
End capping	near-perfect treatment								
Carbon content	approx. 16%	approx. 17%	approx. 10%	approx. 10%	approx. 11%	approx. 18%	approx. 9%	approx. 8%	approx. 20%

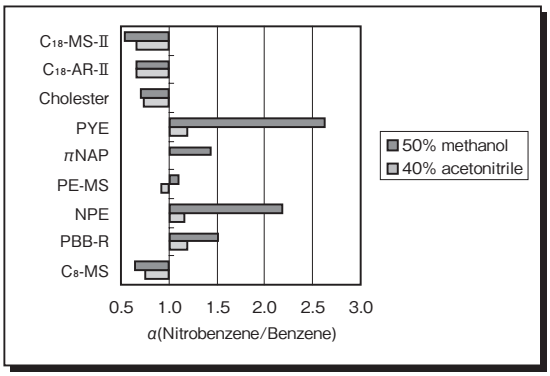
1) Selectivity for polar functional group

Selectivity

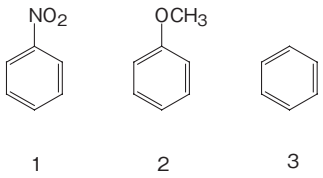
Selectivity for polar functional group is evaluated based on the separation of benzene, nitrobenzene, which has a nitro group, and anisole, which has a methoxy group. The chromatograms below show separation of the three compounds on four COSMOSIL columns : C_{18}-MS-Ⅱ, PE-MS, πNAP and PYE. Elution order on the C_{18} column is as following : nitrobenzene, anisole and benzene. Elution orders on the aromatic columns are reversed. Separation on the C_{18} column is based on hydrophobic interaction only. On the other hand, the packing materials on the other three columns have aromatic rings and reverse the elution order by π-π interaction.

The graph of selectivity for polar functional group is shown below. Among nine COSMOSIL columns, PYE and NPE columns have the highest selectivity factors for polar groups. As to mobile phases, methanol is more effective than acetonitrile for separation using π-π interaction.





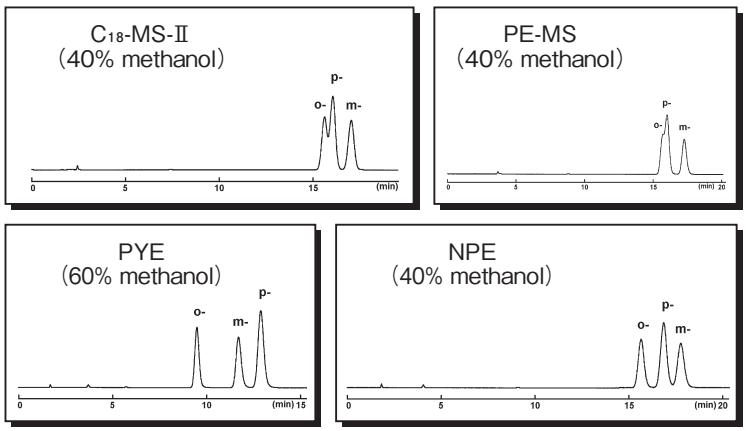
Column size	4.6 mm I.D. × 150 mm	
Flow rate	1.0 ml/min	
Temperature	30℃	
Detection	UV 254 nm	
Sample	1. Nitrobenzene	(0.13 μg)
	2. Anisole	(1.5 μg)
	3. Benzene	(4.0 μg)



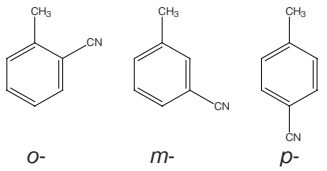
Application

・Separation of tolunitrile position isomers

Tolunitriles have three position isomers. It is difficult to separate ortho and para isomers by C_{18} or PE-MS column because of lack of poor π-π interaction. On the other hand, the isomers are well separated on PYE or NPE column which has strong π-π interaction.



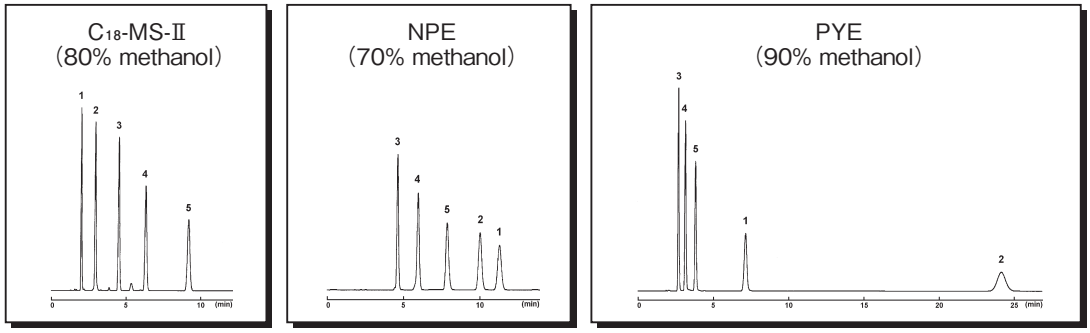
Column size	4.6 mm I.D. × 150 mm	
Flow rate	1.0 ml/min	
Temperature	30℃	
Detection	UV 254 nm	
Sample	1. *o*-Tolunitrile	(2.0 μg)
	2. *m*-Tolunitrile	(2.0 μg)
	3. *p*-Tolunitrile	(1.0 μg)

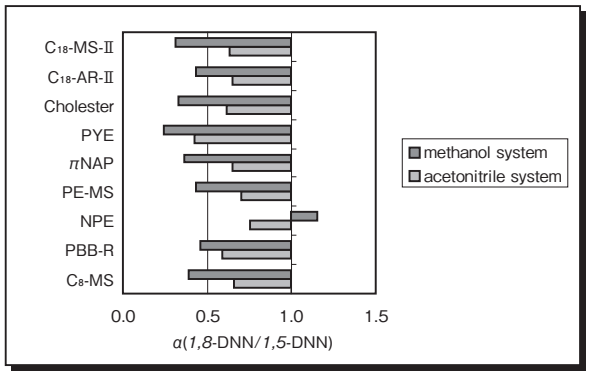


2) Selectivity for dipole

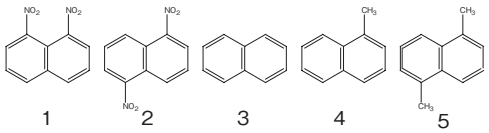
Selectivity

Selectivity for dipole is evaluated based on the separation of *1,5*-dinitronaphthalene and *1,8*-dinitronaphthalene. Dinitronaphthalenes (peak 1 and 2) were strongly retained on PYE and NPE because of π-π interaction compared with dimethylnaphthalenes. However, there is a slight difference between these two columns. While *1,5*-dinitronaphthalene (peak 2) was preferentially retained on PYE, *1,8*-dinitronaphthalene (peak 1) was retained longer on NPE. The results with NPE indicate the presence of strong dipole-dipole interaction. The two nitro group dipoles in *1,8*-dinitronaphthalene are aligned for a much greater dipolar coupling with the bonded nitrophenyl group in NPE than *1,5*-dinitronaphthalene.





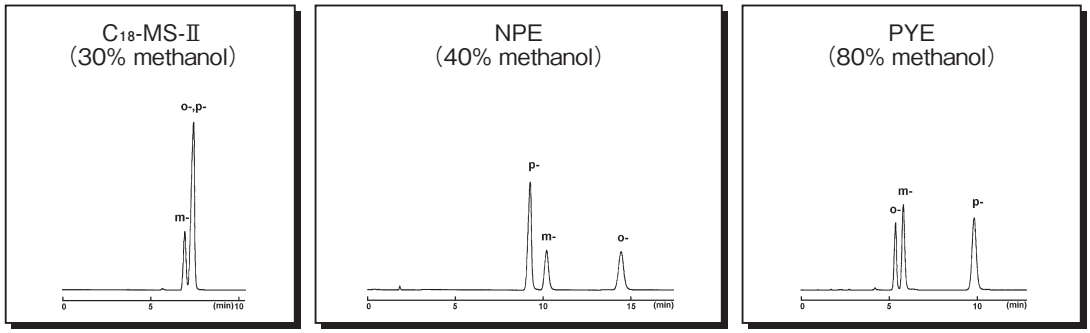
Column size	4.6 mm I.D. × 150 mm	
Flow rate	1.0 ml/min	
Temperature	30℃	
Detection	UV 254 nm	
Sample	1. *1,8*-Dinitronaphthalene (*1,8*-DNN)	(0.21 μg)
	2. *1,5*-Dinitronaphthalene (*1,5*-DNN)	(0.11 μg)
	3. Naphthalene	(0.25 μg)
	4. *1*-Methylnaphthalene	(0.35 μg)
	5. *1,5*-Dimethylnaphthalene	(0.42 μg)



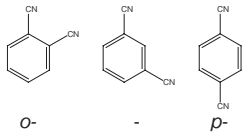
Application

・Separation of phthalonitrile position isomers

Phthalonitriles have three position isomers. NPE or PYE completely separates these compounds due to π-π interaction. Furthermore, NPE strongly retains o-phthalonitrile due to dipole-dipole interaction.



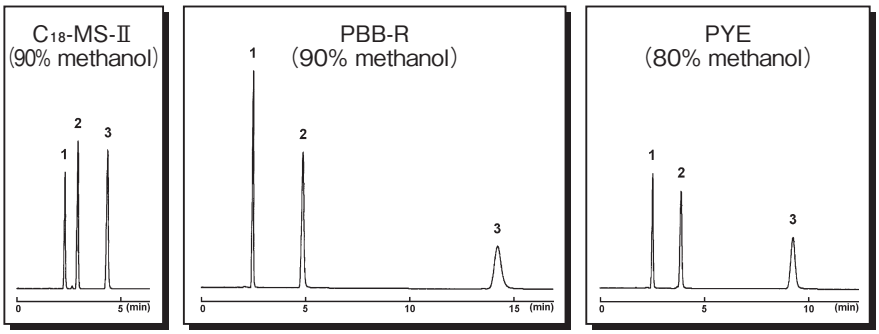
Column size	4.6 mm I.D. × 150 mm	
Flow rate	1.0 ml/min	
Temperature	30℃	
Detection	UV 254 nm	
Sample	1. *o*-Phthalonitrile	(0.3 μg)
	2. *m*-Phthalonitrile	(3.0 μg)
	3. *p*-Phthalonitrile	(0.15 μg)

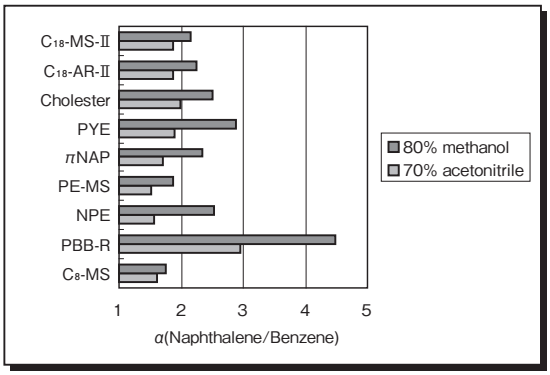


3) Selectivity for polyaromatic compounds

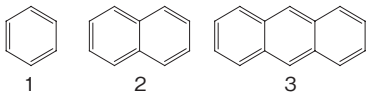
Selectivity

Selectivity for polyaromatic compounds is evaluated based on the separation of benzene, naphthalene and anthracene. The elution orders in all columns are the same : benzene, naphthalene and anthracene. Retention increases in all columns with increasing number of aromatic rings. In addition, highly dispersive packing materials such as PBB and PYE show much stronger retention for polyaromatic compounds due to dispersion interaction.





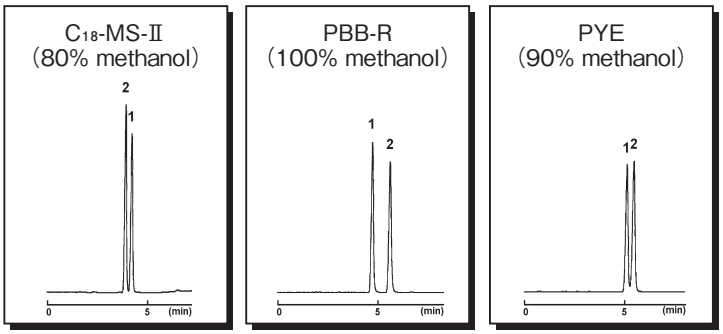
Column size	4.6 mm I.D. × 150 mm	
Flow rate	1.0 ml/min	
Temperature	30℃	
Detection	UV 254 nm	
Sample	1. Benzene	(1.67 μg)
	2. Naphthalene	(0.11 μg)
	3. Anthracene	(0.0063 μg)

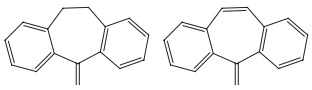


Application

・Separation of dibenzosuberone and dibenzosuberenone

C_{18} retains dibenzosuberone (peak 1) longer than dibenzosuberenone (peak 2). On the other hand, PBB-R and PYE retain dibenzosuberenone (peak 2), which has a π-electron conjugated system, longer than dibenzosuberone (peak 1).



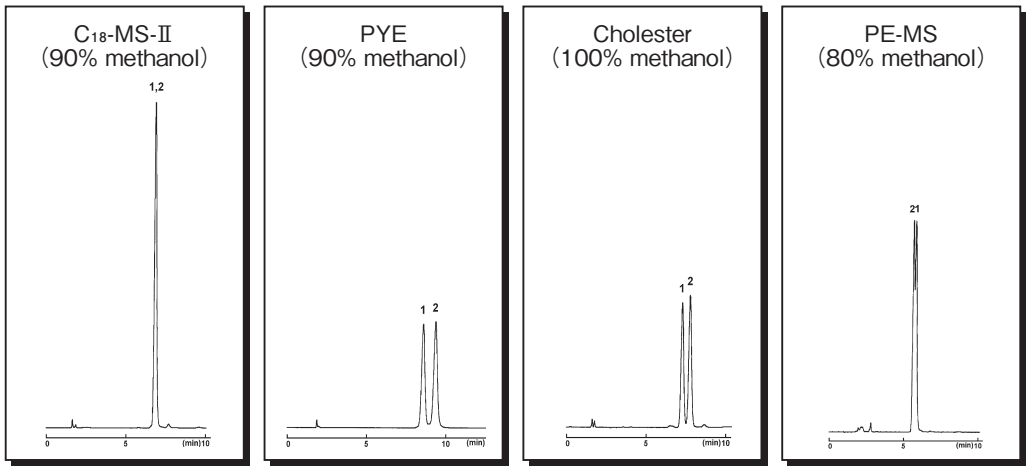


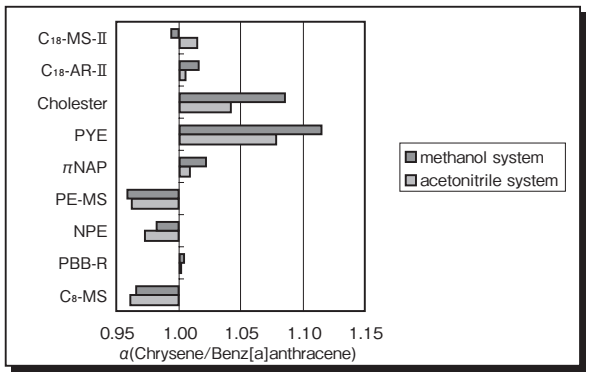
Column size	4.6 mm I.D. × 150 mm	
Flow rate	1.0 ml/min	
Temperature	30℃	
Detection	UV 254 nm	
Sample	1. Dibenzosuberone	(0.1 μg)
	2. Dibenzosuberenone	(0.025 μg)

4) Selectivity for molecular shape

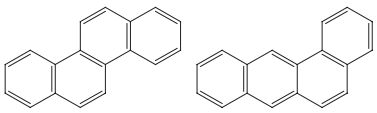
Selectivity

Selectivity for molecular shape is evaluated based on the separation of chrysene and benz[a]anthracene. The isomers of two polyaromatic hydrocarbons, which consist of four benzene rings, are difficult to separate because of the similar hydrophobicity or aromaticity. However, PYE and Cholester columns, which recognize molecular shape, enable them to separate chrysene and benz[a]anthracene.





Column size	4.6 mm I.D. × 150 mm	
Flow rate	1.0 ml/min	
Temperature	30℃	
Detection	UV 254 nm	
Sample	1. Chrysene	(0.04 μg)
	2. Benz[a]anthracene	(0.04 μg)

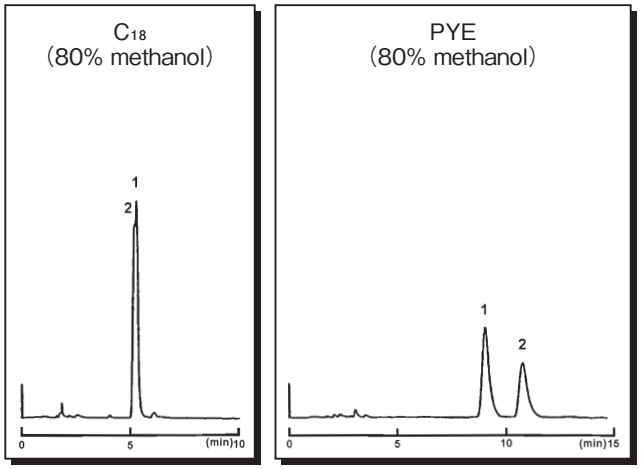


1 2

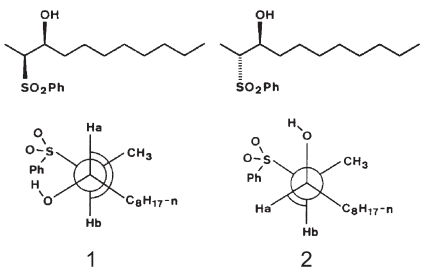
Application

・Separation of diastereomers (threo- and erythro-)

C_{18} cannot separate the threo and erythro forms. On the other hand, PYE retains the planar erythro form longer than the threo form.



Column size	4.6 mm I.D. × 150 mm
Flow rate	1.0 ml/min
Temperature	30℃
Detection	UV 254 nm
Sample	1. *threo* form
	2. *erythro* form



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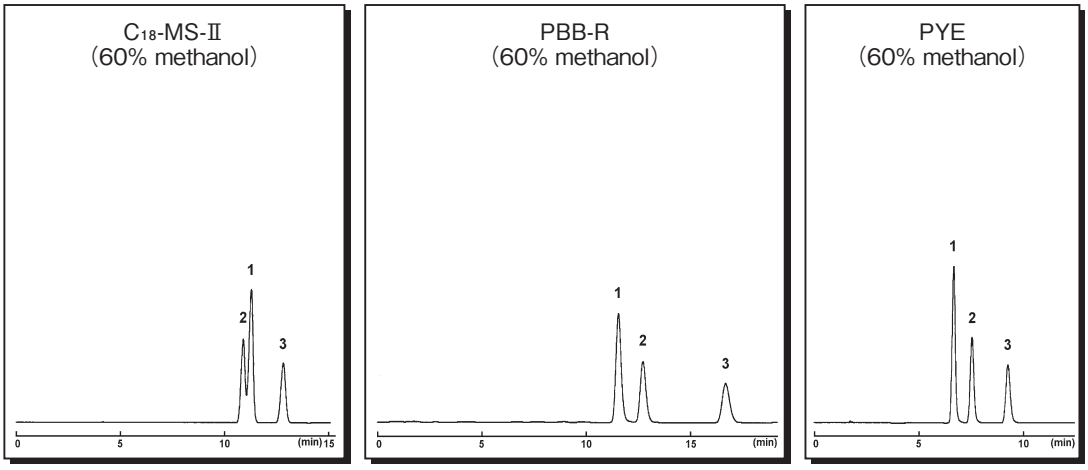
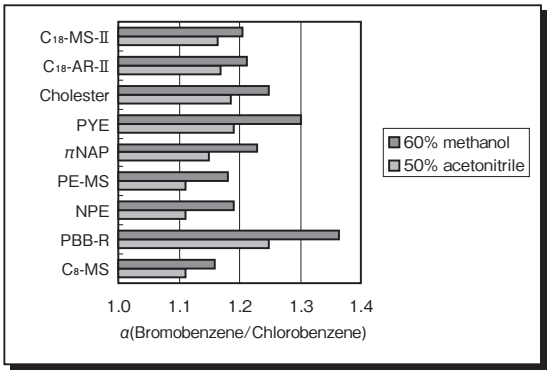
5) Selectivity for halide

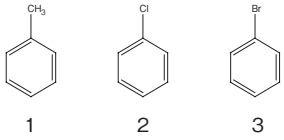
Selectivity

Selectivity for halide is evaluated based on the separation of chlorobenzene and bromobenzene. PBB-R shows the highest selectivity factor due to dispersion interaction of the five bromine atoms.





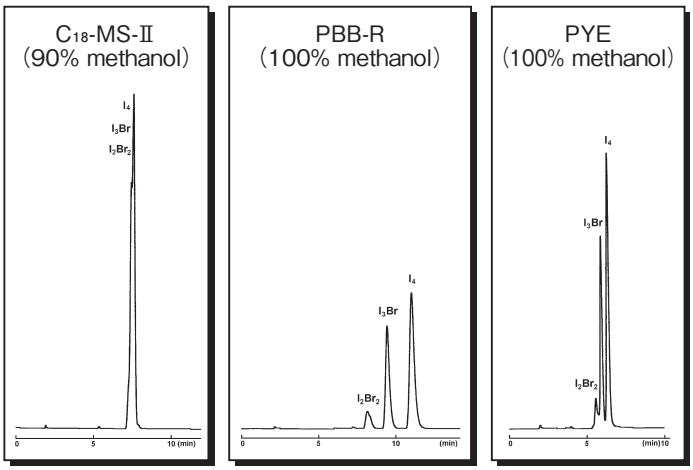
Column size	4.6 mm I.D. × 150 mm
Flow rate	1.0 ml/min
Temperature	30℃
Detection	UV 254 nm
Sample	1. Toluene (3.3 μg)
	2. Chlorobenzene (3.3 μg)
	3. Bromobenzene (3.3 μg)



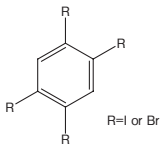
Application

・Separation of halogen exchange reaction products

PYE and PBB-R retain dispersed iodine atom longer than bromine atom. As a result, PYE and PBB-R can separate the complicated bromine and iodine compounds that C18 cannot separate.



Column size	4.6 mm I.D. × 150 mm
Flow rate	1.0 ml/min
Temperature	30℃
Detection	UV 254 nm
Sample	halogen exchange reaction product

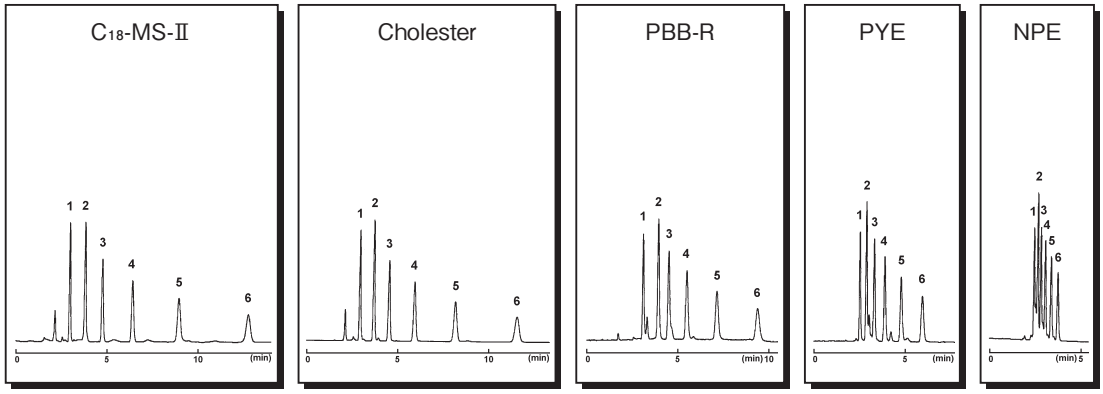


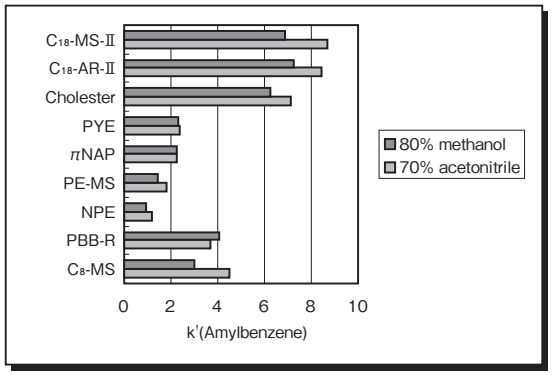
Sample courtesy of Dr. H. Yamamoto,
RIKEN, Condensed Molecular Materials Laboratory

6) Selectivity for hydrophobicity

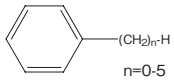
Selectivity

Selectivity for hydrophobicity is evaluated based on the separation of alkylbenzenes. Two C_{18} and Cholester show similar high selectivity for hydrophobicity. Other columns show less hydrophobic selectivity than C_{18}.

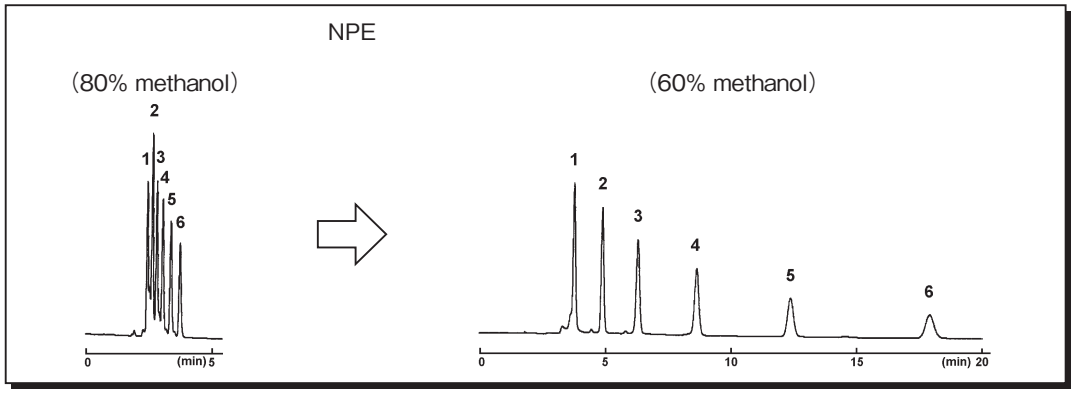




Column size	4.6 mm I.D. × 150 mm	
Mobile phase	methanol : water = 80 : 20	
Flow rate	1.0 ml/min	
Temperature	30℃	
Detection	UV 254 nm	
Sample	1. Benzene	(1.67 μg)
	2. Toluene	(1.67 μg)
	3. Ethylbenzene	(1.67 μg)
	4. Propylbenzene	(1.67 μg)
	5. Butylbenzene	(1.67 μg)
	6. Amylbenzene	(1.67 μg)



Lower concentration of organic solvent in mobile phase leads to much retention in reversed phase chromatography. In case of NPE, when methanol concentration is reduced to 60%, the retention times increase to those similar to C_{18} with 80% methanol.



TECHNICAL NOTE
2. Preparation of mobile phase for HPLC

1) Organic solvent/ aqueous mixed mobile phase

1)-1. Preparation of methanol : water = 70 : 30 (v/v) 1L

① Measure 700 ml of methanol in a measuring cylinder.
② Measure 300 ml of distilled water in a measuring cylinder.
③ Mix ① and ② thoroughly and degas.

The better approach is to prepare the mobile phase gravimetrically rather than volumetrically. Following is example of preparation.

Composition table for mobile phase 1L using methanol and water

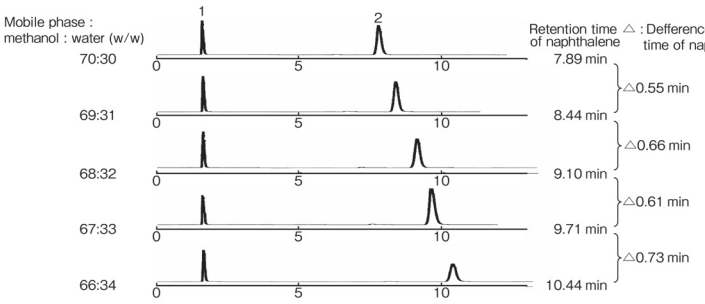
Methanol / Water	Methanol (g)	Distilled water (g)
90/10 (v/v)	711.9	99.8
80/20 (v/v)	632.8	199.6
70/30 (v/v)	553.7	299.5
60/40 (v/v)	474.6	399.3
50/50 (v/v)	395.5	499.1
40/60 (v/v)	316.4	598.9
30/70 (v/v)	237.3	698.7
20/80 (v/v)	158.2	798.6
10/90 (v/v)	79.1	898.4

Composition table for mobile phase 1L using acetonitrile and water

Acetonitrile / Water	Acetonitrile (g)	Distilled water (g)
90/10 (v/v)	707.4	99.8
80/20 (v/v)	628.8	199.6
70/30 (v/v)	550.2	299.5
60/40 (v/v)	471.6	399.3
50/50 (v/v)	393.0	499.1
40/60 (v/v)	314.4	598.9
30/70 (v/v)	235.8	698.7
20/80 (v/v)	157.2	798.6
10/90 (v/v)	78.6	898.4

Caution : Methanol and acetonitrile are hazardous substances, do not use for medical purpose. Always process in a laboratory hood and wear an eye protection and a mask.

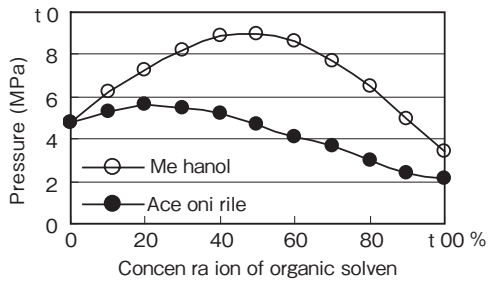
Influence of organic solvent composition in mobile phase on the retention time.
1% difference in the composition significantly changes the retention.

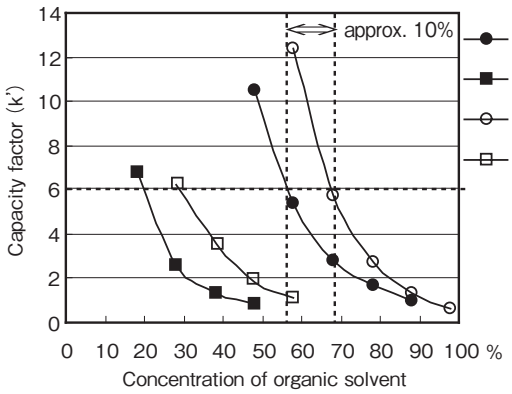


Column COSMOSIL 5C18-MS-Ⅱ
Column size 4.6 mm I.D. ×150 mm
Flow rate 1.0 ml/min
Detection 254 nm 0.16 AUFS
Temperature 30℃
Sample 1 uracil
2. naphthalene

Special attention should be paid to measure correct amount of organic solvent as the retention time is significantly changed by 1% different composition.

Differences between acetonitrile and methanol in reversed phase liquid chromatography

	Acetonitrile (for HPLC)	Methanol (for HPLC)
Pressure		Column COSMOSIL 5C18-MS-Ⅱ Column size 4.6 mm I.D. ×150 mm Flow rate 1.0 ml/min Temperature 30℃ Back pressure differs depending on species of organic solvents and mixing ratio. Back pressure of acetonitrile is lower than that of methanol at the same concentration.

	Acetonitrile (for HPLC)	Methanol (for HPLC)
Elution strength	 Column COSMOSIL 5C18-MS-Ⅱ Column size 4.6 mm I.D. ×150 mm Flow rate 1.0 ml/min Temperature 30℃ Elution strength of acetonitrile/water is stronger than methanol/ water at the same concentration. Within the range of about 30%-80% in the concentration of the organic solvent, elution strength of acetonitrile/water is almost equivalent to that of methanol/water which was raised methanol concentration about 10% (ex : acetonitrile/water = 60/40 → methanol/water = 70/30)	
Absorbance	Acetonitrile has a lower UV absorbance in far UV region (less than 250 nm) .	Methanol has a higher UV absorbance than acetonitrile in far UV region (less than 250 nm).
Degas of mobile phase	When acetonitrile is mixed with water, it is endothermic. It is difficult to degas.	When methanol is mixed with water, it is exothermic. It is easy to degas.

2) Organic solvent/ buffer mixed mobile phase

2)-1. Preparation of methanol : 20 mmol/l phosphate buffer (pH2.5) = 80 : 20 (v/v) 1L

・Preparation of 20 mmol/l phosphate buffer (pH2.5)

① Prepare 20 mmol/l sodium dihydrogenphosphate aqueous solution.

② Prepare 20 mmol/l phosphoric acid aqueous solution.

③ Adjust the pH to 2.5 by mixing ① with ② .

④ Filter ③ under reduced pressure to remove insoluble substance that may deteriorate pump-seal and clog columns (0.45 μm or smaller pore size is recommended).

(Easy method)

① Dissolve 1.31 g of sodium dihydrogenphosphate and 1.05 g of phosphoric acid in distilled water to make 1 L solution.

② Filter the solution under reduced pressure to remove insoluble substance (0.45 μm or smaller pore size is recommended).

③ Confirm that the solution is pH2.5.

・Preparation of methanol : 20 mmol/l phosphate buffer (pH2.5) = 80 : 20 1L

① Measure 800 ml of methanol in a measuring cylinder.

② Measure 200 ml of 20 mmol/l phosphate buffer (pH2.5) in a measuring cylinder.

③ Mix ① and ② thoroughly and degas.

2)-2. Preparation of methanol : 20 mmol/l phosphate buffer (pH7.0) = 80 : 20 (v/v) 1L

・Preparation of 20 mmol/l phosphate buffer (pH7.0)

① Prepare 20 mmol/l sodium dihydrogenphosphate aqueous solution.

② Prepare 20 mmol/l di-sodium hydrogenphosphate aqueous solution.

③ Adjust the pH7.0 by mixing ① with ② .

④ Filter ③ under reduced pressure to remove insoluble substance that may deteriorate pump-seal and clog columns (0.45 μm or smaller pore size is recommended).

(Easy method)

① Dissolve 1.14 g of sodium dihydrogenphosphate and 1.49 g of di-sodium hydrogenphosphate in distilled water to make 1L solution.

② Filter the solution under reduced pressure to remove insoluble substance (0.45 μm or smaller pore size is recommended).

③ Confirm that the solution is pH7.0.

・Preparation of Methanol : 20 mmol/l phosphate buffer (pH7.0) = 80 : 20 1 L

① Measure 800 ml of methanol in a measuring cylinder.

② Measure 200 ml of 20 mmol/l phosphate buffer (pH7.0) in a measuring cylinder.

③ Mix ① and ② thoroughly and degas.

The better approach is to prepare the mobile phase gravimetrically rather than volumetrically. Following is example of preparation.

methanol : 20 mmol/l phosphate buffer	methanol (g)	20 mmol/l phosphate buffer (pH2.5) (g)	20 mmol/l phosphate buffer (pH7.0) (g)
90/10 (v/v)	711.9	99.8	99.9
80/20 (v/v)	632.8	199.6	199.8
70/30 (v/v)	553.7	299.4	299.7
60/40 (v/v)	474.6	399.2	399.6
50/50 (v/v)	395.5	499.0	499.5
40/60 (v/v)	316.4	598.8	599.4
30/70 (v/v)	237.3	698.6	699.3
20/80 (v/v)	158.2	798.4	799.2
10/90 (v/v)	79.1	898.2	899.1

Caution : Methanol and acetonitrile are hazardous substances, do not use for medical purpose. Always process in a laboratory hood and wear an eye protection and a mask.

3) Preparation of ion pair reagent containing mobile phase

3)-1.Preparation of 5 mmol/l sodium *1*-butanesulfonate containing 20 mmol/l phosphate buffer (pH2.5)

① Prepare 5 mmol/l sodium *1*-butanesulfonate containing 20 mmol/l sodium dihydrogenphosphate aqueous solution.

② Prepare 5 mmol/l sodium *1*-butanesulfonate containing 20 mmol/l phosphoric acid aqueous solution

③ Adjust the pH to 2.5 by mixing ① with ② .

④ Filter ③ under reduced pressure to remove insoluble substance that may deteriorate pump-seal and clog columns (0.45 μm or smaller pore size is recommended).

(Easy method)

① Dissolve 1.31 g of sodium dihydrogenphosphate, 1.05 g of phosphoric acid and 0.80 g of sodium *1*-butanesulfonate in distilled water to make 1L solution.

② Filter the solution under reduced pressure to remove insoluble substance (0.45 μm or smaller pore size is recommended).

③ Confirm that the solution is pH2.5.

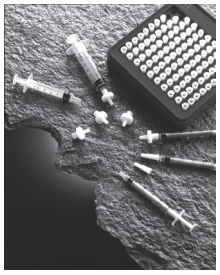
※ 0.5M sodium *1*-butanesulfonate aqueous solution is also available from Nacalai Tesque.

TECHNICAL NOTE
3. Sample pretreatment for HPLC

Pretreatment before HPLC analysis is often required for samples of low concentration or samples containing analytical contaminants. It improves reproducibility and sensitivity in analysis, and protects HPLC columns. The preparation methods are different according to the each sample. The followings are examples of different pretreatments.

1) Filtration

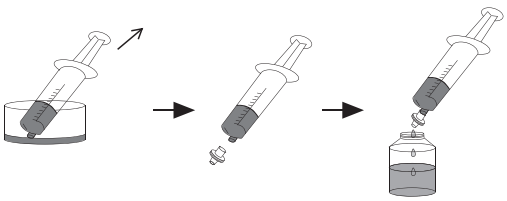
Filtration is a common method used for separating solids from liquids. It extends a column's life by minimizing column damages from solid contaminants such as particles, sediments and colloid substances. It also improves reproducibility of analytical data. We offer both syringe-type and spin-type filters for sample filtration.

■ Syringe filter	■ Centrifugal filter
Cosmonice filter	Cosmospin filter
	
Easy to use. Just attach a filter on top of a syringe.	Easy to use by centrifugation.
・W (aqueous system) ・S (solvent system)	・pore diameter : 0.2 μm ・pore diamater : 0.45 μm
■ Required equipment	
Syringe・Sample bottle	Centrifuge
■ Product information	
Please see page 73	Please see page 73

Cosmonice filter

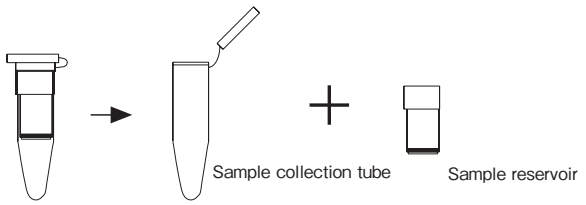
How to use :

① Fill a syringe with the sample you want to filter.
② Attach a Cosmonice filter to the syringe.
③ Push the syringe plunger to filter the sample.
④ Analyze the filtered sample by HPLC.



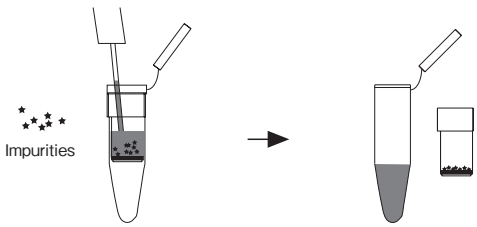
Cosmospin filter

Components : ・Sample reservoir
・Sample collection tube



How to use :

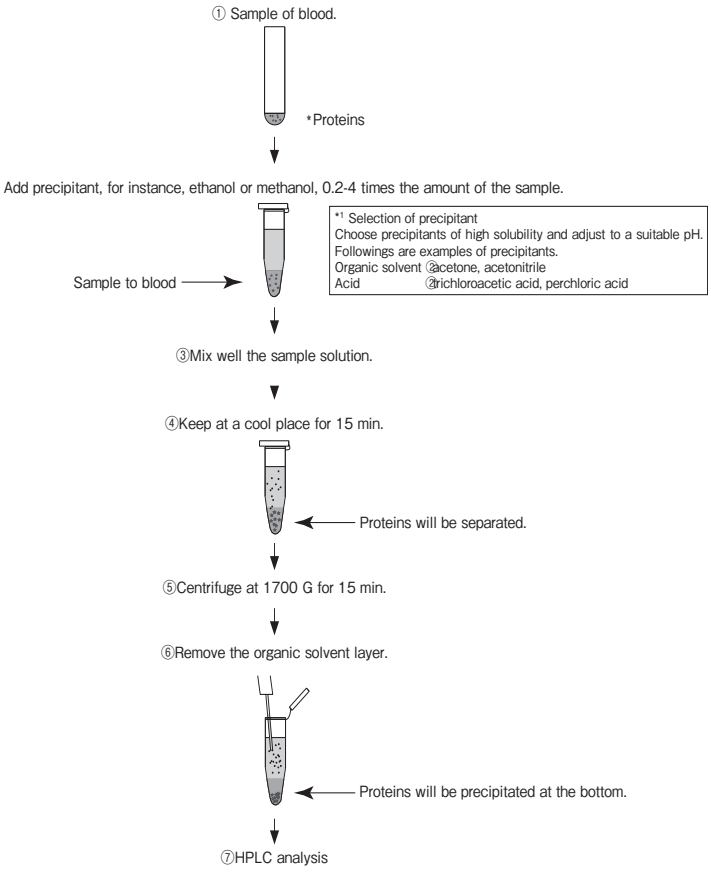
① Insert a Cosmospin sample reservoir into a Cosmospin sample collection tube.
② Add a sample into the Cosmopsin sample reservoir.
③ Close the sample collection tube cap and centrifuge.
④ Remove the sample reservoir and collect the filtered sample in the sample collection tube.
⑤ Analyze the filtered sample by HPLC.



2) Protein precipitation

Protein precipitation is commonly used to remove proteins in samples for downstream analysis. For example, when analyzing drug concentration in blood samples, proteins have to be removed first. Otherwise, proteins may be adsorbed in columns and interfere with the analysis. Common methods for protein precipitation include salting out, isoelectric point precipitation and precipitation with organic solvents. The following shows a general procedure for protein precipitation with organic solvents.

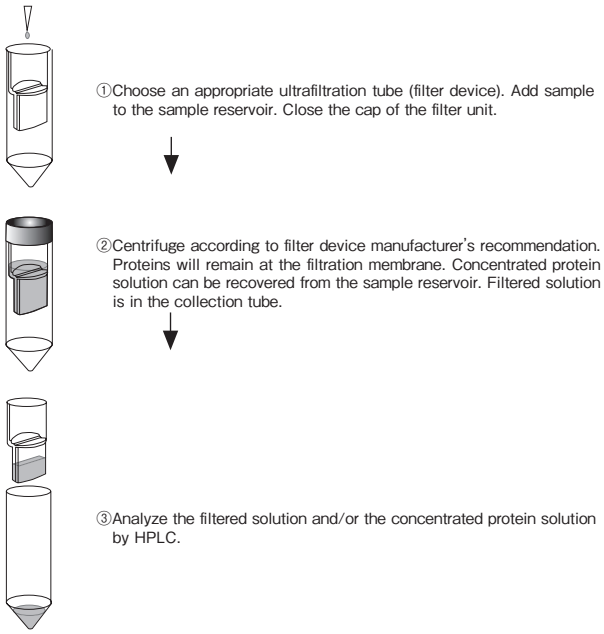
Procedure for protein precipitation :



3) Ultrafiltration

Ultrafiltration is a method to concentrate proteins or other macromolecules through a semi-permeable membrane with defined pores. Ultrafiltration is applicable for sample desalting, concentrating proteins from dilute solution such as urine samples, or deproteinizing samples with high protein concentration (i.e. blood serum or plasma). Following is a general procedure for ultrafiltration.

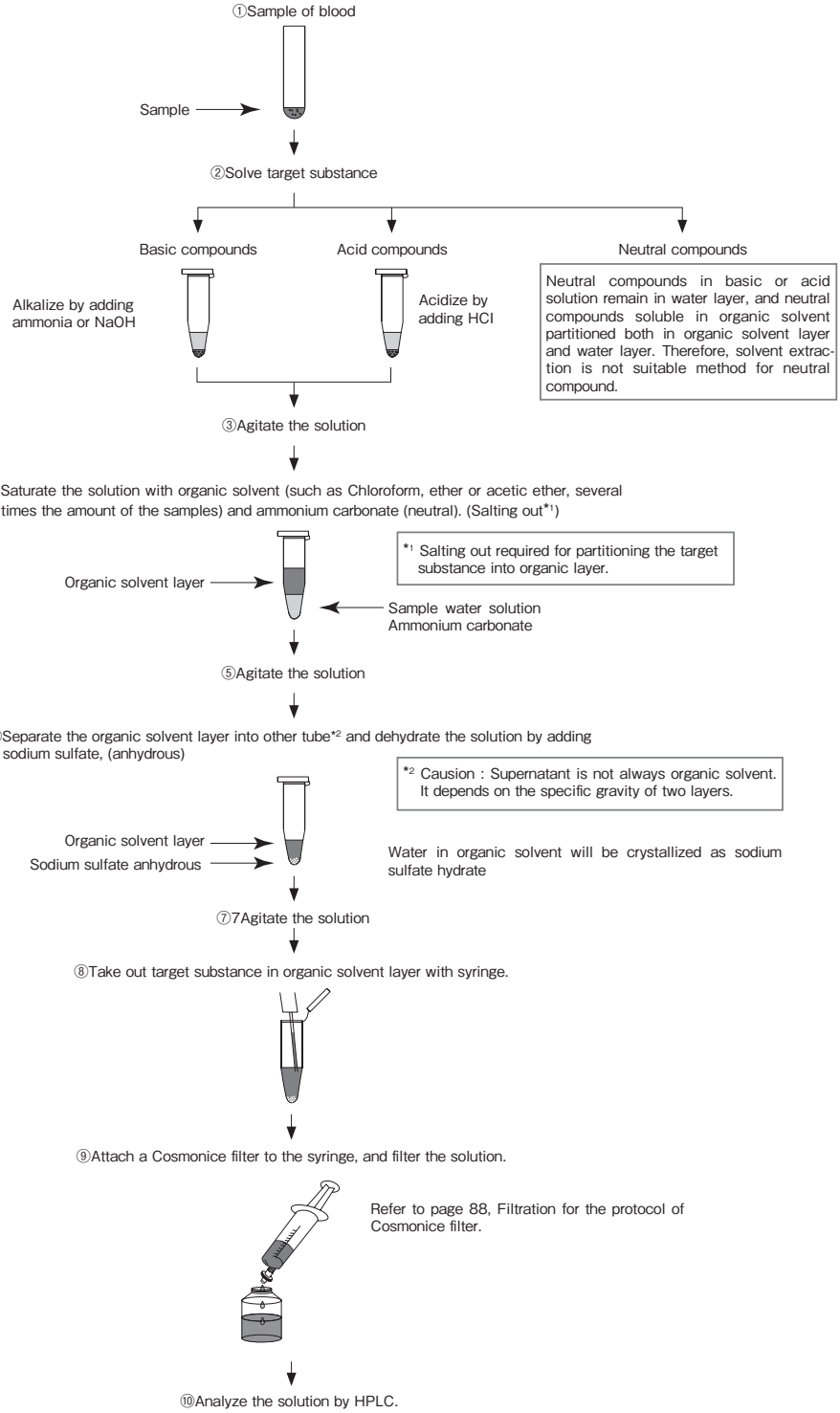
Procedure for ultrafiltration :



4) Solvent extraction method

Solvent extraction is a method to separate compounds due to their unequal solubility in two immiscible liquid phases, usually water and an organic solvent. The method is used to concentrate highly hydrophobic compounds, and consequently increase analytical sensitivity. A buffer solution is added to sample to optimize the pH and target substance is then extracted by an organic solvent such as ether and chloroform. However, when target substance is combined with proteins, solvent extraction may not work well.

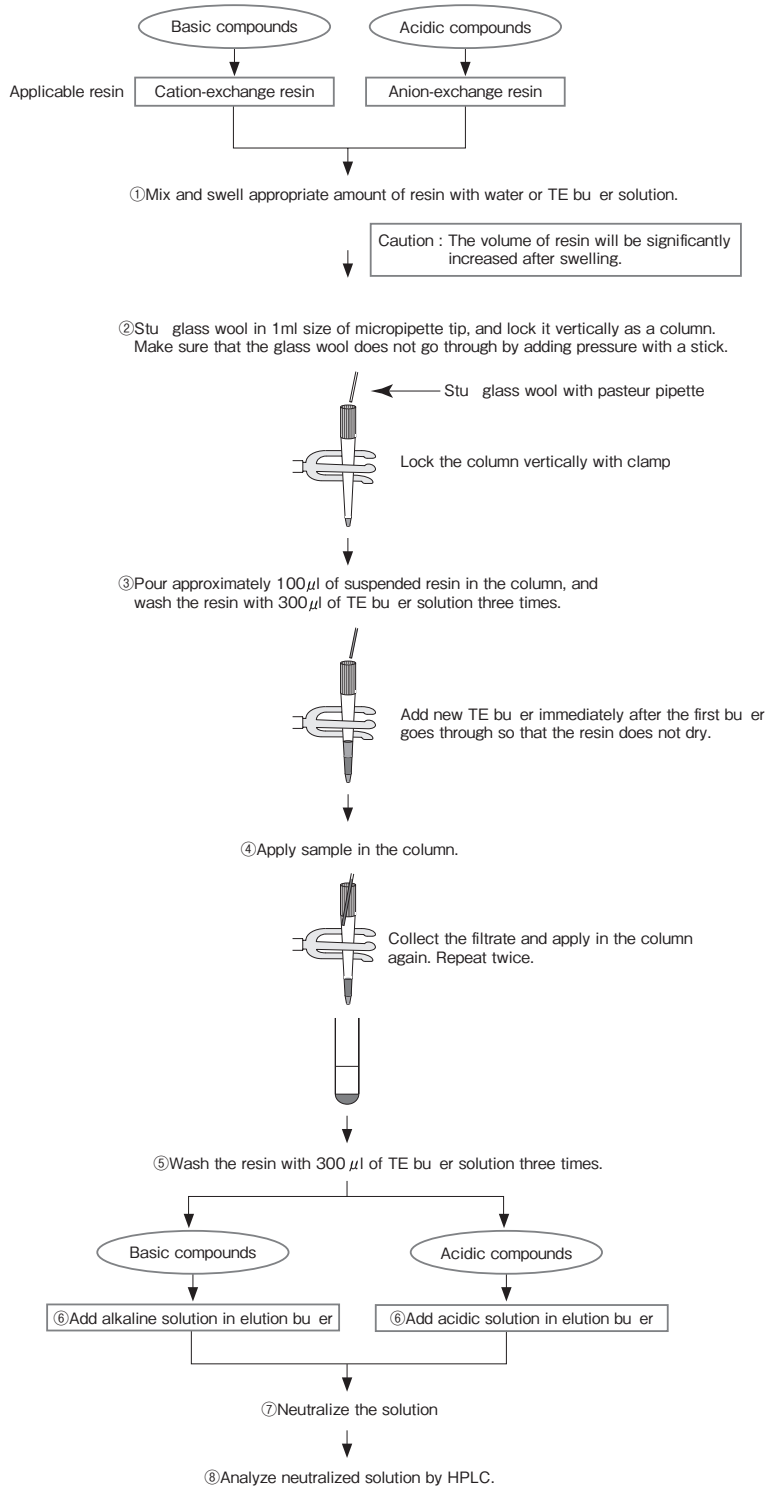
Procedure for solvent extraction method :



5) Ion exchange

Pretreatment by ion-exchange resin may be effective for samples that the solvent extraction method cannot be adapted due to its emulsification. A preliminary experiment may be required for the selection of resin and experimental conditions. For example, a negatively charged compound is strongly adsorbed on an anion-exchange resin such as DEAE cellulose resin. Therefore, the target compound is collected by increasing salt concentration of buffer solution or adjusting pH of elution buffer after washing off other weakly adsorbed undesired substances.

Procedure for ion exchange :



TECHNICAL NOTE
4. Baseline noise in gradient elution

In gradient analysis, incomplete mixing of mobile phases or impurities in water of mobile phase can cause baseline noise. In the former case, it can be improved by using a proper mixer before injector (Baseline 1→2). In the latter case, it can be improved by using a pre-column. Impurities in water are adsorbed on the pre-column (Baseline 2→3). COSMOSIL 5C$_{18}$-AR-Ⅱ 4.6 mm I.D. x 10 mm or 10 mm I.D. x 20 mm as a pre-column.

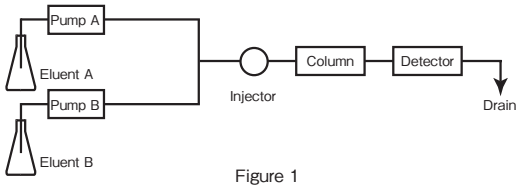
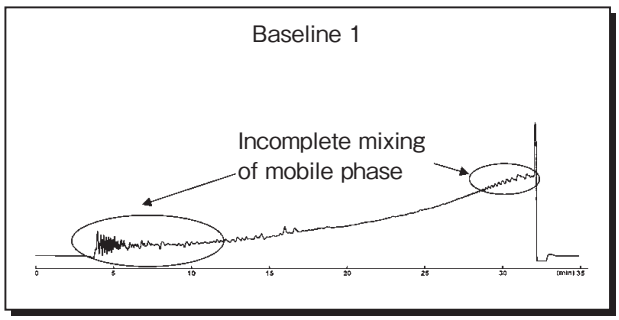


Figure 1



+ Mixer

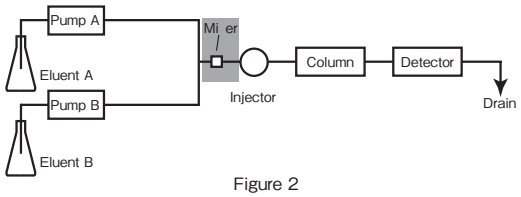
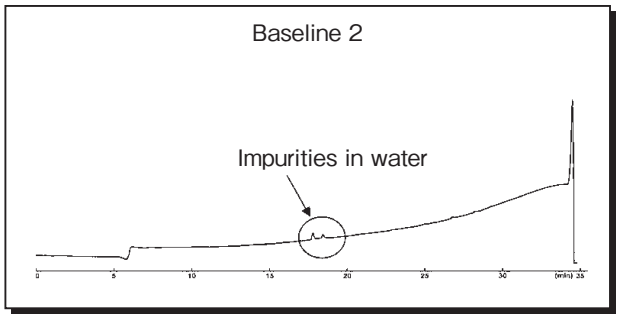


Figure 2



+ Pre-column

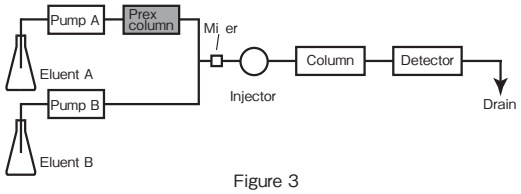
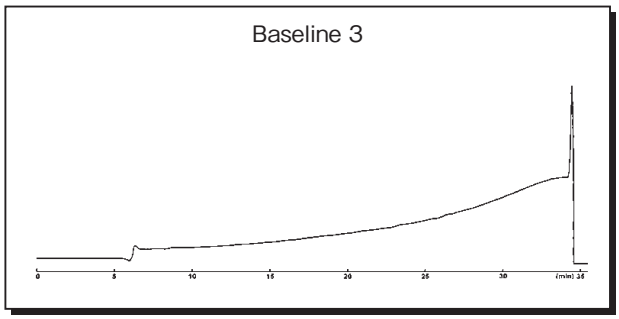


Figure 3



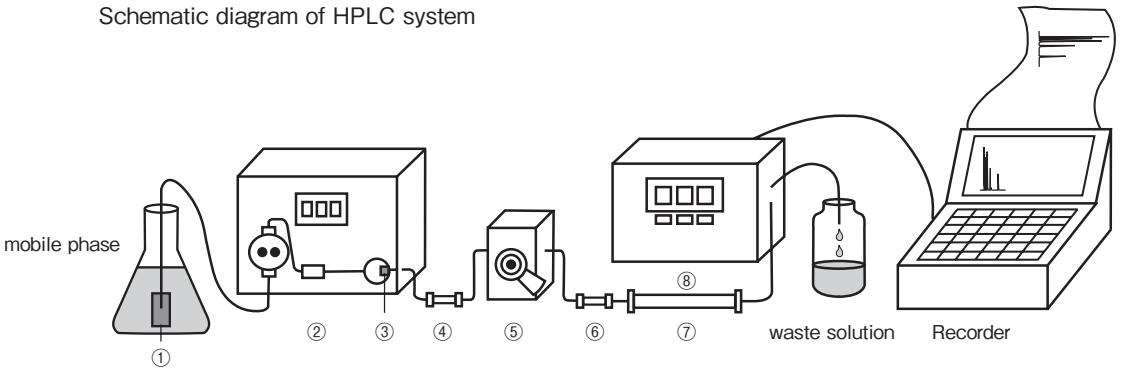
Column	COSMOSIL 5C$_{18}$-AR-300 4.6 mm I.D. × 150 mm
Precolumn	COSMOSIL 5C$_{18}$-AR-Ⅱ 4.6 mm I.D. × 10 mm
Mobile phase	A : 0.1% TFA containing water
	B : 0.1% TFA containing 95% acetonitrile
	B 0% → 100%/30 min linear gradient
Flow rate	1.0 ml/min
Temperature	30℃
Detection	UV 220 nm

TECHNICAL NOTE
5. Troubleshooting for increased pressure

Repeated analysis may increase back pressure. Continuous use of HPLC columns under high pressure can cause deterioration and overload of the equipment. Therefore, it is important to monitor column back pressure regularly and solve the problem timely.

The back pressure increase can be due to clogging of a column or clogging of the equipment. First of all, identify the clogging site.

Schematic diagram of HPLC system



①Suction filter → ②Pump → ③Line filter → ④Pre-column → ⑤Injector → ⑥Guard column → ⑦Analytical column → ⑧Detector

Remove analytical column first and connect the plumbing from the back of HPLC system directly to the detector. Measure the pressure of flowing mobile phase without an HPLC column. Generally equipment should hardly generate any pressure. If there is significant flow pressure, disconnect the system components one by one to identify the clogged component(s). Possible causes and solutions of clogged equipment are discussed in section II below.

If the flow pressure without a column is normal, then pressure increase is due to clogging of a column. In this case, one needs to determine the causes and whether it is time to replace the column. Possible causes and solutions of clogged column are discussed in greater details in sections I and III.

Symptom				Possible Cause
Pressure increase rapidly in short-term use	→ Yes	Flow pressure without a column is 0-0.3 MPa	→	Clogging of column Refer to section Ⅰ
		Flow pressure with a column is 0.3 MPa or higher	→	Clogging of equipment Refer to section Ⅱ
Pressure increase gradually in long-term use	→ Yes	Deterioration of column due to long-term use Refer to section Ⅲ		

Ⅰ. Solution in case an HPLC column is clogged in short-term use.

Select the possible cause of clogging according to the following flow chart.

Step1	・Salt deposition ・Use mobile phase of high concentration organic solvent right after using buffer	→ Yes	Cause 1
	↓ NO		
Step2	・Forget to filter mobile phase ・Sample is not dissolved enough	→ Yes	Cause 2
	↓ NO		
Step3	・Analyzing samples which tend to absorb to a column (i.e. protein samples) ・Sample deposition in column	→ Yes	Cause 3

Cause 1 Salt is deposited on a column.
Solution : Wash columns for 30 minutes at half of the analytical flow rate with 10% organic solvent (methanol or acetonitrile) in water to dissolve deposited salt. If the situation is not improved, wash with 100% water under the same condition.
Prevention : To switch to high concentration organic solvent after using a buffer, first wash a column with a mobile phase not containing salt (with the same concentration of organic solvent as the buffer), then switch to the mobile phase of higher organic solvent concentration. Example : To change mobile phase from 10/90 (v/v) acetonitrile/20mmol/l phosphate buffer (pH2.5) to 90/10 (v/v) acetonitrile/water, first wash for 15 minutes with 10/90 (v/v) acetonitrile/water, and then switch to 90/10 (v/v) acetonitrile/water.

Cause 2 Column filter is clogged by sample or impurities.
Solution : Connect the column in reverse direction, and then wash the column for 30 minutes at half of the usual analytical flow rate with the mobile phase used for analysis. If the situation is not improved, change the end fitting in the front of column. (We can replace end fittings with a paid service fee.)
Prevention : We recommend filtering sample and/or mobile phase. For more information, please see page 88 TECHNICAL NOTE 3. Sample pretreatment for HPLC 1) filtration.

Cause 3 Sample may be adsorbed to packing material or deposited in a column.
Solution : Wash for 30 minutes at half of analytical flow rates with a solvent which adsorbed substances are dissolved in. The followings are how to wash each type of columns.
[Reversed phase columns]
a) When an absorbed substance is not protein, wash with methanol or tetrahydrofuran.
b) When an absorbed substance is protein, wash with 50-70% of acetonitrile/water (containing 0.1% of trifluoroacetic acid). However proteins may be deposited in high concentration of organic solvent depending on varieties.
[COSMOSIL Sugar-D/NH_2/HILIC columns]
Wash with 50/50 (v/v) acetonitrile/water for NH_2-MS and 100% water for Sugar-D and HILIC columns.
[COSMOSIL SL-Ⅱ]
Wash with methanol, tetrahydrofuran or ethanol.
Prevention : Choose appropriate pretreatment for each sample. For more information, please see page 88 TECHNICAL NOTE 3. Sample pretreatment for HPLC 1) filtration. We also recommend using guard column. For more information for guard columns, please see page 96 TECHNICAL NOTE 6. Effect of guard columns.
Caution :
- When wash columns, do not connect column exit and let the solution through.
- Long term of washing may deteriorate the performance of columns.
- Do not use strongly alkaline solution (more than pH 7.5) or strongly acidic solution (less than pH 1.5) for silica gel base packing material.
- Store columns with manufacturer recommended storage solvent after washing,

When the situation is not improved, replace the column.

Ⅱ. Solutions in case pressure is too high because of clogged equipment.

First off, identify the specific clogging site by disconnecting the components in the system one by one and checking the flow pressure. The followings are possible common causes.

Cause 1 Salt is deposited in plumbing.
Solution : Flow water to the plumbing without connecting a column and any other equipment. Washing out the plumbing in a reversing connection is also an effective way. If the situation is not improved, replace it with a new one.

Cause 2 Check-valve of pump is clogged by stain
Solution : Wash the check-valve with a stain dissolving solvent. Take apart the washable part, soak it in the solvent, then clean in an ultrasonic cleaner.

Cause 3 Manual injector is clogged with stain
Solution : Wash with a stain dissolving solvent. Soak rotor seal and line filter in water and clean them in an ultrasonic cleaner. If the situation is not improved, replace the injector.
Prevention : It extends the life time of an HPLC system to maintain regular wash of the system. Wash the

system the same as wash an HPLC column. When the mobile phase contains salt, wash for 10-15 minutes with a mobile phase which has the same composition but not containing salt. For example, when using 50/50 (v/v) methanol/20mmol/l phosphate buffer, wash with 50/50 (v/v) methanol/water. When the mobile phase contains halogen, acid and/or base, wash for 10-15 minutes with mobile phase which has the same composition but not containing halogen, acid and/or base.

Ⅲ. Solutions in case a column is damaged from long term use

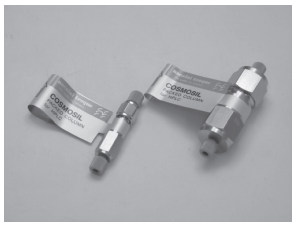
Every column will have to be replaced eventually. Performance of a column is expected to deteriorate slowly after long term use. One has to decide whether it is time to replace the column.

Cause 1 Column deterioration result from long term use.
Solution : Wash according to Solution to Section Ⅰ, Cause 3.
Prevention : Same as Prevention in Section Ⅰ, Cause 3. When the column condition is not improved, you could continue to use the column if peak shapes do not change and the maximum pressure is less than 20 MPa. However, we recommend replacing the column because it place extra burden on the equipment.

Cause 2 Silica gel in the column may be cracked because of long term use.
Solution : Replace the columns.

TECHNICAL NOTE
6. Effect of guard column

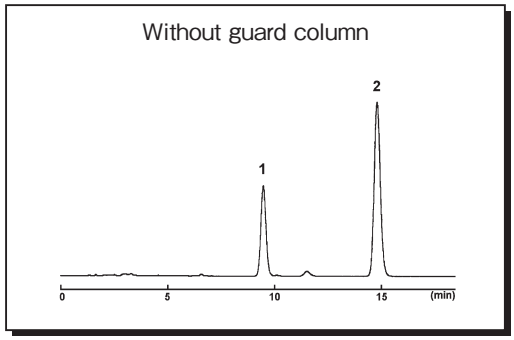
The use of guard columns to protect both analytical and preparative columns is highly recommended.
COSMOSIL guard columns are packed with packing materials identical to that used in analytical and preparative columns. As a result, the COSMOSIL guard columns do not contribute to any decrease in the performance of the main column.

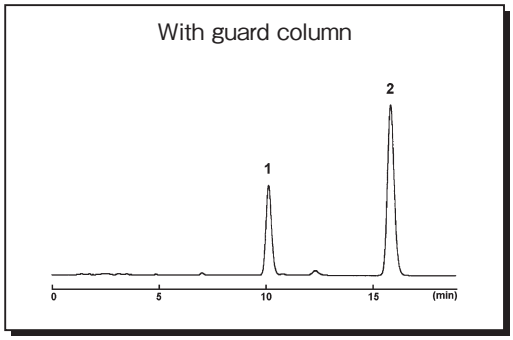

Guard columns


Guard cartridges

Example of using guard columns

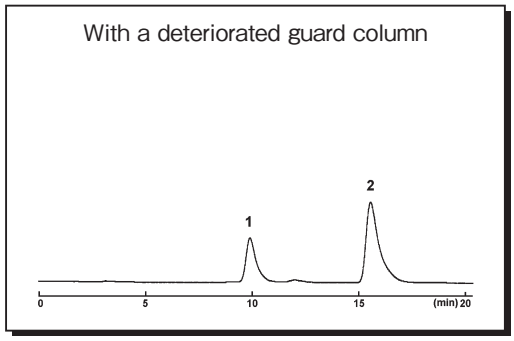
The following chromatograms show analysis examples using a COSMOSIL 5C$_{18}$-MS-Ⅱ analytical column (4.6 mm I.D. × 150 mm) and the same column connected with its guard column (4.6 mm I.D. × 10 mm). There is no change in separation characteristics since the packing material of the guard column is identical to that used in the main column.

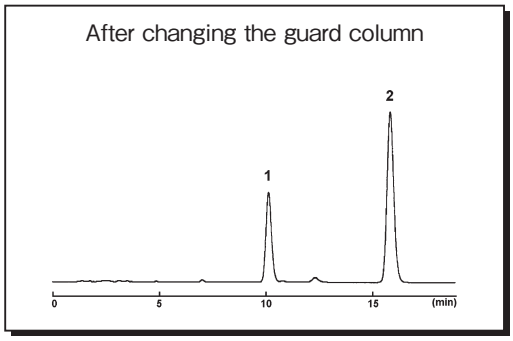




Inspection of columns

We recommend that the performance of a column be examined regularly. Deteriorated columns shall be replaced timely. If a deteriorated guard column continues to be used, the packed column will also deteriorate.





Column	COSMOSIL 5C$_{18}$-MS-Ⅱ 4.6 mm I.D. × 150 mm	Temperature	30℃
	COSMOSIL 5C$_{18}$-MS-Ⅱ 4.6 mm I.D. × 10 mm	Detection	UV 254 nm
Mobile phase	methanol : water = 70:30	Sample	1. Betamethasone *17*-Valerate (0.25 μg)
Flow rate	1.0 ml/min		2. Isoamyl Benzoate (2.5 μg)

Ordering information

Please see respective pages of each column.

Please see page 75 for the parts necessary to use guard columns or guard column cartridges.

TECHNICAL NOTE

7. Troubleshooting for normal phase chromatography

Q 1 : How can I convert from reversed phase mode to normal phase mode or vice versus using the same HPLC equipment?

A 1 : To convert from reversed phase mode to normal phase mode, or vice versus, flush the equipment with a solvent that is miscible with both the current mobile phase and the intended mobile phase. Connect the HPLC pump directly with the detector, and replace the solvents according to following instructions.

Solvent conversion from reversed phase to normal phase

① To convert a mobile phase without buffer solution in reversed phase to normal phase, replace solvents according to the following steps :
   1) Flush the equipment with a solvent for reversed phase. For example, methanol/H_2O (v/v=50/50).
   2) Flush the equipment with a solvent miscible to both mobile phases. For example, tetrahydrofuran, ethanol.
   3) Flush the equipment with a solvent for normal phase. For example, hexane/ethyl acetate

② To convert a mobile phase with buffer solution in reversed phase to normal phase, replace solvents according to the following steps :
   1) Flush the equipment with a solvent with buffer solution for reversed phase. For example, methanol/phosphate buffer (v/v=50/50).
   2) Flush the equipment with a solvent with the composition same as 1) and without salt, for example methanol/H_2O (v/v=50/50).
   3) Flush the equipment with a solvent miscible to both mobile phases. For example, tetrahydrofuran, ethanol.
   4) Flush the equipment with a solvent for normal phase. For example, hexane/ethyl acetate.

Solvent conversion from normal phase to reversed phase

① To convert from normal phase to a mobile phase without buffer solution in reversed phase, replace solvents according to following steps :
   1) Flush the equipment with a solvent for normal phase. For example, hexane/ethyl acetate.
   2) Flush the equipment with a solvent miscible to both mobile phases. For example tetrahydrofuran, ethanol.
   3) Flush the equipment with a solvent for reversed phase. For example, methanol/H_2O (v/v=50/50).

② To convert from normal phase to a mobile phase with buffer solution in reversed phase, replace solvents according to following steps :
   1) Flush the equipment with a solvent for normal phase. For example, hexane/ethyl acetate.
   2) Flush the equipment with a solvent miscible to both mobile phases. For example tetrahydrofuran, ethanol.
   3) Flush the equipment with a solvent with the composition same as 4) and without salt, for example methanol/H_2O (v/v=50/50).
   4) Flush the equipment with a solvent for reversed phase. For example, methanol/phosphate buffer (v/v=50/50).

Q 2 : My flow rate is not stable. How can I troubleshoot?

A 2 : Possible causes for unstable flow rate can be a malfunctioning check valve or air in a mobile phase. Wash the check valve thoroughly by ultrasonic cleaner. Solvents with a low boiling point such as *n*-Hexane and *n*-Heptane generate air easily. To prevent air generation, degas the mobile phase sufficiently.

Q 3 : In spite of using the same condition, the retention time is different. How can I solve the problem?

A 3 : One possible cause is unstable flow rate. Please refer to Q2 section.
Another possible cause is variation of polar component in mobile phases. In normal phase chromatography, the retention time depends on the concentration of small amounts of very polar constituents in the mobile phase. This is especially true for water content in a mobile phase. In this case, always use fresh solvents in a mobile phase. If sample solvent includes water, change to a solvent without water or decrease injection volume. If a column contains water, remove water from the column by washing it with ethanol.

Q 4　How can I wash the COSMOSIL SL-Ⅱ column?

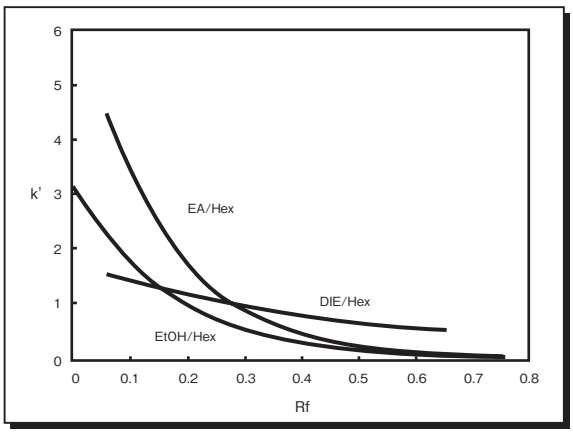
A 4　The SL-Ⅱ column can be washed with tetrahydrofuran, methanol, ethanol, methylene chloride, *n*-Hexane or *n*-Heptane.

Q 5 : How can I store the COSMOSIL SL-Ⅱ column?

A 5 : Store the SL-Ⅱ column with the shipping screw tighten in order to prevent the solvent in the column from volatilization. In case where a solvent containing halogens is used, replace the solvent in the column with a solvent without halogens such as *n*-Heptane before storing.

Q 6 : Could I choose a mobile phase based on TLC data?

A 6 : The retention times of HPLC can be deduced from the Rf values of TLC. Refer to the figure below.



EA/Hex　: ethyl acetate/hexane system
EtOH/Hex : ethanol/hexane system
DIE/Hex　: diisopropyl ether/hexane system

Q 7 : I get peak tailing in my run. What can I do about it?

A 7 : ① In case where the sample contains acidic compounds, add approx. 0.5% of acetic acid to the mobile phase.
② In case where the sample contains basic compounds, add approx. 0.5% of triethylamine to the mobile phase.

Q 8 : I get no peaks. How can I troubleshoot?

A 8 : First off, make sure that there is no problem with the system. If the problems are with the sample, sample solvent or mobile phase, try following check list.

1. The analyte may not be eluted from a column because the retention of the analyte is too strong. In this case, use a stronger eluent (mobile phase).

2. The sample contains chelating compounds or basic compounds. They may be adsorbed to the packing materials. In this case, add 0.1% - 1% acid (trifluoroacetic acid or acetic acid) to the mobile phase.

TECHNICAL NOTE

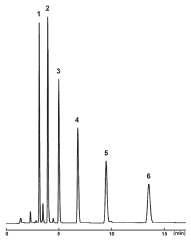
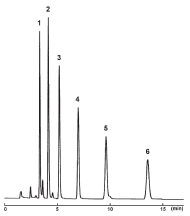
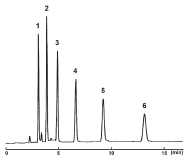
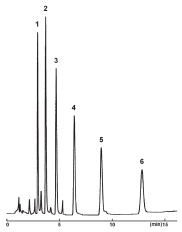
8. Inner diameter of column (scale down and scale up)

The figure below shows general parameters for 1.0 mm to 50 mm I.D. COSMOSIL columns : flow rate, equipment, inner diameter of pipe, application, surface ratio (compared with 4.6 mm I.D.) and particle size. It may help to scale up or down from the most commonly used 4.6 mm I.D. column.

Inner diameter (mm I.D.)	1.0	2.0	3.0	4.6	10	20	28	50
Flow rate (ml/min)	0.05	0.2	0.4	1.0	5.0	18	37	70
Detector cell・Injector	for semi-micro		for analytical			for preparative		
Inner diameter of pipe (mm)	0.05	0.1	0.2-0.3			1.0		
Application	LC-MS solvent saving		solvent saving with standard system	standard	preparative (small scale)	preparative (medium scale)	preparative (large scale)	preparative (super large scale)
Surface ratio with 4.6 mm I.D.	0.05	0.19	0.43	1.00	4.73	18.90	37.05	118.15
Particle size (μm)	3 or 5				5		15 or more	

Scale down

When scaling down from the most commonly used analytical column (4.6 mm I.D.) to a semi-micro or 3.0 mm I.D. analytical HPLC column (of the same column length), sample loading dose is proportionate to the cross section of column. The 3.0 mm I.D. columns provide high sensitivity and solvent saving without the need to change the existing equipment settings. Semi-micro columns (2.0 mm I.D. and 1.0 mm I.D.) provide higher sensitivity and enable analysis of minor components, but one needs to change the piping of HPLC equipment, the injector and the detector cell for semi-micro columns.

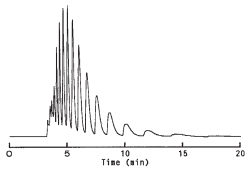
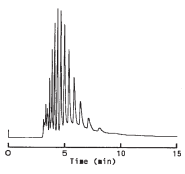
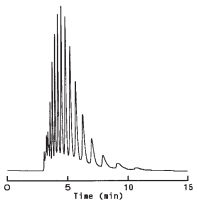
Column size	4.6 mm I.D. × 150 mm	3.0 mm I.D. × 150 mm	2.0 mm I.D. × 150 mm	1.0 mm I.D. × 150 mm
Chromatogram				
Flow rate (ml/min)	1.0	0.4	0.2	0.05
Pressure (MPa)	3.4	3.6	3.8	3.6
Injection volume(μl)	1.0	0.4	0.2	0.05
Detector Cell・Injector	for analytical		for semi-micro	
Detector sensitivity(AUFS)	0.08		0.04	
Inner diameter of pipe (mm)	0.25		0.10	0.05

Column COSMOSIL 5C$_{18}$-MS-Ⅱ
Mobile phase acetonitrile : water = 70 : 30
Flow rate 1.0 ml/min
Temperature 30℃
Detection UV 254 nm

Sample
1. Benzene
2. Toluene
3. Ethylbenzene
4. Propylbenzene
5. Butylbenzene
6. Amylbenzene

Scale up

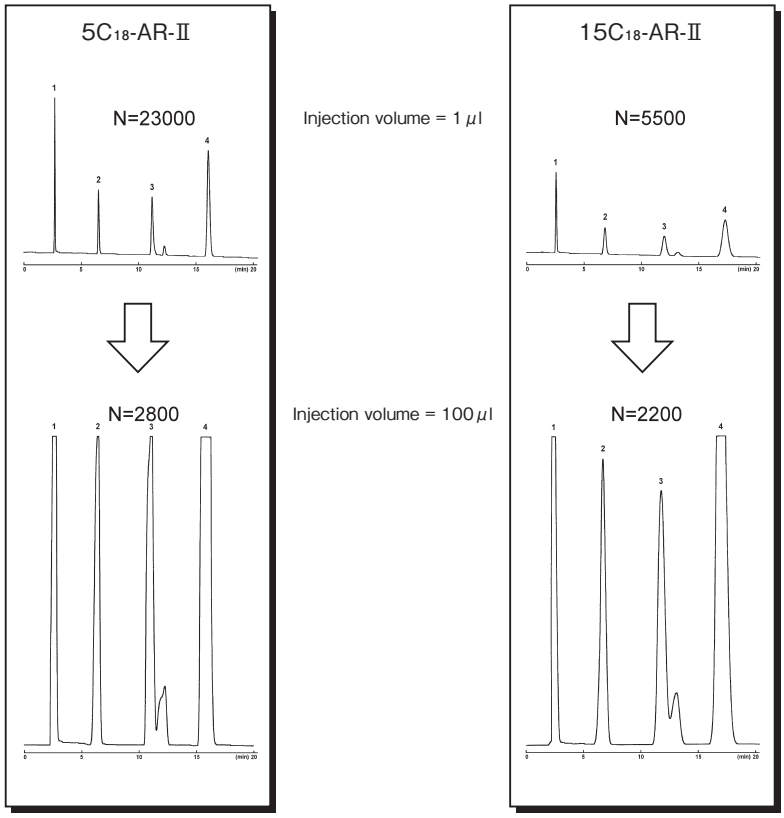
When scaling up from analytical column (4.6 mm I.D.) to preparative HPLC (of the same packing material (particle size) and length), sample loading capacity is proportionate to the cross section of column.

Column size	4.6 mm I.D. × 250 mm	10 mm I.D. × 250 mm	20 mm I.D. × 250 mm
Chromatogram			
Flow rate (ml/min)	1.0	5.0	19.8
Pressure (MPa)	5.5	5.9	5.8
Injection volume(μg)	125	625	2,500
Detector Cell・Injector	for analytical		for preparative
Inner diameter of pipe (mm)	0.25		1.0

Column COSMOSIL 5SL-Ⅱ
Mobile phase acetic ether : ethanol = 4 : 1
Flow rate 1.0 ml/min
Temperature 30℃
Detection UV 254 nm
Sample Triton X-100

Comparison of particle size

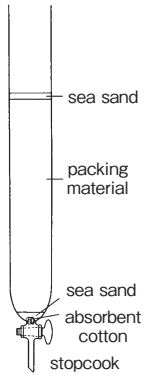
When change particle size of packing material from 5 μm to 15 μm, the number of theoretical plate (N) is reduced by one-third, and the pressure is reduced by one-ninth. As shown in the figures below, when a small amount of sample is injected, there is a big difference in the number of theoretical plates between 5 μm and 15 μm. However, when a large amount of sample is injected, there is not much difference between the two. Therefore the low pressure packing material (particle size 15 μm) is recommended for preparative columns (28 mm I.D. or more).



The number of theoretical plate of N=4. Naphthalene peak.

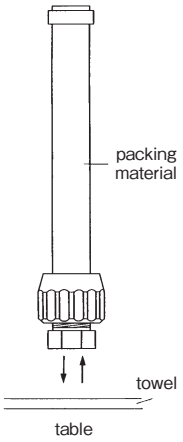
Column size 4.6 mm I.D. × 250 mm
Mobile phase methanol : water = 70 : 30
Flow rate 1.0 ml/min
Temperature 30℃
Detection UV 254 nm
Pressure $5C_{18}$-AR-Ⅱ : 10.9 Mpa
$15C_{18}$-AR-Ⅱ : 1.8 MPa
Sample 1. Uracil
2. Methyl Benzoate
3. Toluene
4. Naphthalene

TECHNICAL NOTE
9. Packing instruction



Slurry packing

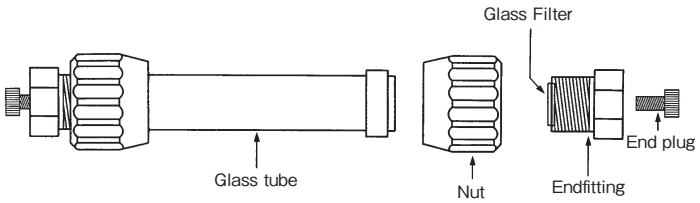
1. Use a standard open glass column, close the stopcock, pack a small amount of absorbent cotton in the bottom of the column and add solvent to approximately 1/3 of the column length.
2. Add a thin layer (5 mm) of sea sand to the surface of the absorbent cotton.
3. Prepare a slurry solution of the packing material (30% w/v) with solvent right before packing. (Make sure to prepare enough slurry solution to form a column bed sufficient to separate the compounds of interest.)
4. Simultaneously open the stopcock and add the slurry solution to the column to form the column bed.
5. After packing the column, wash the newly packed column bed with 5-10 column volumes of solvent. Allow the bed to stabilize overnight in solvent.
6. Add a thin layer (5 mm) of sea sand to the top of the bed in order to prevent disturbance of the top of the column bed during sample or solvent addition.



Dry packing

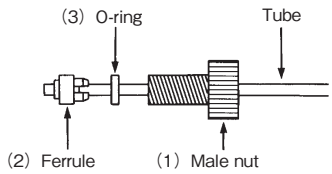
1. Wash the column in methanol and mount the end fitting on the bottom of the dried glass column as shown in the diagram to the left.
2. Add dry packing material while gently tapping the column frequently up and down to insure even distribution.
3. Continue to add packing material while tapping until the last 5 cm of the column length.
4. For the last 5 cm, add 1 cm of dry material at a time while tapping continuously.
5. After having filled the column, wet the surface of the bed with solvent to form a flat surface.
6. Remove all material adhering to the glass rim. Mount and tighten the end fitting.
7. Pump 5-10 column volumes of solvent through the column to remove all air and to stabilize the bed.

The structure of columns



Max. Pressure

Column I.D. (mm)	Pressure (MPa)
8	5.0
20	3.0
30	2.0
50	2.0



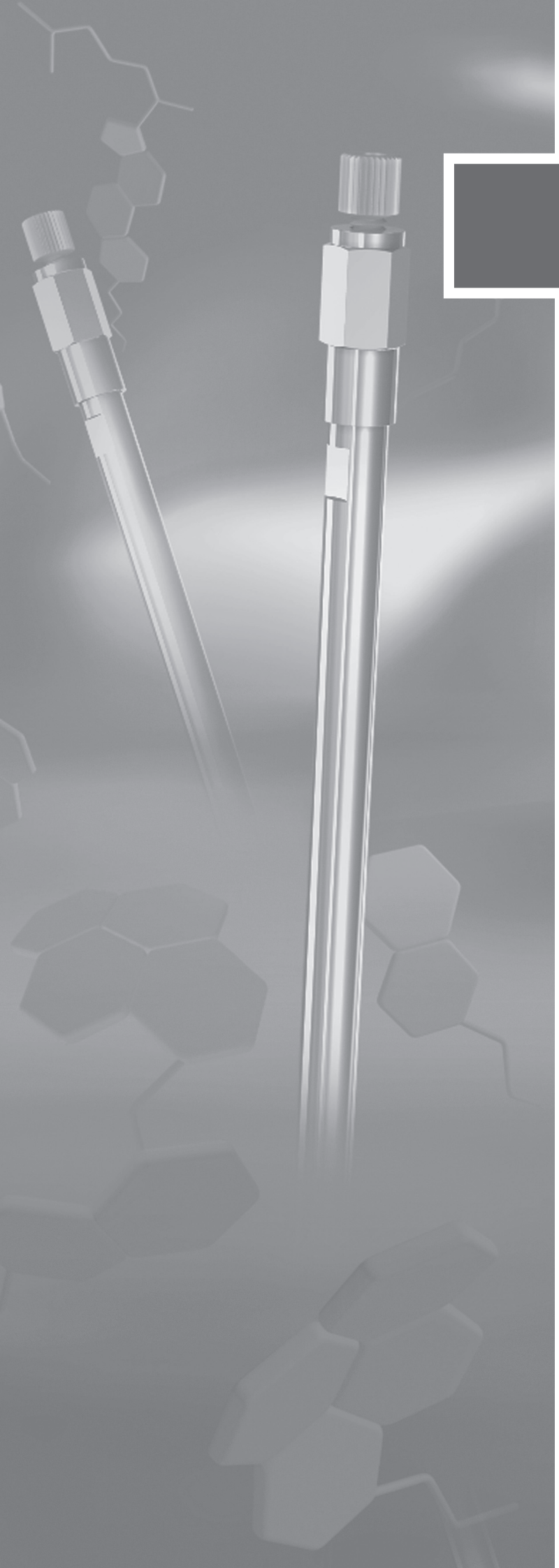
Connecting method

Insert (1), (2), (3) into a 1/16 inches long Teflon tube and then tighten the ferrule properly.

Column size and proper flow rate for medium pressure column chromatography

Column I.D. (mm)	Column length (mm)	Flow rate (ml/min)
8 - 10	150 - 500	1 - 2
20	150 - 500	4 - 10
30	150 - 500	10 - 25
50	150 - 500	25 - 60

Note : To avoid high pressure at high flow rate, use tubing with an I.D. of 0.5 mm or more. If the flow rate becomes very high, keep the pressure under 2.0 MPa.



Ⅳ

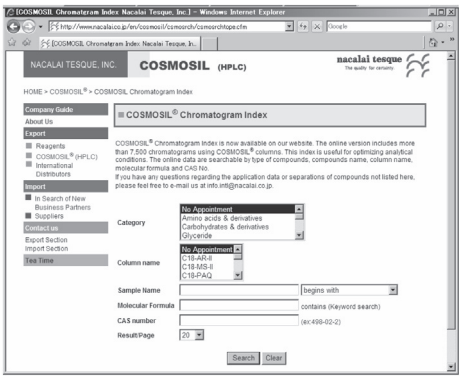
APPLICATION DATA

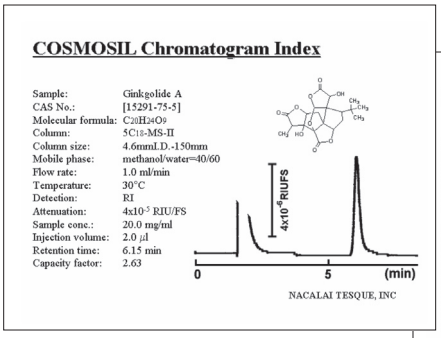
1. Chromatogram index/Application data
2. Reference list

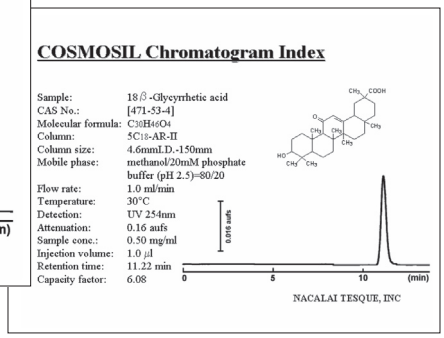
1. Chromatogram Index/Application Data

●COSMOSIL Chromatogram Index

More than 5,700 single compound elution profiles with full chromatographic condition description are available. They are not only an incredible help for chromatographers, but also can be used for reference in choosing conditions for similar compounds.

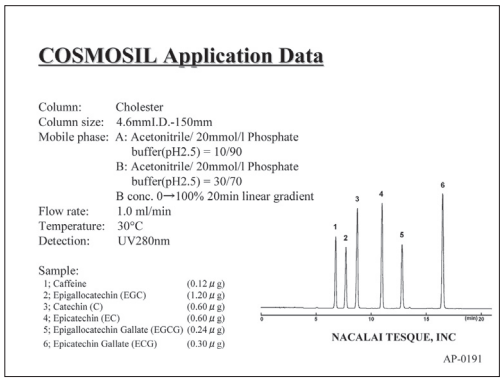


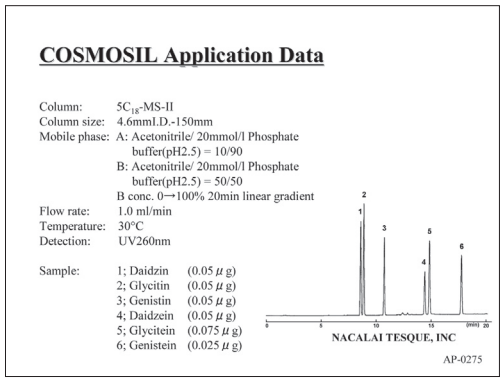




●COSMOSIL Application Data

COSMOSIL Application data is now available on our website. The online version includes more than 1,000 application data using COSMOSIL columns. The online data is searchable by name of sample and column. If you have any questions regarding the application data or separations of compounds not listed here, please feel free to e-mail us at info.intl@nacalai.co.jp.





These data are available at our web site : http://www.nacalai.co.jp/en/cosmosil/index.html

2. Reference List

1. Reversed phase chromatography – Octadecyl types (5C18-MS-Ⅱ・5C18-AR-Ⅱ・5C18-PAQ)

No.	Column	Title	Author	Journal, Vol. (Issue), Year, Page
1	5C18-MS-Ⅱ	Availability of Partially Milled Rice as a Daily Source of γ-Aminobutyric Acid	K. Iwaki and Y. Kitada	Food Science and Technology Research, 13(1), 2007, 41-44
2	5C18-MS-Ⅱ	Molecularly imprinted polymers for triazine herbicides prepared by multi-step swelling and polymerization method Their application to the determination of methylthiotriazine herbicides in river water	H. Sambe, K. Hoshina and J. Haginaka	Journal of Chromatography A, 1152(1-2), 2007, 130-137
3	5C18-MS-Ⅱ	Screening of ACE-inhibitory peptides from a random peptide-displayed phage library using ACE-coupled liposomes	Y. Kumada, N. Hashimoto, F. Hasan, M. Terashima, K. Nakanishi, A. Jungbauer and S. Katoh	Journal of Biotechnology, 131(2), 2007, 144-149
4	5C18-MS-Ⅱ	Characterization of the Tritium-Labeled Analog of L-threo-β-Benzyloxyaspartate Binding to Glutamate Transporters	K. Shimamoto, Y. Otsubo, Y. Shigeri, Y. Yasuda-Kamatani, M. Satoh, S. Kaneko, and T. Nakagawa	Molecular Pharmacology, 71, 2007, 294-302
5	5C18-MS-Ⅱ	New C28 Steroidal Glycosides from Tubocapsicum anomalum	N. Kiyota, K. Shingu, K. Yamaguchi, Y. Yoshitake, K. Harano, H. Yoshimitsu, T. Ikeda and T. Nohara	Chemical & Pharmaceutical Bulletin, 55(1), 2007, 34
6	5C18-MS-Ⅱ	Cu-catalyzed arylation of phosphinic amide facilitated by (±)-*trans*-cyclohexane-1,2-diamine	J. Li, S. Zhang, C. Tao, Y. Fu and Q. Guo	Chinese Chemical Letters, 18(9), 2007, 1033-1036
7	5C18-MS-Ⅱ	Determination of Caffeine and Caffeine-Related Metabolites in Ephedra-Containing Standard Reference Materials Using Liquid Chromatography with Absorbance Detection and Tandem Mass Spectrometry	J. B. Thomas, K. E. Sharpless, S. Mitvalsky, M. Roman, J. Yen, M. B. Satterfield	Journal of AOAC INTERNATIONAL, 90(4), 2007, 934-940
8	5C18-MS-Ⅱ	Medicinal Flowers. XVI. New Dammarane-Type Triterpene Tetraglycosides and Gastroprotective Principles from Flower Buds of Panax ginseng	M. Yoshikawa, S. Sugimoto, S. Nakamura, H. Sakumae and H. Matsuda	Chemical & Pharmaceutical Bulletin, 55(7), 2007, 1034
9	5C18-MS-Ⅱ	Bioactive Constituents from Chinese Natural Medicines. XXIII. Absolute Structures of New Megastigmane Glycosides, Sedumosides A4, A5, A6, H, and I, and Hepatoprotective Megastigmanes from Sedum sarmentosum	K. Ninomiya, T. Morikawa, Y. Zhang, S. Nakamura, H. Matsuda, O. Muraoka and M. Yoshikawa	Chemical & Pharmaceutical Bulletin, 55(8), 2007, 1185
10	5C18-MS-Ⅱ	Bioactive Constituents from Chinese Natural Medicines. XXIV. Hypoglycemic Effects of Sinocrassula indica in Sugar-Loaded Rats and Genetically Diabetic KK-Ay Mice and Structures of New Acylated Flavonol Glycosides, Sinocrassosides A1, A2, B1, and B2	M. Yoshikawa, T. Wang, T. Morikawa, H. Xie and H. Matsuda	Chemical & Pharmaceutical Bulletin, 55(9), 2007, 1308
11	5C18-AR-Ⅱ	Separation and Identification of the Fifteen Constituents in Forsythiae Fructus	W. Huang and S. Sheu	Journal of Food and Drug Analysis, 15(1), 2007, 33-39
12	5C18-AR-Ⅱ	New isoflavones from Belamcandae Rhizoma	M. Moriyasu, Y. Igi, M. Ichimaru, K. Iwasa, J. Kobayakawa, F. Sato-Nishimori, Y. Matsukawa and C. Nagase	Journal of Natural Medicines, 61(3), 2007, 329-333
13	5C18-AR-Ⅱ	Simultaneous determination of anthraquinones in rhubarb by high-performance liquid chromatography and capillary electrophoresis	J. Koyama, I. Morita and N. Kobayashi	Journal of Chromatography A, 1145(1-2), 2007, 183-189
14	5C18-AR-Ⅱ	Two New Megastigmanes from the Leaves of Cucumis sativus	H. Kai, M. Baba and T. Okuyama	Chemical & Pharmaceutical Bulletin, 55(1), 2007, 133
15	5C18-AR-Ⅱ	Three New Monoterpenoids from the Fruit of Genipa americana	M. Ono, N. Ishimatsu, C. Masuoka, H. Yoshimitsu, R. Tsuchihashi, M. Okawa, J. Kinjo, T. Ikeda and T. Nohara	Chemical & Pharmaceutical Bulletin, 55 (4), 2007, 632
16	5C18-AR-Ⅱ	A Novel Oxazolidine Linker for the Synthesis of Peptide Aldehydes	M. Tanaka, S. Oishi, H. Ohno and N. Fujii	International Journal of Peptide Research and Therapeutics, 13(1-2), 2007, 271-279
17	5C18-AR-Ⅱ	Active Site Mapping of Amylo-α-1,6-glucosidase in Porcine Liver Glycogen Debranching Enzyme Using Fluorogenic 6-O-α-Glucosyl-maltooligosaccharides	E. Yamamoto, Y. Makino and K. Omichi	Journal of Biochemistry, 141(5), 2007, 627-634
18	5C18-AR-Ⅱ	Anti-platelet Aggregation Triterpene Saponins from the Galls of Sapindus mukorossi	H. Huang, W. Tsai, C. Liaw, S. Wu, Y. Wu and Y. Kuo	Chemical & Pharmaceutical Bulletin, 55(9), 2007, 1412-1415
19	5C18-AR-Ⅱ	Oxidants in the Gas Phase of Cigarette Smoke Pass Through the Lung Alveolar Wall and Raise Systemic Oxidative Stress	Y. Yamaguchi, F. Nasu, A. Harada and M. Kunitomo	Journal of Pharmacological Sciences, 103(3), 2007, 275-282
20	5C18-AR-Ⅱ	Oxidative coupling of the pyrogallol B-ring with a galloyl group during enzymatic oxidation of epigallocatechin 3-O-gallate	Y. Li, T. Tanaka and I. Kouno	Phytochemistry, 68(7), 2007, 1081-1088
21	5C18-AR-Ⅱ	Gomadalactones A, B, and C: novel 3-oxabicyclo[3.3.0]octane compounds in the contact sex pheromone of the white-spotted longicorn beetle, Anoplophora malasiaca	H. Yasui, T. Akino, T. Yasuda, M. Fukaya, S. Wakamura and H. Ono	Tetrahedron Letters, 48(13), 2007, 2395-2400
22	5C18-AR-Ⅱ	Validation of a Novel HPLC Method for the Determination of Raloxifene and Its Pharmacokinetics in Rat Plasma	Zh. Y. Yang, Zh. F. Zhang, X. B. He, G. Y. Zhao and Y. Q. Zhang	Chromatographia, 65(3-4), 2007, 197-201
23	5C18-AR-Ⅱ	Antimalarial Flavonol Glycosides from Euphorbia hirta	Y. Liu, N. Murakami, H. Ji, Pedro Abreu, S. Zhang	Pharmaceutical Biology, 45(4), 2007, 278-281
24	5C18-AR-Ⅱ	HPLC Determination of Lovastatin in Rat Tissue	Z. Zhang and Z. Yang	Chromatographia, 66(7-8), 2007, 487-491
25	5C18-AR-Ⅱ	Sensitive determination of kojic acid in foodstuffs using PVP (polyvinylpyrrolidone) modified acetylene black paste electrode	X. Yang, and H. Zhang	Food Chemistry, 102(4), 2007, 1223-1227
26	5C18-AR-Ⅱ	Rubusuaviins A—F, Monomeric and Oligomeric Ellagitannins from Chinese Sweet Tea and Their α-Amylase Inhibitory Activity	H. Li, T. Tanaka, Y.Zhang, C.Yang and I. Kouno	Chemical & Pharmaceutical Bulletin, 55(9), 2007, 1325
27	5C18-AR-Ⅱ	Analytical method for determination of dinitropyrenes using gas chromatography–high-resolution mass spectrometry	T. Nakao, O. Aozasa, S. Ohta and H. Miyata	Journal of Chromatography A, 1157(1-2), 2007, 352-357
28	5C18-AR-Ⅱ	Enzymatic Synthesis of Hydroxycinnamic Acid Glycerol Esters Using Type A Feruloyl Esterase from Aspergillus niger	M. Tsuchiyama, T. Sakamoto, S. Tanimori, S. Murata and H. Kawasaki	Bioscience, Biotechnology, and Biochemistry, 71(10), 2007, 2606-2609

No.	Column	Title	Author	Journal, Vol. (Issue), Year, Page
29	5C18-AR-Ⅱ	Efficient Incorporation of Unsaturated Fatty Acids into Volicitin-Related Compounds in Spodoptera litura (Lepidoptera: Noctuidae)	T. Abishi, N. Yoshinaga, K. Noge, R. Nishida and N. Mori	Bioscience, Biotechnology, and Biochemistry, 71(2), 2007, 607-610
30	5C18-AR-Ⅱ	Cloning and characterization of a new hetero-gene cluster of nonribosomal peptide synthetase and polyketide synthase from the cyanobacterium Microcystis aeruginosa K-139	A. Nishizawa, A. B. Arshad, T. Nishizawa, M. Asayama, K. Fujii, T. Nakano, K. Harada and M. Shirai	The Journal of General and Applied Microbiology, 53(1), 2007, 17-27
31	5C18-AR-Ⅱ	Fatty Acid Amides, Previously Identified in Caterpillars, Found in the Cricket Teleogryllus taiwanemma and Fruit Fly Drosophila melanogaster Larvae	N. Yoshinaga, T. Aboshi, C. Ishikawa, M. Fukui, M. Shimoda, R. Nishida, C. G. Lait, J. H. Tumlinson and N. Mori	Journal of Chemical Ecology, 33(7), 2007, 1376-1381
32	5C18-AR-Ⅱ	Changes in pigment content and surface micro-morphology of in cut carnation flower petals under high-temperature conditions	Fukai S., Manabe Y., Yangkhamman P, Takamura T.	The Journal of Horticultural Science and Biotechnology, 82(5), 2007, 769-775
33	5C18-AR-Ⅱ	Protective effect of the Chinese prescription Kangen-karyu against high glucose-induced oxidative stress in LLC-PK1 cells	T. Yokozawa, Y. A. Kim, H. Y. Kim, T. Okamoto and Y. Sei	Journal of Ethnopharmacology, 109(1), 2007, 113-120
34	5C18-AR-Ⅱ	Precursor-feeding strategy for the production of solanine, solanidine and solasodine by a cell culture of Solanum lyratum	M. Lee, J. Cheng, C. Lin, Y. Chen and M. Lu	Process Biochemistry, 42(5), 2007, 899-903
35	5C18-AR-Ⅱ	Anti-inflammatory Effect of Oyaksungisan in Peripheral Blood Mononuclear Cells from Cerebral Infarction Patients	Y. Kim, H. So, J. Kim, C. Park, J. Lee, W. Woo, K. Cho, B. Moon and R. Park	Biological & Pharmaceutical Bulletin, 30(6), 2007, 1037
36	5C18-AR-Ⅱ	2-Geranyl-1,4-naphthoquinone, a Possible Intermediate of Anthraquinones in a Sesamum indicum Hairy Root Culture	T. Furumoto, T. Ohara, T. Kubo, Y. Kawanami and H. Fukui	Bioscience, Biotechnology, and Biochemistry, 71(10), 2007, 2600-2602
37	5C18-AR-Ⅱ	Facile Discrimination of Aldose Enantiomers by Reversed-Phase HPLC	T. Tanaka, T. Nakashima, T. Ueda, K. Tomii and I. Kouno	Chemical & Pharmaceutical Bulletin, 55(6), 2007, 899
38	5C18-AR-Ⅱ	Fengycin antibiotics isolated from B-FS01 culture inhibit the growth of Fusarium moniliforme Sheldon ATCC 38932	Hu, Liang Bin, Shi, Zhi Qi, Zhang, Ting, Yang, Zhi Min	FEMS Microbiology Letters, 272(1), 2007, 91-98
39	5C18-AR-Ⅱ	Detection of Aminophenylnorharman, a Possible Endogenous Mutagenic and Carcinogenic Compound, in Human Urine Samples	R. Nishigaki, Y. Totsuka, H. Kataoka, H. Ushiyama, S. Goto, T. Akasu, T. Watanabe, T. Sugimura and K. Wakabayashi	Cancer Epidemiology Biomarkers & Prevention, 16, 2007, 151-156
40	5C18-AR-Ⅱ	Utilization of hydrophobic bacterium Rhodococcus opacus B-4 as whole-cell catalyst in anhydrous organic solvents	S. Yamashita, M. Satoi, Y. Iwasa, K. Honda, Y. Sameshima, T. Omasa, J. Kato and H. Ohtake	Applied Microbiology and Biotechnology, 74(4), 2007, 761-767
41	5C18-AR-Ⅱ	Mechanism of the Protective Effect of Intraperitoneally Administered Agonists for Formyl Peptide Receptors against Chemotherapy-Induced Alopecia	T. Tsuruki, K. Takahata and M. Yoshikawa	Bioscience, Biotechnology, and Biochemistry, 71(5), 2007, 1198-1202
42	5C18-AR-Ⅱ	Differential Intracellular Signaling through PAC1 Isoforms as a Result of Alternative Splicing in the First Extracellular Domain and the Third Intracellular Loop	M. Ushiyama, R. Ikeda, H. Sugawara, M. Yoshida, K. Mori, K. Kangawa, K. Inoue, K. Yamada, and A. Miyata	Mol Pharmacol 72, 2007, 103-111
43	5C18-AR-Ⅱ	Structure-activity relationships of 1'-acetoxychavicol acetate homologues as new nuclear export signal inhibitors	Y.Liu, N.Murakami, S.Zhang, T.Xu	Pharmazie, 62(9), 2007, 659-662
44	5C18-AR-Ⅱ	Methoxy- and Fluorine-Substituted Analogs of O-1302: Synthesis and in Vitro Binding Affinity for the CB1 Cannabinoid Receptor	S. Tobiishi, T. Sasada, Y. Nojiri, F. Yamamoto, T. Mukai, K. Ishiwata and M. Maeda	Chemical & Pharmaceutical Bulletin, 55(8), 2007, 1213
45	5C18-AR-Ⅱ	Reactive Oxygen Scavenging Activity of Matured Whiskey and Its Active Polyphenols	K. Koga, A. Taguchi, S. Koshimizu, Y. Suwa, Y. Yamada, N. Shirasaka and H. Yoshizumi	Journal of Food Science, 72(3), S212-S217
46	5C18-AR-Ⅱ	Total synthesis of miraziridine A and identification of its major reaction site for cathepsin B	H. Konno, K. Kubo, H. Makabe, E. Toshiro, N. Hinoda, K. Nosaka and K. Akaji	Tetrahedron, 63(38), 2007, 9502-9513
47	5C18-AR-Ⅱ	Analysis of Polyamines, 1-Aminocyclopropane-1-carboxylic Acid and their Conjugated Forms in Floral Organs of Hibiscus syriacus L.	S. Seo, I. Shim, K. Usui and S. Fujihara	Journal of the Japanese Society for Horticultural Science, 76(2), 2007, 149-156
48	5C18-AR-Ⅱ	Chemical Profiling and Gene Expression Profiling during the Manufacturing Process of Taiwan Oolong Tea "Oriental Beauty"	J. Cho, M. Mizutani, B. Shimizu, T. Kinoshita, M. Ogura, K. Tokoro, M. Lin and K. Sakata	Bioscience, Biotechnology, and Biochemistry,71(6), 2007, 1476-1486
49	5C18-AR-Ⅱ	Bacterial c-di-GMP Is an Immunostimulatory Molecule	D. K. R. Karaolis, T. K. Means, D. Yang, M. Takahashi, T. Yoshimura, E. Muraille, D. Philpott, J. T. Schroeder, M. Hyodo, Y. Hayakawa, B. G. Talbot, E. Brouillette and F. Malouin	The Journal of Immunology, 178, 2007, 2171-2181
50	5C18-AR-Ⅱ	N-glycans of recombinant human acid -glucosidase expressed in the milk of transgenic rabbits	S. P Jongen, G. J Gerwig, B. R Leeflang, K. Koles, M. LM Mannesse, P. HC van Berkel, F. R Pieper, M. A Kroos, A. JJ Reuser, Q. Zhou, X. Jin, K. Zhang, T. Edmunds and J. P Kamerling	Glycobiology, 17, 2007, 600 - 619
51	5C18-PAQ	Medicinal Flowers. XII. New Spirostane-Type Steroid Saponins with Antidiabetogenic Activity from Borassus flabellifer	M. Yoshikawa, F. Xu, T. Morikawa, Y. Pongpiriyadacha, S. Nakamura, Y. Asao, A. Kumahara and H. Matsuda	Chemical & Pharmaceutical Bulletin, 55(2), 2007, 308
52	5C18-PAQ	Production of 2-Phenylethanol in Roses as the Dominant Floral Scent Compound from L-Phenylalanine by Two Key Enzymes, a PLP-Dependent Decarboxylase and a Phenylacetaldehyde Reductase	M. Sakai, H. Hirata, H. Sayama, K. Sekiguti, H. Itano, T. Asai, H. Dohra, M. Hara and N. Watanabe	Bioscience, Biotechnology, and Biochemistry, 71(10), 2007, 2408-2419

2. Reversed phase chromatography – Special columns

No.	Column	Title	Author	Journal, Vol. (Issue), Year, Page
1	Cholester	Metachromins L–Q, new sesquiterpenoid quinones with an amino acid residue from sponge Spongia sp.	Y. Takahashi, T. Kubota, J. Fromont and J. Kobayashi	Tetrahedron, 63(36), 2007, 8770-8773
2	Cholester	Peptide Thioester Synthesis via an Auxiliary-Mediated N – S Acyl Shift Reaction in Solution	K. Nakamura, H. Mori, T. Kawakami, H. Hojo, Y. Nakahara and S. Aimoto	International Journal of Peptide Research and Therapeutics, 13(1-2), 2007, 191-202
3	5PYE	Comparative Assessment of Organochlorine Concentrations in Porpoise Melon and Blubber	S.C. Gardner, G.M. Ylitalo, U. Varanasi	Marine Mammal Science, 23(2), 2007, 434-444
4	5PYE	Application of In-Line Liquid Chromatography-Accurate Radioisotope Counting-Mass Spectrometry (LC-ARC-MS) to Evaluate Metabolic Profile of [3H]-Mefenamic Acid in Rat Plasma	W. Lam, C. Loi, J. Atherton, W. Stolle, J. Easter, A. Mutlib	Drug Metabolism Letters, 1(3), 2007, 179-188
5	5PBB-R	An assessment of the retention behaviour of polycyclic aromatic hydrocarbons on reversed phase stationary phases—Thermodynamic behaviour on C18 and phenyl-type surfaces	S. Kayillo, G. R. Dennis and R. A. Shalliker	Journal of Chromatography A, 1145(1-2), 2007, 133-140

3. Reversed phase chromatography – Others

No.	Column	Title	Author	Journal, Vol. (Issue), Year, Page
1	5C$_8$-MS	Enantioselective synthesis of d- and l- α -methylcysteine with hydantoinase	T. Ohishi, H. Nanba, M. Sugawara, M. Izumida, T. Honda, K. Mori, S. Yanagisawa, M. Ueda, N. Nagashima and K. Inoue	Tetrahedron Letters, 48(19), 2007, 3437-3440
2	5C$_8$-MS	Decreased Intestinal CYP3A and P-glycoprotein Activities in Rats with Adjuvant Arthritis	S. Uno, A. Kawase, A. Tsuji, T. Tanino and M. Iwaki	Drug Metabolism and Pharmacokinetics, 22(4), 2007, 313-321
3	5C$_8$-MS	Paclitaxel-2′ -Ethylcarbonate Prodrug Can Circumvent P-glycoprotein-mediated Cellular Efflux to Increase Drug Cytotoxicity	T. Tanino, A. Nawa, E. Kondo, F. Kikkawa, T. Daikoku, T. Tsurumi, C. Luo, Y. Nishiyama, Y. Takayanagi, K. Nishimori, S. Ichida, T. Wada, Y. Miki and M. Iwaki	Pharmaceutical Research, 24(3), 2007, 555-565
4	5TMS-MS	Biodegradation of nonylphenol polyethoxylates under Fe(III)-reducing conditions	J. Lu, Q. Jin, Y. He, and J. Wu	Chemosphere, 69(7), 2007, 1047-1054

4. Normal phase chromatography

No.	Column	Title	Author	Journal, Vol. (Issue), Year, Page
1	5SL-Ⅱ	Prenylterphenyllin and Its Dehydroxyl Analogs, New Cytotoxic Substances from a Marine-derived Fungus Aspergillus candidus IF10	H. Wei, H. Inada, A. Hayashi, K. Higashimoto, P. Pruksakorn, S. Kamada, M. Arai, S. Ishida and M. Kobayashi	The Journal of Antibiotics, 60(9), 2007, 586-590
2	5SL-Ⅱ 5C$_{18}$-AR-Ⅱ	Two New Steroid Glycosides and a New Sesquiterpenoid Glycoside from the Underground Parts of Trillium kamtschaticum	M. Ono, C. Takamura, F. Sugita, C. Masuoka, H. Yoshimitsu, T. Ikeda and T. Nohara	Chemical & Pharmaceutical Bulletin, 55(4), 2007, 551

5. Saccharide analysis columns

No.	Column	Title	Author	Journal, Vol. (Issue), Year, Page
1	Sugar-D	Oxidative coupling reaction of arbutin and gentisate catalyzed by horseradish peroxidase	T. Kiso, M. Shizuma, H. Murakami, T. Kiryu, K. Hozono, T. Terai and H. Nakano	Journal of Molecular Catalysis B: Enzymatic, 45(1-2), 2007, 50-56
2	Sugar-D	Structural Basis for Recognition of High Mannose Type Glycoproteins by Mammalian Transport Lectin VIP36	T. Satoh, N. P. Cowieson, W. Hakamata, H. Ideo, K. Fukushima, M. Kurihara, R. Kato, K. Yamashita, and S. Wakatsuki	J. Biol. Chem., 282(38), 2007, 28246-28255
3	Sugar-D	Improvement in lactic acid production from starch using α -amylase-secreting Lactococcus lactis cells adapted to maltose or starch	K. Okano, S. Kimura, J. Narita, H. Fukuda and A. Kondo	Applied Microbiology and Biotechnology, 75(54), 2007, 1007-1013
4	Sugar-D	Cucurbitane Glycosides from Unripe Fruits of Siraitia grosvenori	D. Li, T. Ikeda, T. Nohara, J. Liu, Y. Wen, T. Sakamoto and G. Nonaka	Chemical & Pharmaceutical Bulletin, 55(7), 2007, 1082
5	5NH$_2$-MS	Blood Lysophosphatidylcholine (LPC) Levels and Characteristic Molecular Species in Neonates: Prolonged Low Blood LPC Levels in Very Low Birth Weight Infants.	Takatera A., Takeuchi A., Saiki K., Morioka I., Yokoyama N., Matsuo M.	Pediatric Research., 62(4), 2007, 477-482

6. Protein separation wide pore columns

No.	Column	Title	Author	Journal, Vol. (Issue), Year, Page
1	5C$_{18}$-AR-300	Porcine germinal angiotensin I-converting enzyme: Isolation, characterization and molecular cloning	K. Takeuchi, H. Araki, T. Sakaue, Y. Yamamoto, M. Fujiwara, K. Nishi and I. Ohkubo	Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 146(2), 2007, 215-226
2	5C$_{18}$-AR-300	A Novel Arginine Kinase with Substrate Specificity Towards d -arginine	K. Uda and T. Suzuki	The Protein Journal, 26(5), 2007, 281-291
3	5C$_{18}$-AR-300	Therapeutic Effects of a ^{186}Re-Complex–Conjugated Bisphosphonate for the Palliation of Metastatic Bone Pain in an Animal Model	K. Ogawa, T. Mukai, D. Asano, H. Kawashima, S. Kinuya, K. Shiba, K. Hashimoto, H. Mori and H. Saji	Journal of Nuclear Medicine, 48(1), 2007, 122-127
4	5C$_{18}$-AR-300	Chemical design of a radiolabeled gelatinase inhibitor peptide for the imaging of gelatinase activity in tumors.	H. Hanaoka, T. Mukai, S. Habashita, D. Asano, K. Ogawa, Y. Kuroda, H. Akizawa, Y. Iida, K. Endo, T. Saga	Nuclear Medicine and Biology, 34(5), 503-510
5	5C$_{18}$-AR-300	Recombinant human antithrombin expressed in Chinese hamster ovary cells shows *in vivo* efficacy on rat DIC model similarly to plasma-derived antithrombin regardless of different N-glycosylation	M. Hirose, M. Tsukada, F. Hirayama, Y. Kubo, M. Kajii, S. Mochizuki, N. and H. Ohi	Thrombosis Research, 119(5), 2007, 631-641
6	5C$_{18}$-AR-300	Assessment of ^{186}Re chelate-conjugated bisphosphonate for the development of new radiopharmaceuticals for bones.	T. Uehara, Z. Jin, K. Ogawa, H. Akizawa, K. Hashimoto, M. Nakayama, Y. Arano	Nuclear Medicine and Biology, 34(1), 2007, 79-87
7	5C$_8$-AR-300	Assembly of two distinct dimers and higher-order oligomers from full-length tau	Sahara N., Maeda S., Murayama M., Suzuki T., Dohmae N., Yen S., Takashima A.	European Journal of Neuroscience, 25(10), 2007, 3020-3029
8	5C$_8$-AR-300	Molecular Cloning of cDNA for Trehalase from the European Honeybee, Apis mellifera L., and Its Heterologous Expression in Pichia pastoris	J. Lee, S. Saito, H. Mori, M. Nishimoto, M. Okuyama, D. Kim, J. Wongchawalit, A. Kimura and S. Chiba	Bioscience, Biotechnology, and Biochemistry, 71(9), 2007, 2256-2265
9	5C$_4$-AR-300	Geranylgeranyl reductase involved in the biosynthesis of archaeal membrane lipids in the hyperthermophilic archaeon Archaeoglobus fulgidus	M. Murakami, K. Shibuya, T. Nakayama, T. Nishino, T. Yoshimura and H. Hemmi	FEBS Journal, 274(3), 2007, 805-814
10	5Diol-300-Ⅱ	Polypeptide linkers suitable for the efficient production of dimeric scFv in Escherichia coli	Y. Kumada, T. Kawasaki, Y. Kikuchi and S. Katoh	Biochemical Engineering Journal, 35(2), 2007, 158-165

7. Specialty columns for Fullerene

No.	Column	Title	Author	Journal, Vol. (Issue), Year, Page
1	Buckyprep	Production and Characterization of Heteroatom-encapsulated Metallofullerene, CaHo@C$_{82}$	K. Sakaguchi, R. Fujii, T. Kodama, H. Nishikawa, I. Ikemoto, Y. Achiba and K. Kikuchi	Chemistry Letters, 36(7), 2007, 832
2	Buckyprep	Synthesis and characterization of difluoromethylene-homo[60]fullerene, C$_{60}$(CF$_2$)	A. S. Pimenova, A. A. Kozlov, A. A. Goryunkov, V. Yu. Markov, P. A. Khavrel, S. M. Avdoshenko, I. N. Ioffe, S. G. Sakharov, S. I. Troyanov and L. N. Sidorov	Chem. Commun, 2007, 374 - 376

No.	Column	Title	Author	Journal, Vol. (Issue), Year, Page
3	Buckyprep	Biological Effects of C_{60} Fullerenes *in vitro* and in a Model System	S. V. Prylutska, O. P. Matyshevska, I. I. Grynyuk, Yu. I. Prylutskyy, U. Ritter, P. Scharff	Molecular Crystals and Liquid Crystals, 468(1), 2007, 265-274
4	Buckyprep	The enthalpy of formation of fullerene fluoride $C_{60}F_{18}$ and the C-F bond energy	T. S. Papina, V. A. Luk' yanova, A. A. Goryunkov, I. N. Ioffe, I. V. Gol' dt, A. G. Buyanovskaya, N. M. Kabaeva and L. N. Sidorov	Russian Journal of Physical Chemistry A, Focus on Chemistry, 81(10), 2007, 1560-1564
5	Buckyprep	Synthesis and molecular structure of 1,6,11,16,18, 24,27,36-$C_{60}(CF_3)_8$	A. A. Goryunkov, E. I. Dorozhkin, N. B. Tamm, D. V. Ignat' eva, S. M. Avdoshenko, L. N. Sidorov and S. I. Troyanov	Mendeleev Communications, 17(2), 2007, 110-112
6	Buckyprep	Production of endohedral ^{133}Xe-higher fullerenes by ion implantation	S. Watanabe, T. Katabuchi, N. S. Ishioka, S. Matsuhashi, H. Muramatsu	Journal of Radioanalytical and Nuclear Chemistry, 272(3), 2007, 467-469
7	Buckyprep	The former $C_{60}F_{16}$ is actually a double-caged adduct: $(C_{60}F_{16})(C_{60})$	A. A. Goryunkov, I. N. Ioffe, P. A. Khavrel, S. M. Avdoshenko, V. Yu. Markov, Z. Mazej, L. N. Sidorov and S. I. Troyanov	Chem. Commun., 2007, 704 - 706
8	Buckyprep	Formation of single-wall carbon nanotubes in Ar and nitrogen gas atmosphere by using laser furnace technique	S. Suzuki, N. Asai, H. Kataura and Y. Achiba	The European Physical Journal D - Atomic, Molecular, Optical and Plasma Physics, 43(1-3), 2007, 143-146
9	Buckyprep	Two-dimensional hopping motion of encapsulated La atoms in silylated $La_2@C_{80}$	T. Wakahara, M. Yamada, S. Takahashi, T. Nakahodo, T. Tsuchiya, Y. Maeda, T. Akasaka, M. Kako, K. Yoza, E. Horn, N. Mizorogi and S. Nagase	Chem. Commun., 2007, 2680 - 2682
10	Buckyprep	Technology for manufacture of pure fullerenes C_{60}, C_{70} and a concentrate of higher fullerenes	Yu. S. Grushko, V. P. Sedov and V. A. Shilin	Russian Journal of Applied Chemistry, 80(3), 2007, 448-455
11	Buckyprep	Synthesis and Catalytic Activity of 1-Allyl and 3-Allyl, Ethyl, and Hydrido Complexes of Ruthenium-Pentamethyl[60]fullerene	Y. Matsuo, T. Uematsu, E. Nakamura	European Journal of Inorganic Chemistry, 2007(18), 2007, 2729 - 2733
12	Buckyprep	Generation of C_{60} nanoparticle aerosol in high mass concentrations	A. Gupta, W. C. Forsythe, M. L. Clark, J. A. Dill and G. L. Baker	Journal of Aerosol Science, 38(6), 2007, 592-603
13	Buckyprep	X-ray structure and DFT study of C_1-$C_{60}(CF_3)_{12}$. A high-energy, kinetically-stable isomer prepared at 500 °C	I. E. Kareev, N. B. Shustova, D. V. Peryshkov, S. F. Lebedkin, S. M. Miller, O. P. Anderson, A. A. Popov, O. V. Boltalina and S. H. Strauss	Chem. Commun., 2007, 1650 - 1652
14	Buckyprep	Higher trifluoromethylated derivatives of C_{60}, $C_{60}(CF_3)_{16}$ and $C_{60}(CF_3)_{18}$ Synthesis, structure, and theoretical study	S. I. Troyanov, A.A. Goryunkov, E. I. Dorozhkin, D. V. Ignat' eva, N. B. Tamm, S. M. Avdoshenko, I. N. Ioffe, V. Yu. Markov, L. N. Sidorov, K. Scheurel and E. Kemnitz	Journal of Fluorine Chemistry, 128(5), 2007, 545-551
15	Buckyprep 5PBB	Formation of novel Si-fullerene compound materials using a high-density silicon-ion plasma	T. Hirata, R. Suzuki and R. Hatakeyama	Thin Solid Films, 515(9), 4177-4181

8. Packing materials for reversed phase preparative liquid chromatography

No.	Column	Title	Author	Journal, Vol. (Issue), Year, Page
1	75C_{18}-OPN	Compositions of Royal Jelly II. Organic Acid Glycosides and Sterols of the Royal Jelly of Honeybees (Apis mellifera)	T. Kodai, K. Umebayashi, T. Nakatani, K. Ishiyama and N. Noda	Chemical & Pharmaceutical Bulletin, 55(10), 2007, 1528
2	75C_{18}-OPN	Identification and Characterization of Cannabinoids That Induce Cell Death through Mitochondrial Permeability Transition in Cannabis Leaf Cells	S. Morimoto, Y. Tanaka, K. Sasaki, H. Tanaka, T. Fukamizu, Y. Shoyama, Y. Shoyama, and F. Taura	J. Biol. Chem., 282(28), 2007, 20739-20751
3	75C_{18}-OPN	Microbial metabolism of steviol and steviol-16α,17-epoxide	L. Yang, F. Hsu, S. Chang, J. Chen, J. Hsu, C. Hsu, P. Liu and S. Lin	Phytochemistry, 68(4), 2007, 562-570
4	75C_{18}-OPN	α -Glucosidase inhibitors from Devil tree (Alstonia scholaris)	N. J. Anurakkun, M. R. Bhandari and J. Kawabata	Food Chemistry, 103(4), 2007, 1319-1323
5	75C_{18}-OPN	A Galactolipid Possesses Novel Cancer Chemopreventive Effects by Suppressing Inflammatory Mediators and Mouse B16 Melanoma	C.Hou, Y. Chen, J. Wu, C. Huang, S. Wang, N. Yang and L Shyur	Cancer Research, 67, 2007, 6907-6915
6	75C_{18}-OPN	Epicatechin conjugated with fatty acid is a potent inhibitor of DNA polymerase and angiogenesis	K. Matsubara, A. Saito, A. Tanaka, N. Nakajima, R. Akagi, M. Mori and Y. Mizushina	Life Sciences, 80(17), 2007, 1578-1585
7	75C_{18}-OPN	Characterization of a natural inducer of coral larval metamorphosis	M. Kitamura, T. Koyama, Y. Nakano and D. Uemura	Journal of Experimental Marine Biology and Ecology, 340(1), 2007, 96-102
8	75C_{18}-OPN	Tricalysiolide G and tricalysiols A and B: rearranged ent-kaurane-type and ent-kaurane-type diterpenoids from the leaves of Tricalysia dubia (Lindl.) Ohwi	D. He, K. Matsunami, H. Otsuka , T. Shinzato, M. Aramoto, M. Bando and Y. Takeda	Journal of Natural Medicines, 61(1), 2007, 46-50
9	75C_{18}-OPN	A novel noninvasive method for assessing glutathione-conjugate efflux systems in the brain	T. Okamura, T. Kikuchi, K. Fukushi, Y. Arano and T. Irie	Bioorganic & Medicinal Chemistry, 15(9), 2007, 3127-3133
10	75C_{18}-OPN	New prenylated flavones from Artocarpus champeden, and their antimalarial activity *in vitro*	A. Widyawaruyanti, Subehan, S. K. Kalauni, S. Awale, M. Nindatu, N. C. Zaini, D. Syafruddin, P. B. S. Asih, Y. Tezuka and S. Kadota	Journal of Natural Medicines, 31(4), 2007, 410-413
11	75C_{18}-OPN	Two New Sesquiterpenoid Glucosides from the Aerial Parts of Saussurea involucrate	X. Wang, S. Gesang, W. Jiao, X. Liao and L. Ding	Journal of Integrative Plant Biology, 49(5), 2007, 609-614
12	75C_{18}-OPN	Inhibitory Effects of 5, 6, 7-Trihydroxyflavones on Tyrosinase	H Gao, J Nishida, S Saito, J Kawabata	Molecules, 12, 2007, 86-97
13	75C_{18}-OPN	Molecular Cloning, Expression and Properties of an α/β-Galactoside α 2,3-Sialyltransferase from Vibrio sp. JT-FAJ-16	Y. Takakura, H. Tsukamoto and T. Yamamoto	Journal of Biochemistry, 142(3), 2007, 403-412
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