# SHISEIDO HPLC COLUMNS



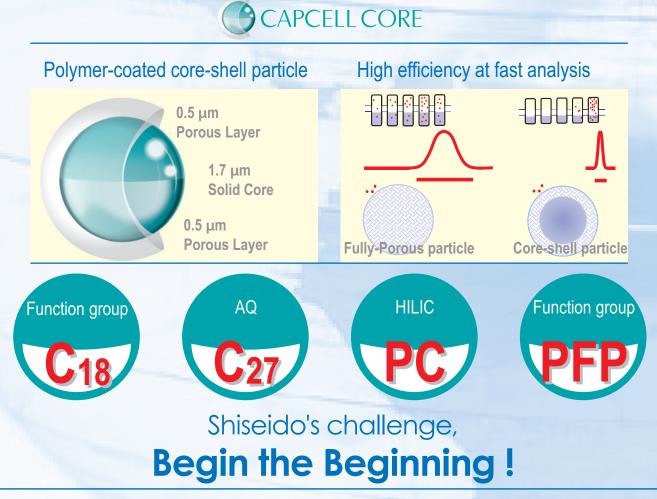
Polymer-coating type core-shell technology



## Core-shell, the new particle geometry of HPLC

Since 1987 Shiseido has been pursuing the best LC separation by creating new chemistry on the surface of powdery materials.

Fusing Shiseido's chemistry and a new particle geometry together, the beginning of new LC separation is ready to present! Polymer-coating type Core-shell technology

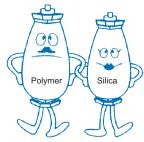


In Diversity, Strength In Challenge, Growth In Heritage, Excellence

# CAPCELL PAK

CELL PA

The revolutionary polymer-coated "capsule type" HPLC column with combined advantages of silica-based and polymer-based columns





**CAPCELL PAK** columns show tremendous durability and reproducibility, being free from undesirable secondary effects typical of other silica-based columns. Their unique synthetic process consists of two steps.

- 1) Surface coating of the silica using a silicone monomer by vapor deposition, resulting in a homogenous polymeric mono-layer.
- 2) Attaching alkyl groups to the coated surface that shields acidic silanols (Fig.1).

This imparts superior mechanical strength as well as extended pH stability, and provides excellent peak profiles for acidic, basic and chelating compounds. CAPCELL PAK exhibits excellent separation and chemical stability, hence the combined benefits of silica supports.

### Table 1

	silica type	capsule type	polymer type
pressur durability	(20MPa)	(20MPa)	× (3.5-7MPa)
basic resistance	∆ (pH2-7)	O (pH1-10)	(pH1-12)
separation	0	O	Δ
validation	0		×

 $\bigcirc$ : exellent  $\bigcirc$ : good  $\triangle$ : marginal  $\times$ : bad

### Spherical silica Spherical si

Fig.1

### FEATURES

- Polymer coating deactivates residual silanols which cause peak tailing
- Extended pH range (1-10) provides longer lifetime
- Durable and reproducible
- Low column pressure
- Excellent selectivity
- Available in many popular phases
- GLP/GMP validation supported (UG, MG series)

Through its precisely-controlled manufacturing process, **CAPCELL PAK** is a perfectly inert column that neither contains nor releases impurities.



- High-purity silica Metal impurities in silica, the starting material, used for SG, UG, MG Series, ACR, and IF AQ type are less than 5ppm.
- Precisely controlled pore size and distribution

Advantages of CAPCELL PAK

• Spherical silica with an extremely narrow distribution of diameter, which leads to low column pressure.

### Low pressure, easy-to-use

CAPCELL PAK analytical columns (4.6 mm i.d. x 250mm) typically show lower pressures, compared to conventional ODS columns (Fig. 2).

Mobile phase : 70vol% CH<sub>3</sub>OH,

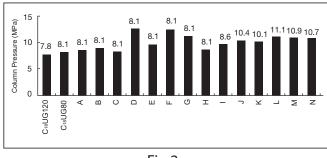


Fig.2

120

100

60

40

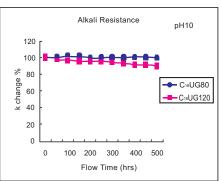
20

80

k change

### Durable over a wide pH range (pH 2-10) (ACR, pH 1-10)

1.0mL/min, 25°C



Column : CAPCELL PAK C10 UG80/120 S5 4.6 mm i.d. x 150mm

Mobile phase : CH<sub>3</sub>OH/10mmol/L Phosphate buffer=70/30,

0 100 200 300 400 500 Flow Time (hrs)

Acid Resistance

pH2

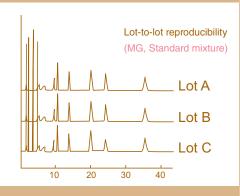
- C18UG80

- C18UG120

Column : CAPCELL PAK C18 UG80/120 S5 4.6 mm i.d. x150mm Mobile phase : CH8OH/H2O (pH1.0, H8PO4)=70/30, pH2.0

### Excellent Peak profile and Lot-to-Lot reproducibility

- Symmetrical peaks are obtained even for basic compounds since the undesired effects of residual silanols and metal impurities are kept minimal.
- Excellent lot-to-lot reproducibility.





### Stability of Retention Factor K'

# **Evolution of Capcell Pak Technology**

Ever since the first CAPCELL PAK was introduced in 1987 (AG type), the product line has evolved in terms of the quality of the silica support as well as the polymer coating technology (Table 2). To meet the increased demands in high-throughput analysis, low background analysis, and analysis using harsh mobile phases (e.g., pH<2), CAPCELL PAK with acid resistance (ACR) and different geometries (Mini and capillary) were released to the market. In 2004, Capcell Pak MGII, was launched as a new milestone of CAPCELL PAK, which was developed for generic HPLC method of basic compounds even under neutral condition. In response to the increasing needs of LC-MS and UHPLC analysis, novel modification in particle size and surface bonding with keeping the conventional performance of CAPCELL PAK, gave birth of MGIII, MGIII-H, and IF2 (sub2µm) Now, upon the new challenge in core-shell technology, CAPCELL CORE columns have been launched to extend the CAPCELL PAK product line.

### Table 2

Polymer-coating Type	Base silica	Polymer coating	pH range	Separation	retention of polar compounds
AG	conventional grade	Mono-layer	2-10	Good	Fair
SG	High purity (metal content: <5ppm)	Mono-layer	2-9	Good	Fair
UG	High purity (metal content: <5ppm)	Homogeneous mono-layer	2-10	Excellent	Fair
MG, MGII MGIII, IF2	High purity (metal content: <5ppm)	Controlled homogeneous mono-layer	2-10	Excellent	Strong
ACR	High purity (metal content: <5ppm)	Reinforced homogeneous mono-layer	1-10	Excellent	Fair
AQ	AQ High purity (metal content: <5ppm)		2-9	Excellent	Excellent (100% water)

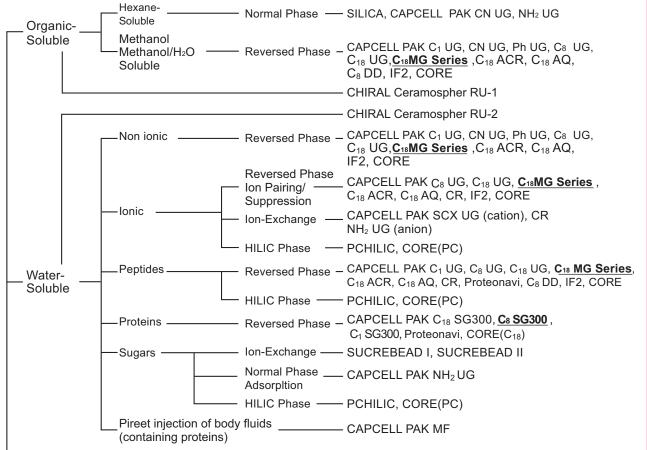
### **Column List**

-	1 A A 4 4 4 4			
Core-sh	ell Tı	vne (		umns
COLC SIL	<b>C</b> 11 1		-01	annis

•	CAPCELL CORE (C18, AQ, PC, PEP) <b>NEW!</b>
	Core-shell type columns for improved LC, LC-MS and UHPLC
	General Columns
•	CAPCELL PAK C <sub>18</sub> IF2 <i>NEW!</i>
	A Sub2-µm column in response to ultrahigh-pressure analysis
•	CAPCELL PAK C <sub>18</sub> MG Series (Capillary, MGII, MGIII, Minimini)9-18
	For improved retention of polar compounds with reduced peak tailing
•	CAPCELL PAK UG Series (C18, C8, Ph, CN, NH2, SCX)
	For fast separation of basic and polar compounds
•	CAPCELL PAK ACR (C18, Capillary, cartridge)
	C18 column with unprecedented acid resistance
•	CAPCELL PAK AQ (C18, Capillary, cartridge)
	C18 column operable in 100% water
•	CAPCELL PAK CR (1:50, 1:20, 1:4) <i>NEW!</i>
	A mixing mode of strong cation - exchange and reversed phase.
•	CAPCELL PAK C <sub>8</sub> DD
	C <sub>8</sub> , yet resistant to acid and alkali
•	PC HILIC NEW!
	A silica based HILIC column bound with Phosphorycholine (PC) group
	Wide-Pore Columns
•	PROTEONAVI <b>NEW!</b>
	For analytical and preparative separation of protein
	Specialty Columns
•	CAPCELL PAK MF (SCX, C <sub>8</sub> , Ph)
	For direct injection of serum or plasma without pretreatment
•	CHIRAL columns (Ceramospher Ru-1/Ru-2, CD-Ph)
•	SUCREBEAD I, SUCREBEAD II
	For separation of sugars
•	REDUCTION Column

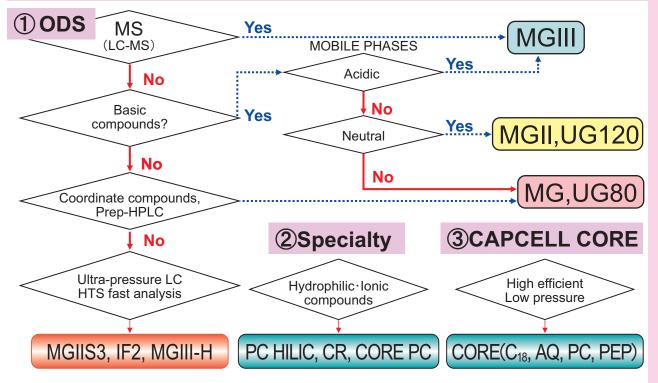
# CAPCELL PAK SELECTION

Capcell Pak columns provide reversed-phase, normal-phase and HILLC (Reverse of reversed-phase) modes ion-exchange separation modes. The following guide will help chromatographers to choose a suitable column that best fits their applications.



- PC HILIC

# Column selection guide



**JHIJEIDO** 

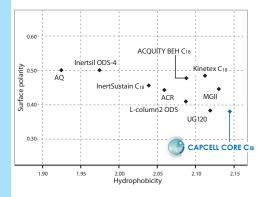
# CAPCELL CORE C18

### Polymer-coating type core-shell column

CAPCELL CORE is a polymer-coating type core-shell column of 2.7-µm particle with 1.7-µm solid core and 0.5-µm porous layer. CAPCELL CORE provides high-speed and improved separation in UHPLC as well as conventional HPLC.

### **Characteristics**

Function	Micro pore	Particle size	Specific surface	С%	Operational pH	Pressure
group	diameter (nm)	(µm)	area (m <sup>2</sup> /g)		range	resistance (MPa)
C <sub>18</sub>	9	2.7	150	7	1.5 – 10	60

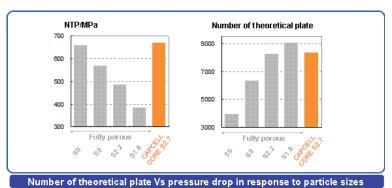


### Evolution of polymer coating technology in core-shell!

CAPCELL CORE is a column with minimized undesirable second effect of the silanols by applying polymer coating on the surface of core-shell base material. CAPCELL CORE phase is developed by aiming at full play to high performance of separation derived from the unique structure of core-shell.

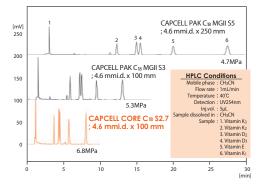
### High efficient separation with lower back pressure

core-shell type CAPCELL CORE overcome the separation impedance of sub 2-um porous particles with similar high efficiency under a lower back pressure.



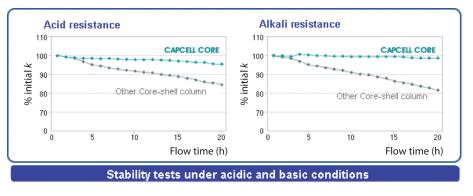
### High-speed high-efficient analysis

CAPCELL CORE  $C_{18}$  is suggested the improved way to gain the highest separation efficiency at fast analysis even in conventional HPLC.



### Excellent stability under acidic and basic conditions (pH1.5-10)

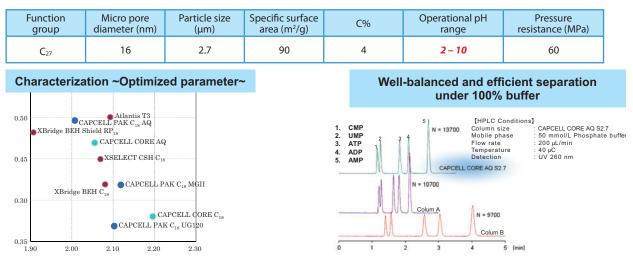
Polymer coating technology applied on Capcell Core leads to an excellent stability under acid and basic conditions. Clear differences from other core-shell products can be observed



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# CAPCELL CORE AQ

CAPCELL CORE AQ is C<sub>27</sub> column developed for improved retention of high hydrophilic compounds under 100% aqueous mobile phase at fast analysis.



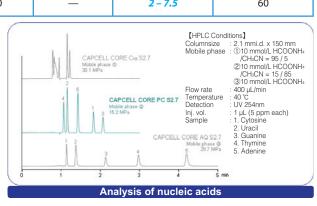
# CAPCELL CORE PC

CAPCELL CORE PC is developed by sophisticated bonding of phosphorylcholine group (PC). The PC column provides HILIC-mode retention of very polar compounds.

Function group	Micro pore diameter (nm)	Particle size (µm)	Specific surface area (m <sup>2</sup> /g)	C%	Operational pH range	Pressure resistance (MPa)
РС	9	2.7	150	—	2 - 7.5	60

### Synergy of PC technology and CAPCELL CORE

CAPCELL CORE PC retains high hydrophilic compounds under organic solvent-rich mobile phase where  $C_{18}$  has no retention. Core-shell type PC is a good alternative for UHPLC (sub 2- $\mu$ m ) HILIC mode and provides improved LC-MS for high hydrophilic compounds.

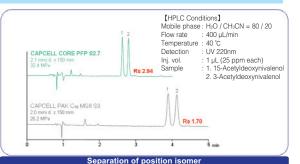


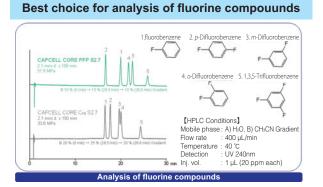
# CAPCELL CORE PFP

CAPCELL CORE PFP is a novel phase with function group of pentafluorophenyl group. It provides improved separation capacity by specific retention of fluorine compounds and position isomers.

Function group	Micro pore diameter (nm)	Particle size (µm)	Specific surface area (m <sup>2</sup> /g)	C%	Operational pH range	Pressure resistance (MPa)
PFP	9	2.7	150	5	2 - 9	60

### Specific selectivity of position isomer in UHPLC





6

AG

(PC

( PFP

# UHPLC column for Ultra-High-Speed High-Pressure Separation

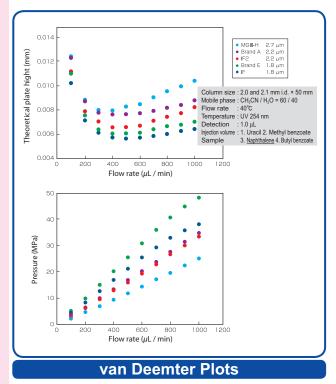
Durability is an indispensable quality to columns for ultra-high-speed high-pressure separation. A short lifetime under high pressure, and therefore, a high column cost would make high-pressure separation less attractive to chromatographers.

# CAPCELL PAK C18 IF2

In response to such customer's needs, CAPCELL PAK C<sub>18</sub> IF2 has been developed. IF2 enables excellent peak shape of basic compounds as well as the separation efficiency, while showing unsurpassed durability, hence offers the best choice for real ultra-high-speed, high-pressure separation

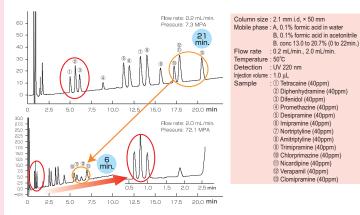
### **Properties**

Function group	Pore size (nm)	Average Particle size (µm)	Specific sur- face area (m²/g)	C%	Density (µmol/m²)	Applicable pH range
C <sub>18</sub>	8	2.2	480	15.5	1.5	2-9



### Quick and Sharp Elution of Basic Compounds

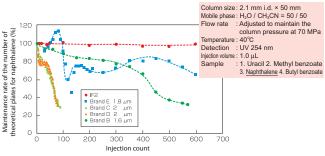
The CAPCELL PAK IF2 showing sharp peaks for basic analytes, provides excellent peak shape and separation efficiency even at tentime greater flow rate, while enables the analytes to elute quickly.



### Unsurpassed Durability against Pressure up to 100 MPa

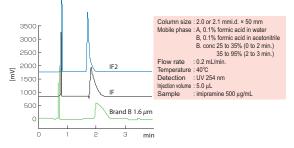
The graph below shows the durability comparison measured under a pressure of 70 MPaby adjusting the flow rate of each column. Among the columns whose specifications of withstand pressure are known as more than 70Mpa, CAPCELL PAK IF2 with pressure resistance up to 100 MPa, shows outstanding durability as a UHPLC column truly ideal for ultra-high-speed high-pressure separation.

Packing technology is an important process that determines the quality of the column. To achieve such excellent durability, we used considerable ingenuity in the packing of the CAPCELL PAK IF2.



### Higher Loadability than Existing Products

We injected 50-time more concentrated imipramine to compare the peak shape. The CAPCELL PAK IF2, which is suitable for the separation of basic compounds, sharply elutes even high concentrations of basic compounds.



### CAPCELL PAK C<sub>18</sub> IF2 S2

Catalog No	Inner Diameter (mm)	Length (mm)
92883	2.1	20
92885	2.1	50
92887	2.1	100

IF2

# **UHPLC column for Ultra-High-Speed LC-MS analysis**

With the improved performance of mass spectrometers, the requirements for columns are divided into two properties: capability of fast crude separation and a superior separation efficiency that enables highlysensitive analysis of a smaller amount of samples. Both of them require high pressure resistance.

### \_18 **\** APCE

CAPCELL PAK MGIII-H is an evolution of MGIII in response to such needs. MGIII-H enables operation pressure up to 50 MPa, showing outstanding durability, hence offers improved high-speed LC-MS of basic compounds with flexible pressure resistance.

100

80

60

40

20

0

0

Properties							
Function group	Pore size (nm)	Average Particle size (µm)	Specific sur- face area (m²/g)	C%	Density (µmol/m²)	Applicable pH range	
C <sub>18</sub>	10	2.7	300	15	2.3	2-9	



The durability is determinated under 50 MPa by adjusting the flow rate of each column. The CAPCELL PAK MGIII-H maintains the number of theoretical plates even after 600 times of injection, so can be used with confidence.

MGIII-H

Column size : 2.0 mm i.d. × 50 mm Mobile phase : H<sub>2</sub>O / CH<sub>3</sub>CN = 40 / 60

40°C

UV 254 nm

600

Adjusted to maintain th

column pressure at 50 MPa

3. Naphthalene 4. Butyl benzoate

700

Flow rate

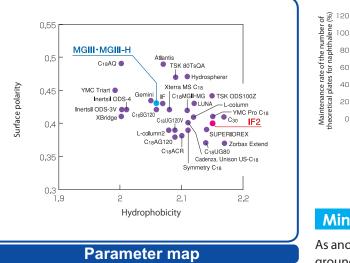
Temperature

Detection

Sample

500

400



### **Specifications for MG Series Columns**

We conduct strict control by adding the symmetry of the peak shape and the retention coefficient for amitriptyline, which is sensitive to silanol, to the specifications.

Column Test item	MGIII	MGII	MG	
Pyridine/Pheno	As (Py): 0.90-1.50 As (Ph): 0.90-1.20	Shipment standard specified	Shipment standard specified	
Quinizarin	As: 0.90-1.40	-	-	
Amitriptyline (neutral condition)			-	
Amitriptyline (acidic condition)	As: 0.90-1.30 k: 1.3-1.6	-	_	

### **Excellent Reproducibility for Basic Compounds under Acidic Conditions**

The lot-to-lot reproducibility is an issue for isocratic analysis under acidic conditions, which is often conducted as the LC-MS analysis of basic compounds. The MGIII-H is developed with special conditioning similarly to that of the MGIII during the manufacturing processes, therefore can provide promised performance with confidence.

### **Minimized Bleeding**

100

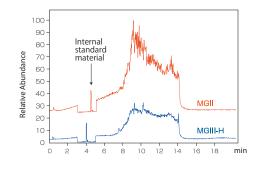
MGIII

200

300

Injection times

As another effect of the special preconditioning, the background (bleeding) most affecting the high sensitivity range has been drastically reduced.



### CAPCELL PAK C<sub>18</sub> MGIII-H S3

Catalog No	Inner Diameter (mm)	Length (mm)
92782	2.0	20
92784	2.0	50
92786	2.0	100

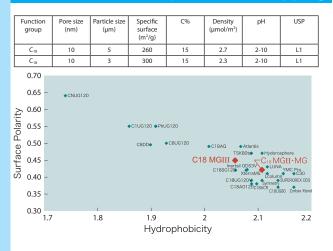


**/HI/EIDO** 

# CAPCELL PAK C<sub>18</sub> MGIII

The CAPCELL PAK C<sub>18</sub> MGIII, the third generation of the MG series, is developed to overcome the column-to-column variation in retention of basic compounds under an acidic condition. The quality of MGIII will help develop improved methods in various LC-MS applications.

### Characteristics and parameter mapping



### Comparison of Shiseido HPLC columns

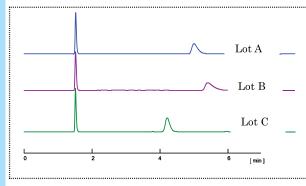
Feature	MGIII	MGII	MG	UG120	ACR	AQ	
High-purity silica		~~			~~		
Highly basic compounds (under acidic mobile phases)	~~~	~~	~~	~~	~	~~	
Highly basic compounds (under neutral mobile phases)	~	~~~	n/a	n/a	n/a	n/a	
LC-MS/MS	~~~	~~	~	~	~	~	
Multi-component analysis	~~	~~	~~	~	~~	~~	
High-polarity compounds	~~	~~	~~	~	~	~~~	
Pyridine/phenol test	~~	~~	~~	~~	~~	~~	
Quinizarin	~~	-	-	-	-	-	
Acidic durability	~~	~~	~~	~~	~~~	~~	
Basic durability	~~	~~~	~~~	~~~	~~~	~~	
Semi-preparative scale	-	~~	~~	~~	~~	~~	
3-µm particle type	~~	~~	~~	~~	~~	~~	

✓✓✓: excellent ✓✓: good(appliable) n/a: not applicable

### The problem in the analysis of basic compounds under an acidic condition

9

The following figure is an example of columnto-column variation under an acidic condition. The compound used here was amitriptyline, a highly basic compound used for the USP evaluation method. The results were obtained under an isocratic condition (0.1% formic acid/methanol). Retention times of the three columns (Column A, anonymous) were found very different under the mobile phase that was one of the most common in LC-MS. The similar tendency was observed in many other columns. Based on our previous studies, we found the responsible factor was related to the synthetic byproducts and the residues of impurities from reagents used for the synthetic process. We accordingly applied a special pre-conditioning process to the column production, which provided the stable retention to both amitriptyline and naphthalene.



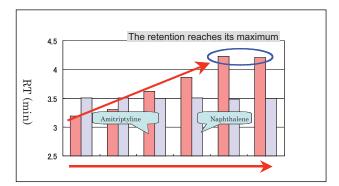
### Lot variation under an acidic condition

HPLC Conditions Column Mobile phase Flow rate

Sample dissolved in

Temperature

Detection Ini.vol. :Column A 4.6 mm i.d. x 150 mm :CH<sub>3</sub>OH / H<sub>2</sub>O / HCOOH = 500 / 500 / 1 :1 mL/min :40 °C :UV, 254 nm :5 μL :H<sub>2</sub>O(50 μg/mL)

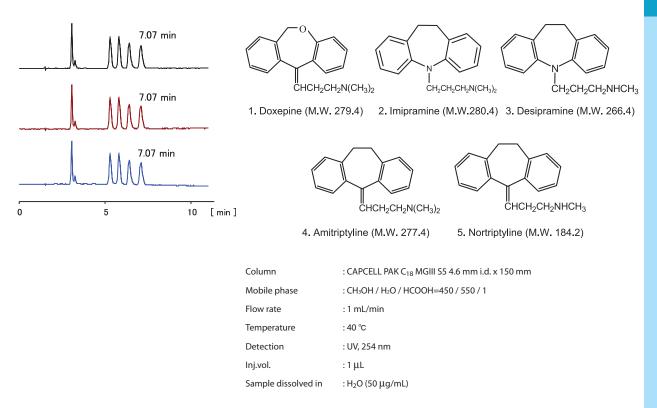


Optimization of pre-conditioning (PC) time

### **JHIJEIDO**

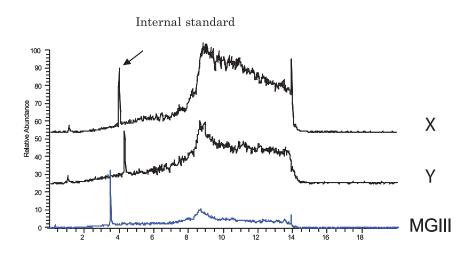


### MGIII---excellent reproducibility under an acidic condition



### **MGIII**...low-bleeding column

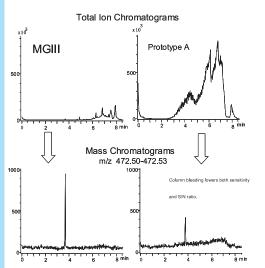
Total ion chromatograms (TICs) were compared among different Shiseido columns under a validated gradient condition. The PC process was found to reduce the amount of column bleeding to a large extent, especially in the region for highly hydrophobic compounds.

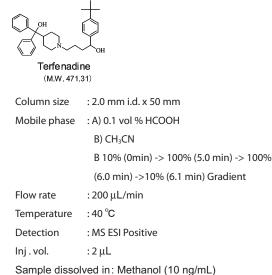


Comparison of column bleeding

### Column bleeding" influences LC-MS sensitivity (Ion suppression)

Column bleeding not only interferes an analyte signal by its components with close m/z values, but may lower an intensity of the analyte peak itself. The effect of column bleeding has been extensively studied throughout the development of the MGIII series.

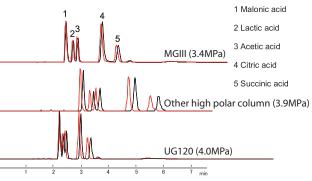




Influence of column bleeding on ion intensity of analyte (Ion suppression)

### MGIII...wide usage from high-polarity compounds to low-polarity ones

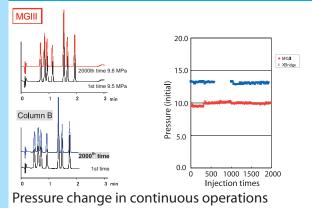
MG Series columns used to be categorized as "medium polar". MGIII, being treated with the PC process, has a higher polarity than previous MG columns. Separation of small organic acids was attempted to evaluate the polarity of MGIII, in comparison with another Shiseido column and one of the competitor's high-polarity columns.



Column size	: 2.0&2.1 mm i.d. x 150 mm
Mobile phase	: 0.1 vol% $H_3PO_4$ / $CH_3CN = 99.5$ / 0.5
Flow rate	: 200 μL/min
Temp.	: 40 °C
Detection	: UV 210 nm
Inj. vol.	: 2.0 μL
Sample dissolved in	: Mobile phase ( 500 µg/mL )

Separation of organic acids

### MGIII...low pressure and high durability



- Low pressure and high durability. A similar separation efficiency to be obtained with 25% less pressure.
- A new end fitting design (filter pore size, shape of through pore) to meet the new column specifications.
- A new process to finish the inner wall of the empty column.

http://hplc.shiseido.co.jp/e/

### LC-MS analysis of fourteen nucleic acid-related compounds

MGIII is suitable for separations of polar compounds. Nucleic acid-related compounds, which are generally considered hard to retain in  $C_{18}$  columns, were also well separated with MGIII.

### LC-MS Conditions

 Column
 : CAPCELL PAK C18 MGIII S5 ; 2.0 mm i.d. x 150 mm

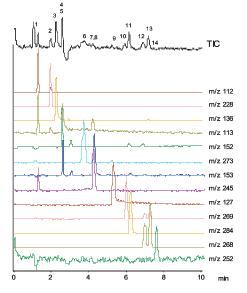
 Mobile phase
 : A) 0.1 vol% CH<sub>3</sub>COOH
 B) CH<sub>3</sub>OH

 B 2% (0 min) -> 20% (10 min) -> 2% (10 min) Gradient

Flow rate : 200 µL/min Temperature : 40 °C Detection : MS ESI Positive

Inj.vol. :2 μL Sample : guan

: guanine (1000 µg/mL in 0.1 mol/L KOH ), xanthin (1000 µg/mL in 0.1 mol/L NaOH), uridine (500 µg/mL in 1% HCOOH), deoxyadenos ine (1000 µg/mL in 1% HCOOH) and other solutions (1000 µg/mL in 1% HCOOH) were mixed together, and diluted to 1 mL with water. Sonication (30 min) is necessary to dissolve some of the compounds.

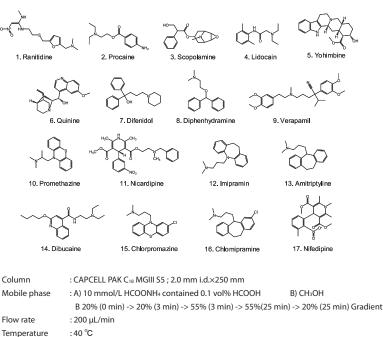


1. Cytosine (MW. 111.1)

2. Deoxycytidine (MW. 227.2)

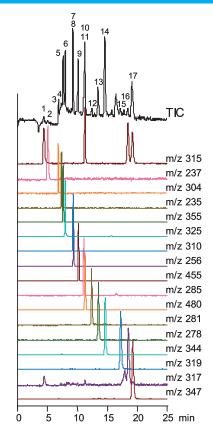
- 3. Adenine (MW. 135.1)
- 4. Uracil(MW. 112.1)
- 5. Guanine (MW 151.1)
- 6. Hypoxanthine (M.W. 136.1)
- 7. Xanthine (M.W. 152.1)
- 8. Uridine (MW.244.2)
   9. Thymine (MW.126.1)
- 10. Inosine (MW. 268.2)
- 11. Guanosine (MW.2832)
- 12. Adenosine (MW.267.2)
- .\_\_\_\_,
- 13. Deoxyguanosine (MW. 267.2)
- 14. Deoxyadenosine (MW. 251.2)

### Simultaneous separation of seventeen basic compounds with MGIII column



Temperature	: 40 °C
Detect	: MS ESI Positive
Inj.vol.	: 2 μL

Sample : Methanol (Diphenhydramine: 10 µg/mL, Other sixteen compounds: 2 µg/mL)





**JHIJEIDO** 

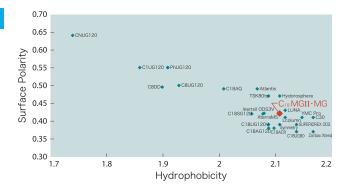
# CAPCELL PAK C18 MGII

CAPCELL PAK MG II is based on high-purity silica support, being one of the MG series columns. MG II is designed to provide excellent peak shapes for basic compounds under neutral mobile phase conditions. Its outstanding " silanol-shielding" material was generated by the original polymer-coating technology.

Characteristics and parameter mapping

The general characteristics of CAPCELL PAK  $C_{18}$  MG II are same as that of MG. MG II is an easy-to-use column with moderate hydrophobicity and moderate surface polarity.

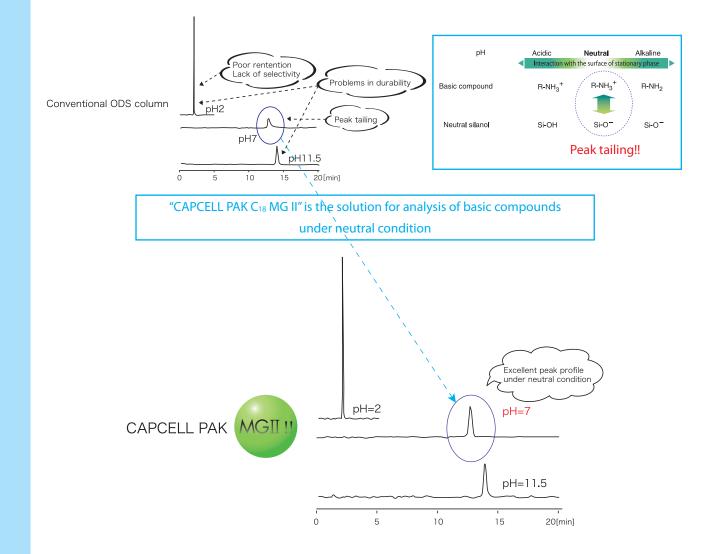
Function group	Pore size (nm)	Particle size (µm)	Specific surface area (m²/g)	Carbon%	Alky group density (µmol/m²)	рН	USP
C <sub>18</sub>	10	5	260	15	2.7	2-10	L1
C <sub>18</sub>	10	3	300	15	2.3	2-10	L1



# Why do we need a good column to be used under neutral mobile phase conditions?

Many physiologically active compounds and their metabolites possess a basic nature. Chromatographers keep on seeking a good column for such compounds, free from peak shape deterioration caused by silica's acidity, the inherent nature of silica-based columns.

<Problems in analysis of basic compounds under acidic, basic, and neutral condition>



13





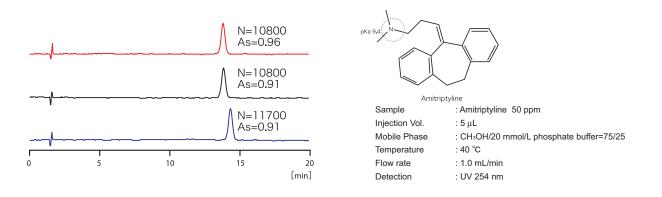
**Excellent reproducibility** 

In addition to "Standard of Silica" and "Standards for Parameters of Packing Materials" (both documented by Shiseido), a test with amitriptyline is used to quality-control the production of MG II.

Advantage 1 -> Possible to avoid lowering pH for compounds unstable at acidic conditions. Advantage 2 -> Beginning a mobile phase optimization at neutral pH makes the process simple,

Advantage 3 - Good for compounds that show the highest ion intensity at neutral pH in LC-MS.

especially in LC-MS.

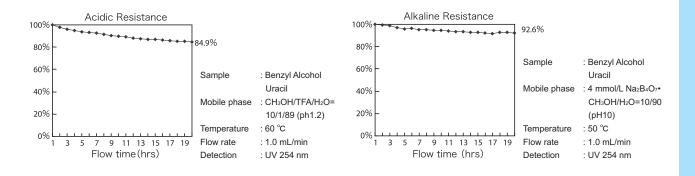


### GLP/GMP Support Column

CAPCELL PAK C<sub>18</sub> MG II, a GLP/GMP support column, is attached with a test chromatogram and a certificate of performance for packing materials used for it. In addition, it is possible to request three columns from three different production lots for a validation purpose.

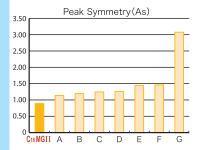
### Wide pH range from 2 to 10

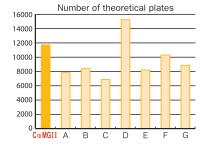
CAPCELL PAK C<sub>18</sub> MG II is a column having excellent performance and durability. The graphs below show the results of the durability test under acidic and basic conditions, indicating that CAPCELL PAK C<sub>18</sub> MG II can be used in a wide pH range.

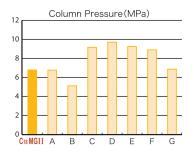


### Shielding silanol groups at the highest level

The graphs below show the comparisons among columns of other suppliers in terms of tailing factor (As), Number of theoretical plates, and pressure (MPa) of amitriptyline, which are the indicators of the influences of residual silanol. As for MG II, good values were obtained in the three comparative factors. The results indicate that MG II is the best choice for analysis of basic compounds, and has achieved the highest level in shielding silanol groups.

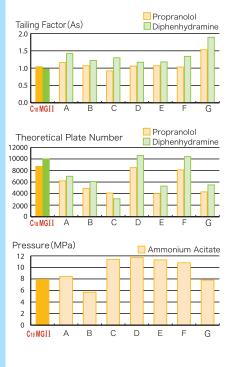


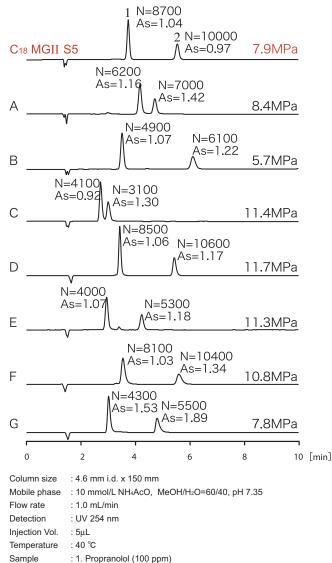




### Analysis using volatile salts

Basic compounds were analyzed with different columns using ammonium acetate, which is a neutral salt often used in LC-MS. Compared with other columns, CAPCELL PAK C<sub>18</sub> MG II showed the top level on peak symmetry and number of theoretical plates. In addition, MG II also showed low column pressure, which is one of the features common to all the CAPCELL PAK columns.





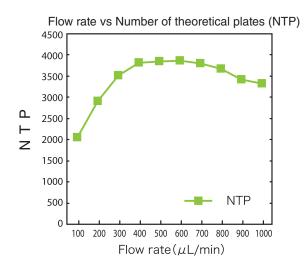
2. Diphenhydramine (200 ppm)

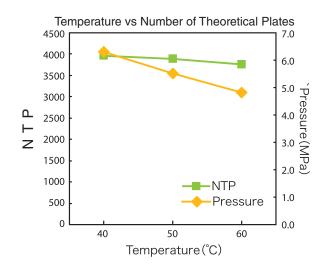
15

### 3-µm particles for high-throughput analysis

### CAPCELL PAK C<sub>18</sub> MGII S3 Provides the solution to meet the high-throughput demand.

The largest number of theoretical plates is obtained around twice the normal flow rate (200µL/min for 2-mm column) and is nearly maintained in the zone of higher flow rates. (Recommended column pressure: 15 MPa or less, Maximum operating pressure: 20 MPa)





Run at more than twice the normal flow rate • The run time is shortened in inverse proportion to the flow rate!

• Number of theoretical plates increases too!

Sample	: Naphthalene
Injection Vol.	: 5 μL
Column	: C <sub>18</sub> MGII S-3
	2.0 mm i.d. × 35 mm
Mobile phase	: CH <sub>3</sub> CN/H <sub>2</sub> O=50/50
Temperature	: 40 °C
Detection	: UV 254 nm

Raise the temperature.

 Lowering pressure and increasing number of theoretical plates!

Sample	: Naphthalene
Injection Vol.	: 5 μL
Column	: C <sub>18</sub> MGII S-3
	2.0 mm i.d. × 35 mm
Mobile phase	: CH <sub>3</sub> CN/H <sub>2</sub> O=50/50
Floe rate	: 400 µL/min
Floe rate Detection	: 400 μL/min : UV 254 nm
11001000	•

### **Popular Column Dimension**

Partial Number	Function Group	Grade	Pore Size (Å)	Particle Size (µm)	Length (mm)	I.D. (mm)
92469	C18	MGII	100	3	100	2.0
92470	C18	MGII	100	3	150	2.0
92479	C18	MGII	100	3	50	4.6
92480	C18	MGII	100	3	75	4.6
92481	C18	MGII	100	3	100	4.6
92482	C18	MGII	100	3	150	4.6

Partial Number	Group	Grade	Pore Size (Å)	Particle Size (µm)	Length (mm)	I.D. (mm)
92519	C18	MGII	100	5	100	2.0
92520	C18	MGII	100	5	150	2.0
92521	C18	MGII	100	5	250	2.0
92531	C18	MGII	100	5	100	4.6
92532	C18	MGII	100	5	150	4.6
92533	C18	MGII	100	5	250	4.6

# CAPCELL PAK C18 MG

MG stands for Miracle Grade. The enhancement of hydrophobicity had previously been considered to conflict with the enhancement of surface polarity. This miraculous packing material, however, achieved an exquisite balance through the fine control of polymer coating and alkyl group introduction.

A wide range of compounds (acidic, neutral, and basic compounds, low-polarity to high-polarity compounds, and others) can be efficiently separated. This column is the optimum "first choice", being free of specific selectivity, easyto-use, available in the pH range 2-10, and extremely durable.

Column

Flow Rate

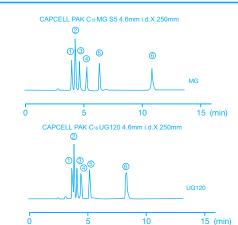
Detection

Sample

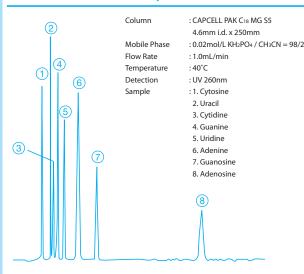
Mobile Phase

Temperature

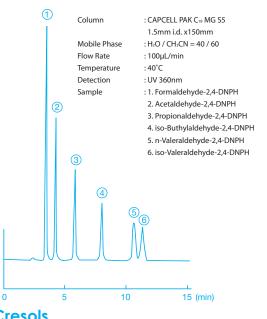
### **Organic** acids



### Nucleic acid bases, Nucleosides



### **DNPH-aldehydes**



: 4.6mm i.d. x 250mm

: 1.0mL/min

: UV 210nm

: 1. Malonic acid 2. Lactic acid

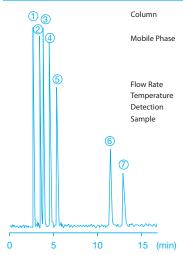
3. Acetic acid 4. Citric acid

5. Succinic acid 6. Propionic acid

:40°C

:  $0.1vol\% H_3PO_4 / CH_3CN = 97.5 / 2.5$ 

### 0 10 15 5 (min) Analysis of Arsenic Compounds by LC/ICP



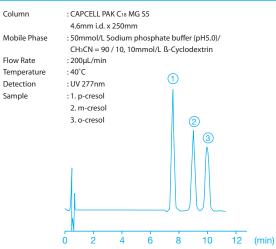
: CAPCELL PAK C18 MG S5 4.6mm i.d. x 250mm : 10mmol/L Sodium butanesulfonate, 4mmol/L Tetramethylammonium hydroxide, 4mmol/L Malonic acid, 0.05vol% CH<sub>3</sub>OH : 1.0mL/min : R.T. : ICP : 1. Sodium arsenate 2. Sodium arsenite 3. Methylarsonic acid 4. Dimethylarsinic acid 5. Arsenobetaine

- 6. Trimethylarsine oxide
- 7. Arsenocholine

### Cresols

Column

Sample



### **J**HIJEIDO

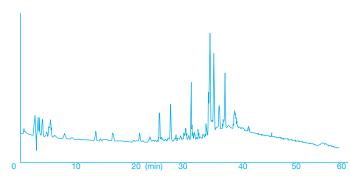
### **Capillary Columns**

### The MG S5 and S3 are now available in 0.5mm and 0.3mm i.d. !!

The MG phase is now available in capillary columns to meet the highest sensitivity and resolution, demanded typically in the field of proteomics.

At 150mm length, S5 (5µm) and S3 (3µm) offer NTP (number of theoretical plates) of over 10,000 and 14,000 respectively.

### Peptide Mapping with high Resolution

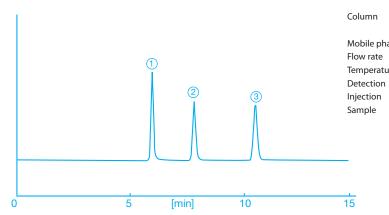




### **HPLC** Conditions

Column	: CAPCELL PAK C18 MG S3 0.3mm i.d. x 150mm	
Mobile phase	: A: 0.05vol% TFA , H2O	
	B: 0.05vol% TFA , CH₃CN	
	B: 10%(0min) → 50%(45min) → 50%(60min)	
Flow rate	: 5μL/min	
Temperature	: RT	
Detection	: UV 210 nm	
Injection	: 60nL	
Sample	: Tryptic digest of casein	

### **High NTP and Excellent Peak Symmetry**



	: CAPCELL PAK C18 MGS3
	0.3mm i.d. x 150mm
ase	: H <sub>2</sub> O / CH <sub>3</sub> CN= 25 / 75(v/v)
	: 5μL/min
ure	: RT
	: UV 254nm
	:60nL
	: 1. Ethyl benzene
	2. n-Propyl benzene
	3. n-Butyl benzene

### **Column Dimension**

phase	type	size	i.d.(mm)	length (mm)		
	18 <b>MG</b>	Eum	0.3			
C <sub>18</sub>		5 µm	ο μπ	5 μπ	0.5	150
<b>U</b> 18		0	0.3	150		
		3 µm	0.5			

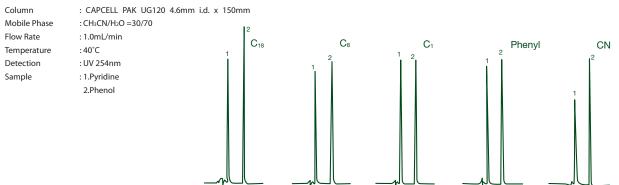


# CAPCELL PAK UG (C18, C8, Ph, CN, NH2, SCX)

The CAPCELL PAK UG type utilizes a high-purity silica with low metal impurity (<5ppm), that gives a fast separation of basic and polar compounds with sharp symmetrical peaks.

### **Excellent surface inertness**

### PYRIDINE/PHENOL TEST



### C<sub>18</sub>UG

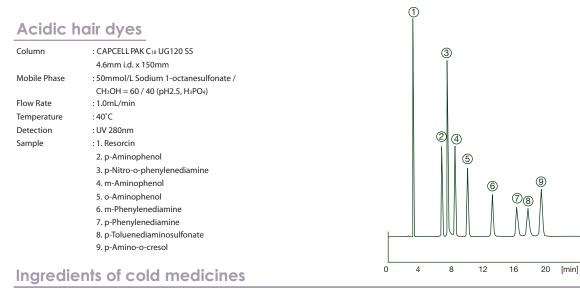
### ١

Water soluble vi	tamins	Antiseptics	
Column	: CAPCELL PAK C18 UG120 S5	Column	: CAPCELL PAK C18 UG120 S5
	4.6mm i.d. x 150mm		4.6mm i.d. x 150mm
Mobile Phase	: (5mmol/L Sodium hexanesulfonate	Mobile Phase	: 0.05mol/L NaH2PO4, pH4.5 /MeOH /
	+ 20mmol/L H <sub>3</sub> PO <sub>4</sub> , pH2.3) /		CH <sub>3</sub> CN = 50 / 35 / 15, 4mmol/L
	CH₃CN = 91 / 9		Cetyltrimethyl ammonium chloride
Flow Rate	: 1.0mL/min	Flow Rate	: 1.0mL/min
Temperature	:40°C	Temperature	: 40°C
Detection	: UV 210nm	Detection	: UV 235nm
Sample	: 1. L-Ascorbic acid	Sample	: 1. Methylparaben
	2. Nicotinic acid		2. p-Hydroxy benzoic acid
	3. Nicotinamide		3. Ethylparaben
	4. Sodium pantothenate		4. Dehydroacetic acid
	5. Pyridoxine hydrochloride		5. n-Propylparaben
	6. Riboflavin phosphate		6. Sorbic acid
	7. Thiamine		7. Benzoic acid
1)	8. Folic acid		8. Iso-Butylparaben
Ϋ́	9. Biotin		9. n-Butylparaben
	(7) 10. Riboflavin (VB <sub>2</sub> )		10. Salicylic acid
	Ŭ		
		6	
3			
5			
24		2	
		1	
		03	
		5 7	
	9	@®	
			10
			()
0 5 10	15 20 min		
0 5 10	15 20 <sub>min</sub>	0 4 8 12	16 20 24 28 <sub>(min)</sub>

19

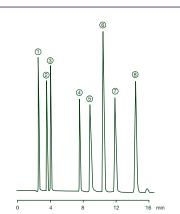
**U**G

**JHIJEIDO** 

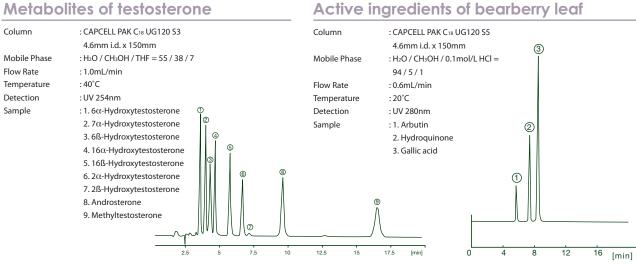


Column	: CAPCELL PAK C18
	4.6mm i.d. x 150
Mobile Phase	: 0.05mol/L NaH <sub>2</sub> F
	20 / 80 (pH2.5, H
Flow Rate	: 1.0mL/min
Temperature	: 40°C
Detection	: UV 280nm
Sample	: 1. Potassium qua
•	2. Acetaminophe
	3. Caffeine
	4. Salicylamide
	5. Chlorpheniran
	6. Phenol(I.S.)
	7 Acpirin

18 UG120 S5 0mm 2PO4 / CH3CN = H3PO4) aiacolsulfonate nen mine maleate Aspirin 8. Ethenzamide

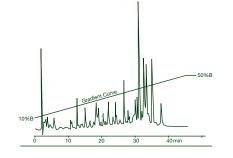


### Metabolites of testosterone



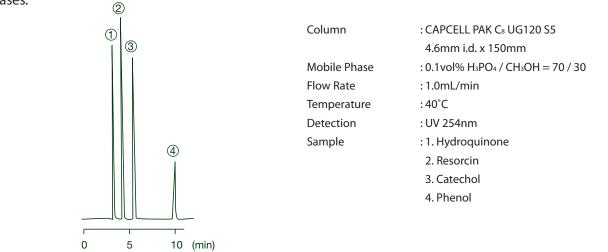
### Tryptic digest of casein (peptide mapping)

Column	: CAPCELL PAK C18 UG120 S5
	1.0 mm i.d. x 250 mm
Mobile Phase	: A : 0.1vol% TFA, H2O
	B: 0.1vol% TFA, CH3CN
	B 10% - 50% (45min) Gradient
Flow Rate	: 70µL/min
Temperature	: 35°C
Detection	: UV 210nm
Sample	: 1. Tryptic digest of casein



### C8 UG120

Suitable for quick separation of polar compounds, which used to be retained too long in other  $C_{18}$  phases.



### Ph UG120

Used for obtaining a different selectivity for analytes possessing an aromatic moiety.

### 1 **Antiepileptics** Column : CAPCELL PAK Ph UG120 S5 2 3 4.6mm i.d.x 150mm Mobile Phase : { 50mmol/L Na<sub>2</sub>HPO<sub>4</sub> + 50mmol/L KH2PO4 (pH6.8) } / CH<sub>3</sub>CN = 70 / 30 Flow Rate : 1.0mL/min Temperature :40°C Detection : UV 254nm Sample : 1. Phenobarbital 2. Carbamazepine 3. Phenytoin 0 4 8 12 (min)

### **CN UG120**

A phase having the least retentive nature of all reversed phases and a different selectivity brought by cyano groups.  $(1)_{|_{1} \otimes 1}$ 

Steroids		
Column	: CAPCELL PAK CN UG120 S5	
	4.6mm i.d. x 150mm	(4)
Mobile Phase	: CH <sub>3</sub> CN / H <sub>2</sub> O = 35 / 65	
Flow Rate	: 1.0mL/min	
Temperature	: 35°C	
Detection	: UV 242nm	
Sample	: 1. Cortisol	
	2. Cortisone	
	3. Corticosterone	
	4. Testosterone	

0

10

(min)

5

UG

Ph

CN

### **JHIJEIDO**

NH

(SC)

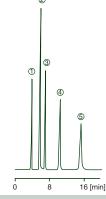
### NH<sub>2</sub> UG80

To be used as a normal phase under a water/organic mobile phase, or a weak anion exchanger under an acidic buffer.  $$_{\odot}$$ 

### Nucleotides

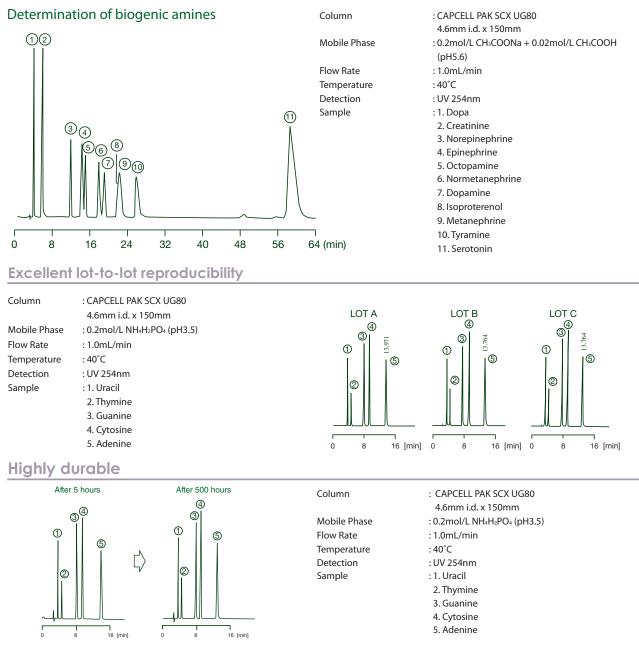
Column
--------

: CAPCELL PAK NH<sub>2</sub> UG80 S5 4.6mm i.d. x 250mm : 0.05mol/L (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> (pH3.0) : 1.0mL/min : 40°C : UV 254nm : 1.5'-CMP 2.5'-AMP 3.5'-UMP 4.5'-IMP 5.5'-GMP



### SCX UG80

A strong cation exchanger used for basic compounds.

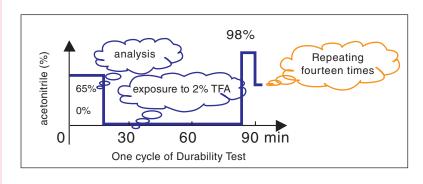


# CAPCELL PAK C18 ACR

**CAPCELL PAK C18 ACR** was synthesized through a modified polymer-coating technique, and intended to show an outstanding durability under an acidic mobile phase. Its performance was proven in the evaluation method originally designed for acidic resistance.

\* ACR Capillary Columns are also available

### Acidic resistance test





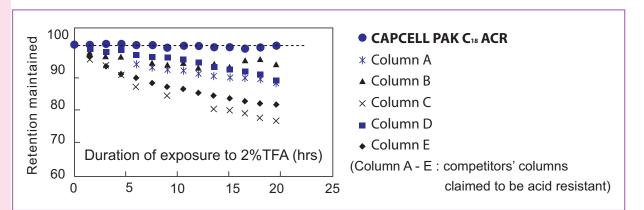
### HPLC conditions Column Mobile phase

B: 65%(20min) - 0%(60min) - 98%(5min) - 65%(5min) : 1.0mL/min : 60°C : UV 254 nm : 7μL : uracil, amylbenzene

: C18 ACR 4.6mm i.d.x150mm

: A: 2vol% TFA in H20 (pH1) B: 2vol% TFA in CH3CN

### **Comparison of Acidic Resistance**



### CAPCELL PAK C18 ACR compared to other CAPCELL PAK C18 phases

T	Surface Area	Pore Volume	Pore Size	Particle Diameter	Carbon Content
Туре	m²/g	mL/g	nm	μm	%
ACR	340	0.8	8	5	18
MG	260	0.9	10	5	15
UG120	300	1.0	12	5	15
UG80	340	0.8	8	5	18

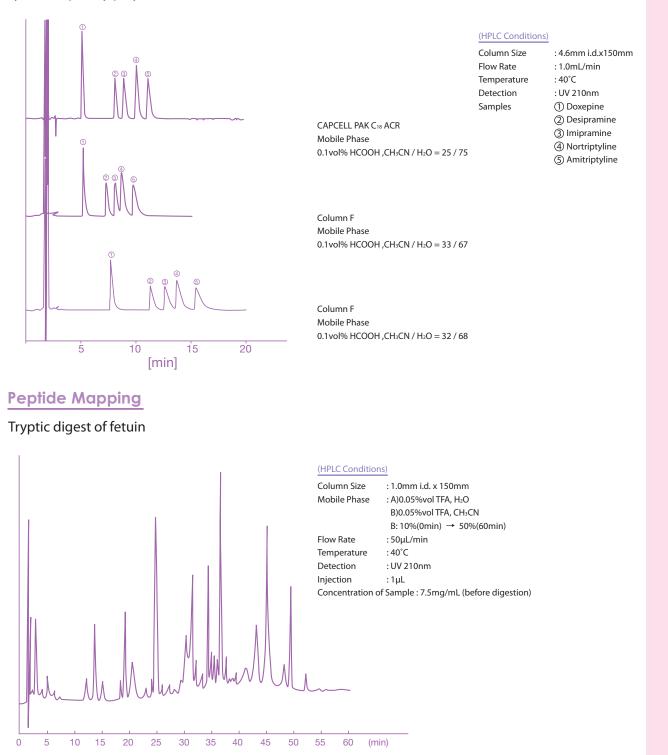
ACF

### **JHIJEIDO**

### **ACR Applications**

### Basic Compounds…Tricyclic antidepressants

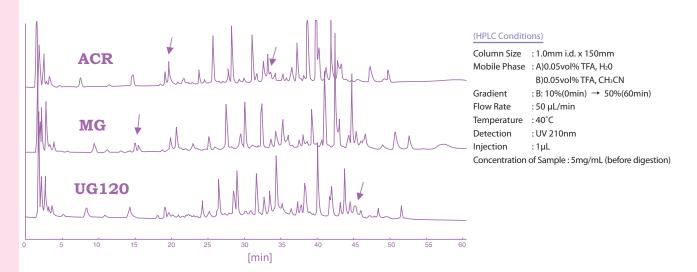
Peaks of tricyclic antidepressants, highly basic compounds, are known to be easily affected by residual silanols of a stationary phase. The tendency is pronouncing under acidic mobile phases commonly used in LC-MS. The following comparison is performed between CAPCELL PAK C<sub>18</sub> ACR and Column F, one of the major commercial columns, using five typical antidepressants. The ACR column shows a good baseline separation for these compounds, while Column F shows a very unstable retention behavior, influenced by a slight change in organic content in a mobile phase. The inertness of the ACR column was explained by its completely polymer-coated surface structure.



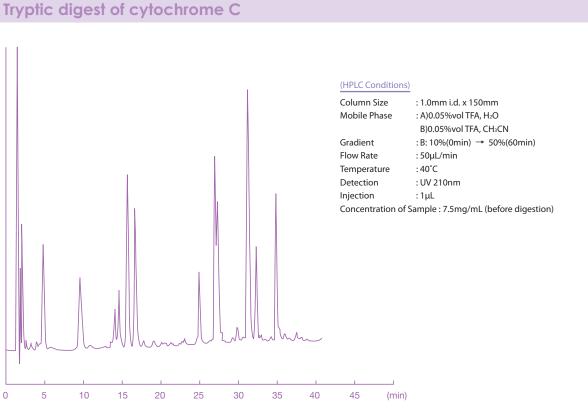


### **Tryptic digest of casein**

Profiles of tryptic digest of casein obtained with CAPCELL PAK ACR, MG, and UG120 are compared as shown below. Acidic mobile phases are commonly used in peptide mapping based on reversed-phase chromatography.



### Some selectivity differences (indicated with arrows) were observed among these columns.



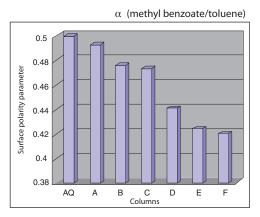
ACR

# CAPCELL PAK C18 AQ

**CAPCELL PAK C**<sub>18</sub> **AQ** was intended for separating highly polar compounds under water (buffer) rich mobile phase. Its C<sub>18</sub> group density was designed to be small, and shows a relatively small carbon content. The surface excess of organic solvent molecules on the stationary phase is adequately limited, and a stable retention of analytes can be obtained even under an aqueous mobile phase.

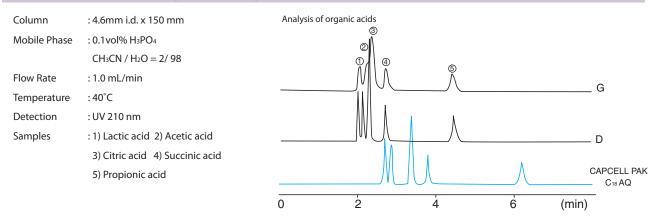
characteristics





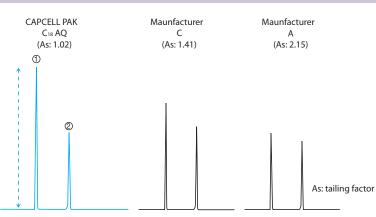
Column	Specific surface area of silica support (m²/g)	Carbon content (C%)
CAPCELL PAK C18 AQ	300	11
CAPCELL PAK C18 MG	260	15
CAPCELL PAK C <sub>18</sub> UG	300	15

### **Excellent retention of polar compounds**



# The peak shape of basic compounds represented by pyridine is almost symmetrical without tailing.

$H_3CN / H_2O = 30 / 70$
mL/min
°C
254 nm
Pyridine
Phenol



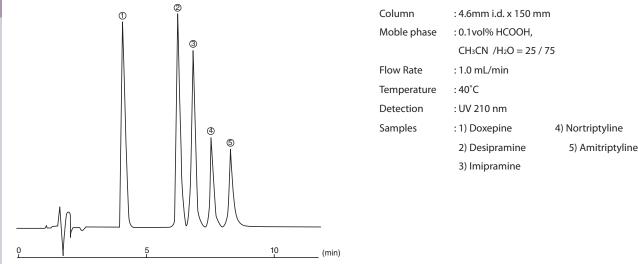
# http://hplc.shiseido.co.jp/e/

# -26 CELCON

**JHIJEIDO** 

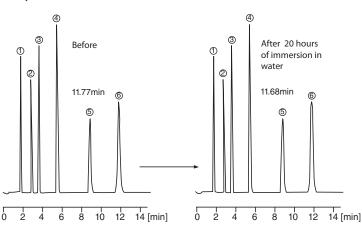
### Good peak shape of basic compounds under slightly acidic conditions.

Analysis of tricyclic antidespressants



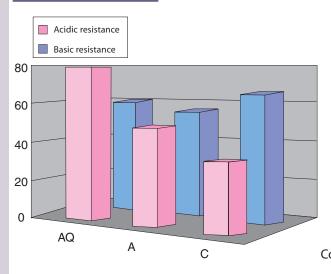
### Compatible with a mobile phase of 100% water

Analysis of nucleic acid base

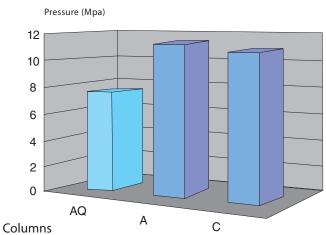


Column	: 4.6 mm i.d. x 150 m	n
Mobile phase	: 20 mmol/L KH2PO4.	
	20 mmol/L k <sub>2</sub> HPO <sub>4</sub>	
Flow tate	: 1.0 mL/min	
Temperature	:40°C	
Detection	: UV 254 nm	
Samples	: 1) Sodium nitrite	4) Guanine
	2) Cytosine	5) Thymine
	3) Uracil	6) Adenine

### Superior resistance to acidic and **Basic conditions**



### Excellent durability due to low column pressure



### **CR...Strong Cation Exchange & Reversed Phase**

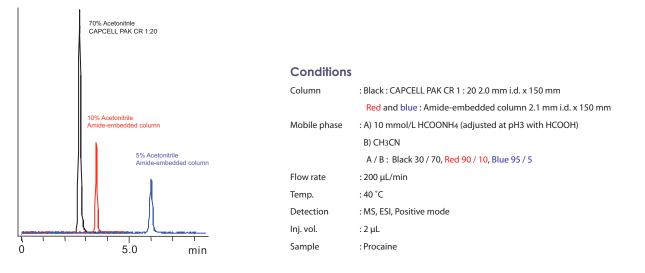
As a method to improve the sensitivity of basic drugs and their metabolites in LC-MS, Shiseido has developed a unique stationary phase.

The new product, "CAPCELL PAK CR," is a single column in which SCX and  $C_{18}$  are mixed inside. The CR column is available with different mixing ratios that were not possible to obtain by connecting two columns; SCX: $C_{18} = 1:50$ , 1:20 and 1:4. Simply choose the optimum column that best suits your separation.

They are intended to elute basic compounds possessing a certain level of hydrophobicity under a mobile phase with a higher organic content than that for  $C_{18}$  phases, for obtaining a higher sensitivity in LC-MS, or simply to obtain an altered separation selectivity.

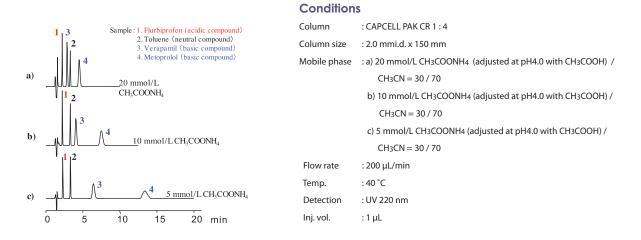
### Sensivity increase in LC-MS

When a very hydrophilic and basic compound is to be analyzed in LC-MS, the choice of mobile phase may not be straightforward. An acidity and a large organic content are preferred to obtain a good ionization efficiency (sensitivity), while an organic content is limited in order to keep an adequate retention on reversed phase. CAPCELL PAK CR makes it possible to use a large organic content in a mobile phase for hydrophilic compounds, such as procain, while only a very small organic content is allowed even for an amide-embedded column, a column considered suitable for such polar compounds.



### Simultaneous analysis of acidic/neutral/basic materials

CAPCELL PAK CR allows the analysis of not only basic compounds but the simultaneous analysis of neutral and acidic compounds. By varying the salt concentration in the mobile phase, it is also possible to independently adjust the retention of the basic compound.



# http://hplc.shiseido.co.jp/e



**JHIJEIDO** 

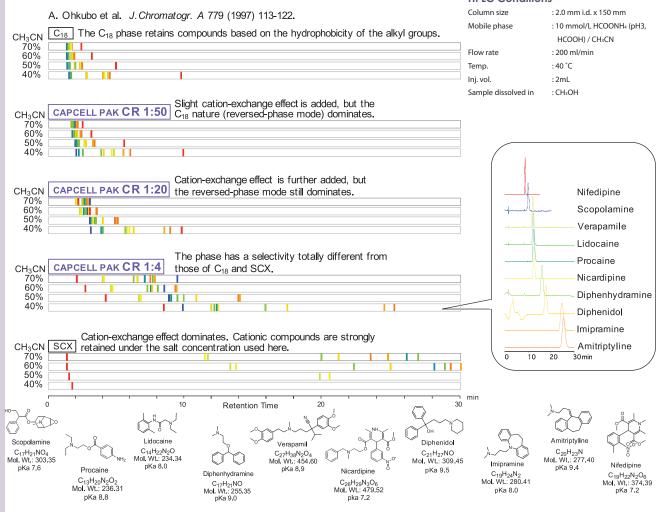
### Choice of three different ratios

Reducing run time and improving the separation profile are possible with the same mobile phase condition by choosing a different mixing ratios available in CAPCELL PAK CR.

		Conditions	
		Column	: Red : CAPCELL PAK CR 1 : 50
Sample : 1. Verapamil 2. Alprenolol 3. Clomipramine 4. Chlorpromazine 5. Ranitidine	2. Alprenolol		Black : CAPCELL PAK CR 1 : 20
	4. Chlorpromazine	omazine	Blue : CAPCELL PAK CR 1 : 4
	5. Kanitidine	Column size	: 2.0 mm i.d. x 150 mm
		Mobile phase	: 10 mmol/L HCOONH4 $$ (adjusted at pH 3.0 with
			HCOOH) / CH <sub>3</sub> CN = 30 / 70
CR 1:20		Flow rate	: 200 μL/min
1 .		Temp.	:40 °C
$1  \frac{23}{M}  \frac{4}{3}$	$\stackrel{5}{\frown}$ CR 1 : 4	Detection	: UV 220 nm
		Inj. vol.	: 2 μL
0 5 10	15 20 25 min	Sample	: Basic compounds 5 types

### **CAPCELL PAK CR** - Atlas-

CAPCELL PAK C<sub>18</sub>, CAPCELL PAK SCX, and three types of CAPCELL PAK CR columns were compared in the separations of ten typical basic compounds. The figures below show structure, pKa value, and change in retention time and selectivity under different mobile phases, for each compound. While CR 1:50 and CR 1:20 generally show selectivity close to those of C<sub>18</sub>, CR 1:4 has selectivity totally different from those of C<sub>18</sub> and SCX. It is advised to utilize the results for method developments of other basic compounds.



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# CAPCELL PAK C<sub>8</sub> DD (Double Durability)

### CAPCELL PAK C<sub>8</sub> DD, Different performances from those of conventional C<sub>8</sub> phases

Alkyl groups attached to silica-based packing material are cleaved when used in an acidic mobile phase for a long period of time. When used in a basic mobile phase, the silica support dissolves thus destroying the column. Durability of reversed phases has a tendency to decrease as the length of the alkyl group decreases. CAPCELL PAK C<sub>8</sub> DD (Double Durability) is a column with unparalleled acidic and basic resistance. The high surface polarity and smaller hydrophobicity, compared to C<sub>18</sub> columns, make this product the best choice for short-time analysis of mixtures with diverse hydrophobicities.

### Excellent durability (pH range : 1.5 – 10)

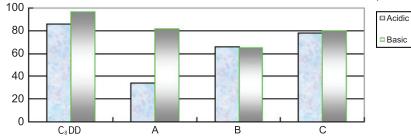
### **Excellent Acidic resistance**

Acidic resistance depends on the concentration of the organic content in a mobile phase. It is known that the higher the concentration of the organic content is, the more difficult it is to cleave the Si-C bond. The test method here uses a mobile phase of pH 1 with no organic solvent, thus representing an extremely harsh acidic condition.

### **Excellent Basic resistance**

Silica is not hydrolysed under acidic conditions, but unstable under neutral to basic conditions. The test method uses a mobile phase of pH 10 which represents an extremely harsh basic condition.

Durability (%)



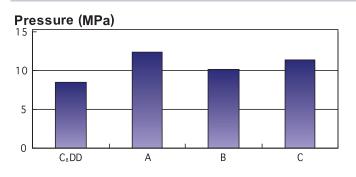
\*Acidic resistance test conditions

Mobile phase	: (A) 2 vol% TFA, H2O, pH1
	(B) 2 vol% TFA, CH <sub>3</sub> CN
	B 65% (20min)>0% (60min)
	>98% (5min)>65% (5min)
Flow rate	: 1.0 mL/min
Temperature	: 60 °C
Detection	: UV 254 nm
Sample	: Uracil, amylbenzene

\*Basic resistance test conditions

Column	: 4.6mm i.d. x150mm
Mobile phase	:4 mmol/L Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> /CH <sub>3</sub> CN=90/10, pH 10.0
Flow rate	: 1.0 mL/min
Temperature	: 50 °C
Detection	: UV 254 nm
Sample	: Uracil, amylbenzene

Low back pressure



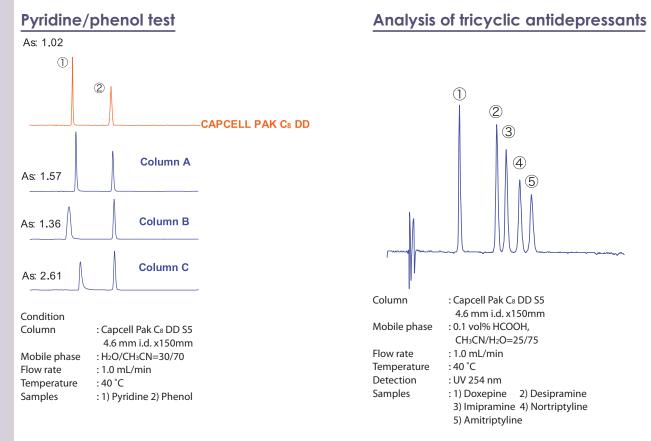
### Conditions

Column	: 4.6mm i.d. x150mm
Mobile phase	:H2O/CH3CN=50/50
Flow rate	: 1.0 mL/min
Temperature	: 40°C



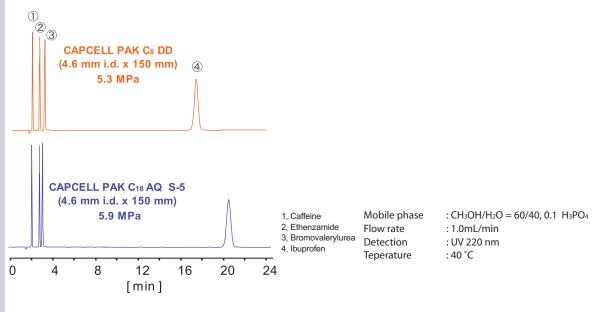
### Excellent peak shape of basic compounds

The polymer coating technology used for CAPCELL PAK C<sub>8</sub> DD resulted in showing excellent peak shapes for basic compounds. The figure down on the left is the comparison with other columns in "pyridine/phenol test", an evaluation method commonly used for silanol effects. A good separation was also obtained for five tricyclic antidepressants, highly basic compounds (down, left).



### Suitable for quickly separating mixtures with diverse hydrophobicity

This is a comparison between CAPCELL PAK  $C_{18}$  AQ, and CAPCELL PAK  $C_8$  DD column. Due to large hydrophobicity corresponding to the long functional group, CAPCELL PAK  $C_{18}$  AQ requires more time to elute ibuprofen that has a relatively high hydrophobicity. On the other hand,  $C_8$  DD column, with its low hydrophobicity, is capable of separating the sample in a much shorter time. In addition, because of the high surface polarity that is equivalent to that of the  $C_{18}$  AQ, highly polar samples are effectively retained.



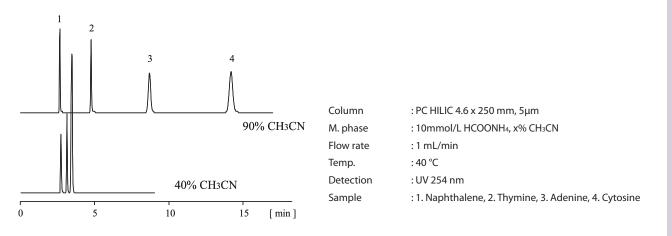
### **Features**

- A silica-based HILIC column with phosphorylcholine (PC) group
- Excellent retention and separation of very polar and hydrophilic compounds
- Large number of theoretical plates and outstanding peak profiles

PC HILIC is a silica-based HILIC column with phosphorylcholine (PC) group. The superhydrophilic character of PC was taken advantage of in preparing an optimum stationary phase for HILIC mode separation. Polar, hydrophilic, and charged compounds, which are hard to handle in reversed-phase mode, are expected to show adequate retention with PC HILIC.

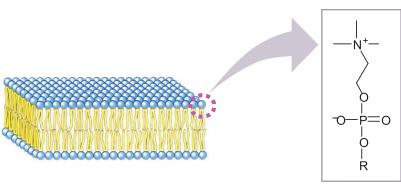
### What is HILIC?

Hydrophilic interaction liquid chromatography (HILIC) is a relatively new LC technique that uses a hydrophilic stationary phase, in most cases, with organic-dominant mobile phase. The elution order of substances in HILIC mode is roughly the reverse of that in reversed-phase mode.



### What is PC?

Phosphorylcholine (PC) is a partial structure of phosphatidylcholine (lecitin), one of the phospholipids forming cell membranes. PC has a betaine structure and shows high hydrophilicity, biocompatibility, and inhibitory effect of protein adhesion. Its superhydrophilic character is suitable to the application as a HILIC phase.



Cell Membrane

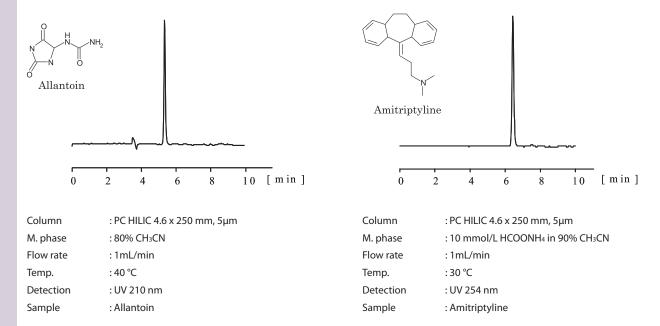
Phosphorylcholine (PC)



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### Strong retention of polar compounds

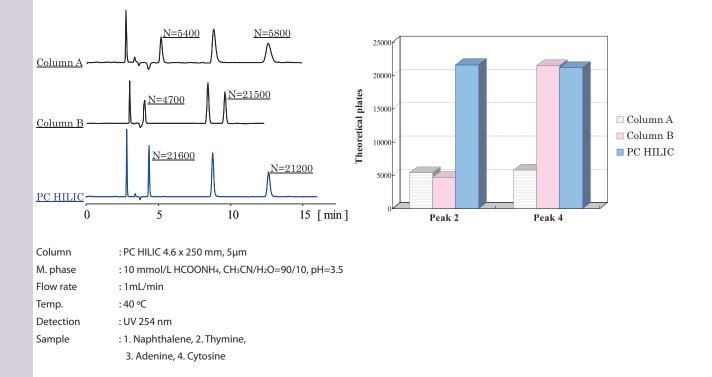
HILIC mode provides another alternative to handle extremely polar and hydrophilic compounds, which are unretainable in reversed-phase (e.g. a chromatogram of allantoin, shown below)



Amitriptyline, a compound with a strong basicity, is often used for discussing the quality of columns. PC HILIC provides excellent peak shapes for basic compounds, too.

### **High Column Efficiency**

PC HILIC shows large numbers of theoretical plates, compared to conventional HILIC columns.



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## **PROTEONAVI** ~For analytical and preparative separation of protein~

### **Features**

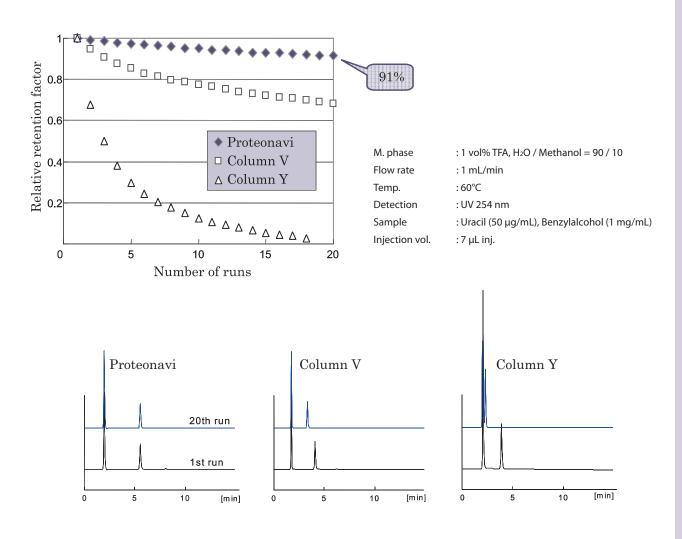
- Excellent acidic durability!
- Minimal protein adsorption! Minimum sample loss!
- Easy to shift from analytical to preparative size!

Adsorption to a stationary phase is one of the most common limiting factors in protein separation in reversed-phase mode. It is generally understood that the irreversible adsorption is caused by denaturing of protein in the hydrophobic phase or a coulombic interaction with silica, a chromatographic support. Proteonavi has overcome the problem by introducing the short four- carbon structure on the silica surface with a unique chemistry. Its synthetic process has already been established for even a large industrial-scale purification.

### **Outstanding Acid Durability**

Acidic hydrolysis is the major cause of loss in performance in reversed phase. Proteonavi's durability under acidic conditions was proven by the accelerated test using 1vol% of trifluoroacetic acid (TFA), a concentration one order of magnitude higher than those used for mobile phases for common protein separations.

**Sequence of process:** After thermal equilibration of column, start the pump. Sixty minutes later, run the sample and record its retention time. Repeat the sequence in every 60 minutes and observe the loss of retention. (For HPLC condition, see below.)

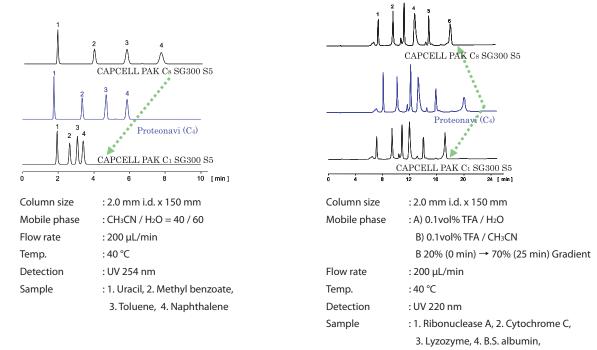


http://hplc.shiseido.co.jp/e/



#### **Specific Retention for Proteins**

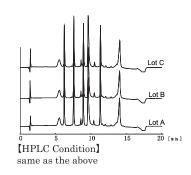
While their retention times of small neutral compounds are supposed to be correlated to amount or length of alykyl chains of stationary phase, that of protein is, in general, governed not only by hydrophobic interaction, but by hydrophilic or ionic interactions. Proteonavi is designed to show large retention specifically for proteins, by precisely controlling its synthetic process.



5. Myoglobin, 6. Ovalbumin

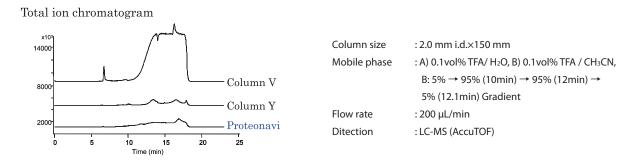
#### **Minimal Lot Variation**

Lot-to-lot variation in separation is often discussed in protein analysis. Proteonavi's silica support and its synthetic procedure are precisely controlled to minimize it.



#### **Reduced Column Bleed**

Total ion chromatograms in LC-MS were compared among competitor's columns under validated gradient conditions. Proteonavi showed the least column bleed, and is expected to provide high purification efficiency in preparative applications, as well as a high sensitivity in LC-MS.



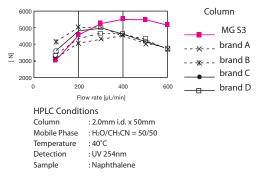
# Columns for LC-MS, High-throughput Screening (HTS) and combinatorial chemistry

A column for LC-MS and HTS should be inert, and background-free. Polymer-coated Capcell Pak was designed to correspond to such a need. In response to different analytes and conditions, Shiseido proudly provides the chromatographers with improved separation tools.

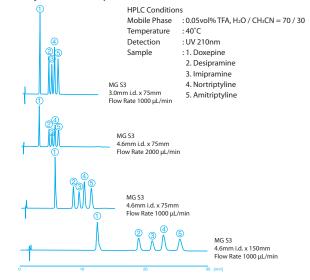
Column	Phases	Characteristics
MGIII	C <sub>18</sub>	Excellent reproducibility of basic compounds under acidic conditions, Low bleeding
MGII	C <sub>18</sub>	Minimized residual silanol, ideal for basic compounds under neutral conditions
MG	C <sub>18</sub>	High-efficiency separation and well-balanced retention for diverse compounds
UG	C <sub>18</sub> , C <sub>8</sub> , Ph, CN	Good retention for hydrophobic compounds, fast separation of basic and polar compounds
ACR	C <sub>18</sub>	Excellent acidic durability (pH 1~10)
AQ	C <sub>18</sub>	Suitable for aqeous mobile phases to be used for polar compounds
DD	C <sub>8</sub>	Superb acidic and basic durability
CR	C <sub>18</sub> +SCX	Provides a large retention for basic compounds
PC HILIC	PC	Excellent retention and separation of very polar and hydrophilic compounds
Proteonavi	$C_4$	Specific retention for proteins and excellent acidic durability

#### High efficiency at higher flow rate, Capcell Pak C18 MG, MGII, MGIII \$3

 $3-\mu m$  CAPCELL PAK shows a large number of theoretical plates at high flow rates



#### Tricyclic antidepressants



#### Semi-microcolumn of 1.0-2.0mm i.d.

Semi-micromcolumn of 1.0-2.0mm i.d. is extremely effective in the high sensitivity analysis. The benefits of reducing the inner diameter of the column are:

- Improvement in absolute sensitivity of a concentration-sensitive detector
- Reduction of mobile phase consumption
- Reduction of baseline noise in LC-MS
- Small Amounts of packing material result in a good dynamic range for substances causing an irreversible adsorption on a stationary phase

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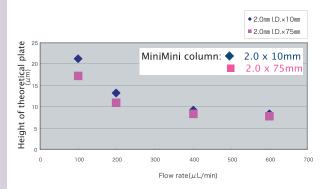


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# MiniMini columns Low pressure•Fast separation•Long lifetime 2.0 mm i.d. x 10 mm

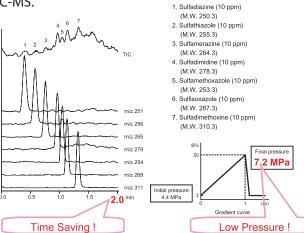
1.5 mm i.d. x **10 mm** 

#### Designed for fast separation



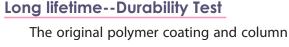
# Low-pressure and Rapid LC-MS of seven sulfa drugs

Simultaneous analysis of seven sulfa drugs was attempted with a MiniMini column (1.5mm i.d. x 10mm), which provided high-resolution separation with a low pressure at a high flow rate in LC-MS.



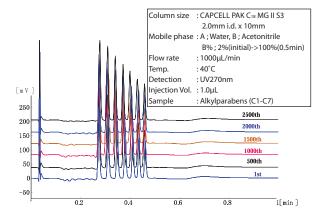
#### Columns for combinatorial chemistry

The unique performance of high efficiency and low pressure is attributed to a narrow distribution of particle diameter. Capcell Pak is suitable to preparative separation in combinatorial chemistry.

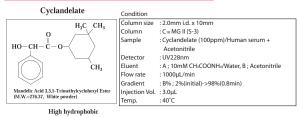


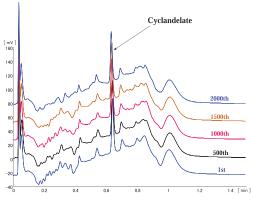
packing technology bring the excellent durability and lifetime.

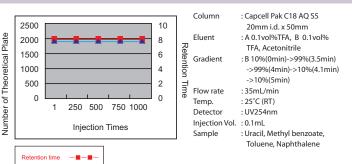
# Durability test by continuous operations of Alkylparabens



#### Durability test by continuous operations of a serum-derived sample







-

1000-time durability test (10mm i.d. x 50mm)

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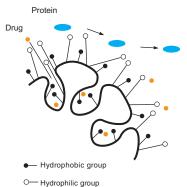
#### CAPCELL PAK MF is a mixed-function phase for direct analysis of drugs contained in serum, plasma or other body fluids.

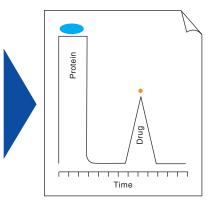
- · Direct injection of body fluids
- High recovery of drugs
- Very reproducible

- Higher sensitivity using column switching method
- Available as analytical columns and guard cartridges
- Available in four different functional groups

Using the same polymer coating technology, CAPCELL PAK MF is designed to allow the direct injection of serum or other biological fluids, without tedious and time-consuming pretreatment procedures. This is done by bonding hydrophilic polyoxyethylene groups and hydrophobic groups (phenyl, C<sub>8</sub>, C<sub>1</sub>, SCX) to the polymer coated silica. This allows proteins to pass through the column and elute in the void volume due to restricted access to the surface of the packing, while retaining a drug of interest on the small hydrophobic phase. CAPCELL PAK MF columns have three main functions.

- 1) Protein removal
- 2) Sample concentration
- 3) Sample analysis

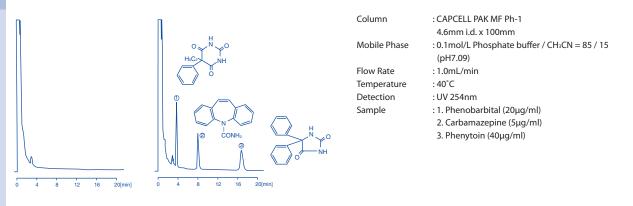




#### **EXCELLENT DURABILITY**

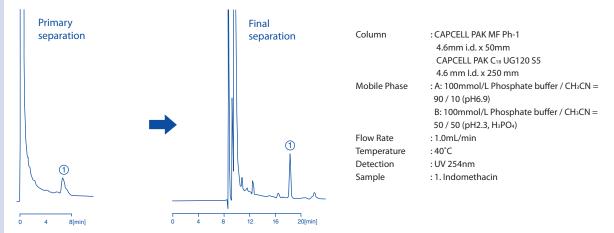
Analytical colu	mn	Cartridge co	blumn
Column	: CAPCELL PAK MF Ph-1 4.6mm i.d. x 100mm	Column	: CAPCELL PAK MF Ph-1 Guard Cartridge 4.0mm i.d. x 10mm
Mobile Phase	Nobile Phase : 0.1mol/L Phosphate buffer / CH <sub>3</sub> CN = 90 / 10 (pH6.98)		: 100mmol/L Phosphate buffer (pH6.9) / CH <sub>3</sub> CN = 90 / 10
Flow Rate	: 1.0mL/min	Flow Rate	: 1.0mL/min
Temperature	:40°C	Temperature	: 40°C
Detection	: UV 254nm	Detection	: UV 254nm
Sample	: Trimethoprim	Sample	: Phenytoin
CH <sub>3</sub> O CH <sub>3</sub> O 1st	5.19 $ \begin{bmatrix} so \\ N \\ NH_2 \end{bmatrix} $ 500th accumulated inje volume=10mL k' = 5.20 $ \begin{bmatrix} k' = 5.20 \end{bmatrix} $	on 	100th (5000µl) 75th (3750µl) 50th (2500µl) 25th (1250µl) 1st (50µl) 2 4 6 8 10 12(min)

#### Direct Injection of Serum or Plasma / Single Column System ANTI-EPILEPTIC DRUGS

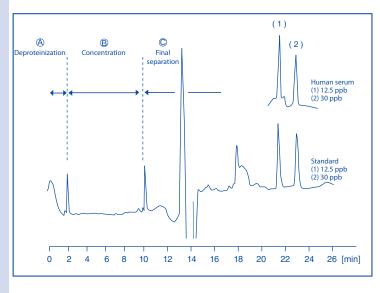


### Direct Injection of Serum or Plasma / Dual Column-Switching System

#### INDOMETHACIN



#### Analysis of Carbamazepine and Phenytoin in Human Serum/Triple Column-Switching System



Conditions of Pi	rimary separation and Focusing			
Column	: CAPCELL PAK MF Ph-1 Cartridge			
	4.0mm i.d. x 10mm			
	CAPCELL PAK C18 UG120 S5			
	1.5 mm l.d. x 35 mm			
Mobile Phase	: 100mmol/L Phosphate buffer (pH7) / CH3CN			
	= 90 / 10			
Flow Rate	: 0.5 mL/min - 0.25mL/min			
Temperature	: 40°C			
Detection	: UV 254nm			
Conditions of Final separation				
Column	: CAPCELL PAK C18 UG120 S5			
	1.5 mm i.d. x 250 mm			
Mobile Phase	: 100mmol/L Phosphate buffer (pH7) / CH₃CN			
	= 70 / 30			
Flow Rate	: 0.1 mL/min			
Temperature	: 40°C			
Detection	: UV 254nm			
Sample	: 1.Carbamazepine			
	2. Phenytoin			

ditions of Primary constation and Eocusing

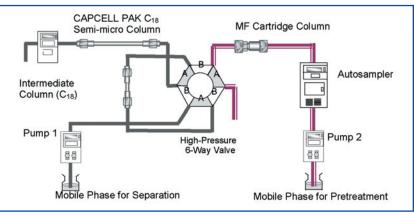
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MF

### Trace Analysis of Drugs in Serum and Plasma/ Triple Column-Switching System

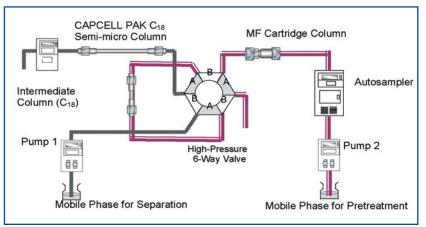
This analytical format consists of a CAPCELL PAK MF cartridge, a small intermediate column and a main analytical column.

#### DEPROTEINIZATION



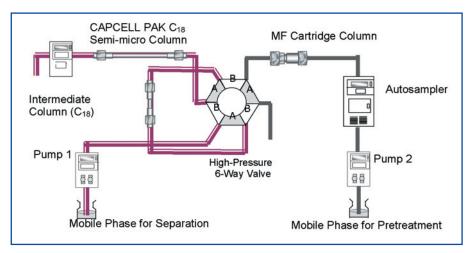
This system using an intermediate column is designed to save run time when a 1.5mm i.d. or smaller column is used. Proteins pass through the MF column, while drugs are retained.

#### FRACTIONATION AND CONCENTRATING



Drugs are transferred and concentrated in the intermediate column.

#### FINAL SEPARATION



The concentrated drug is separated in a final semi-micro column, An increased sensitivity without any loss in chromatographic efficiency can be obtained.



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# SHISEIDO CHIRAL COLUMNS

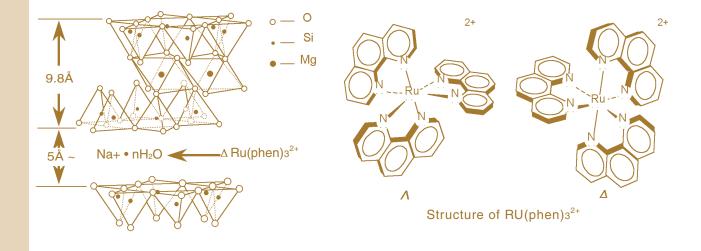
- High efficiency and stability against pressure
- Choice of normal or aqueous mobile phase condition
- Exceptional enantioselectivity for acidic, basic and neutral chiral compounds
- Stable under a wide temperature range
- High loadability combined with long column lifetime



Based on 5-µm spherical sodium magnesium silicate particles, Ceramospher phases RU-1 and RU-2 are novel materials for chiral HPLC separations. Chiral separation is accomplished by an optically-active ruthenium complex that has been ion exchanged with sodium ions in the original clay material. Ceramospher phases show excellent selectivity for a wide variety of chiral samples.

Ceramospher has the remarkable loadability due to its large specific surface area (pore size 4 nm, 300m<sup>2</sup>/g). The advantage is more pronounced when applied at preparative scales. Both phases utilize simple eluents.RU-1 is used under non-aqueous mobile phases, whereas RU-2 is compatible also with aqueous mobile phases.





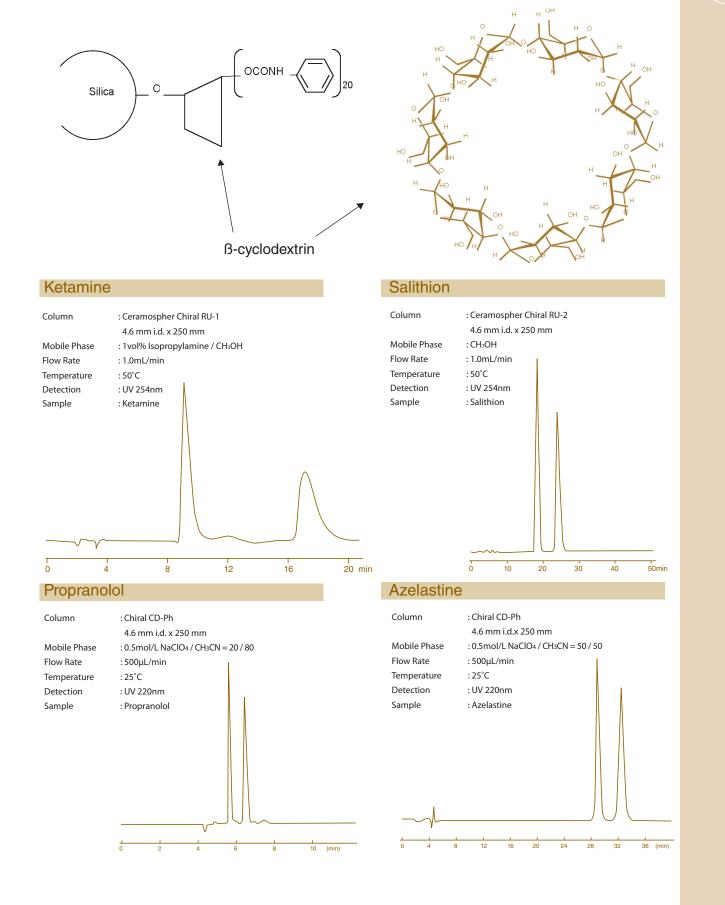


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### CHIRALCD-Ph

The Chiral CD-Ph utilizes precisely classified high-purity silica as its support, to which phenylcarbamated ß-cyclodectrin is chemically bonded. A large number of theoretical plates is usually achieved. The combined use with the Ceramosphers, covers a wide variety of chiral compounds.

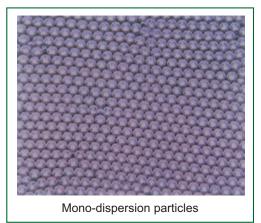


http://hplc.shiseido.co.jp/e/

## SUGAR COLUMNS

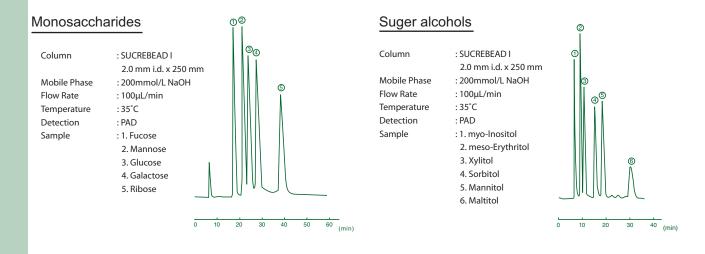
### **SUCREBEAD I**

Sucrebead I is targeted exclusively to carbohydrate analysis. It is based on styrene/divinylbenzene polymer support. Its mono-dispersion character was given by the unique two-step swelling method used for the polymerization, and is advantageous in chromatographic separation.



- Excellent durability
- Efficient peaks at low pressure

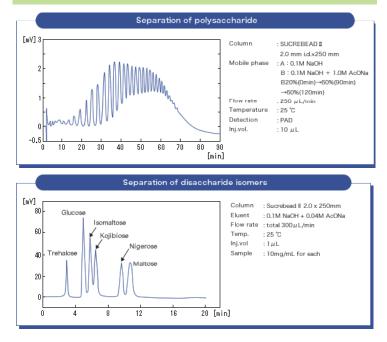
# Sucrebead I, in combination with the pulsed amperometric detector (PAD), provides a high-sensitivity carbohydrate analysis.



### **SUCREBEAD II**

Sucrebead II is developed to analyze carbohydrates by using anion-exchanging polymer as a stationary phase. Sucrebead II enables operation under high pH range and high selectivity with carbohydrates.

#### Optimum for analyzing oligosaccharides and polysaccharides



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#### For analysis of oxidative stress markers

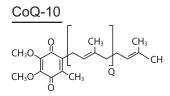
Oxidization stress by active oxygen and free radicals is considered to be closely related to the development of symptoms of various geriatric diseases and the progression of aging. A method of measuring stress accurately and easily is in great demand.

As an index used for diagnosis and treatment, coenzyme Q10, vitamin C, vitamin E, and glutathione that indicate anti-oxidization effect in the human body are receiving great attention.

Among them, reduced coenzyme Q10 is sensitive to oxidization by active oxygen and free radicals, and produces oxidized coenzyme Q10. Therefore, the ratio of oxidized coenzyme Q10 to total coenzyme Q10 may be a sensitive marker for oxidization stress.

Shiseido, with its optimum catalytic column (reduction column) for high-sensitivity analysis of quinone derivatives, developed an analytical system for CoQ10 by combining an electrochemical detector and a reduction column.

1.S.Yamashita,Y.Yamamoto:Simultaneous Detection of Ubiquinol and Ubiquinone in Human Plasma as a Marker of Oxdative Stress,Anal.Biochem.,250,66-73(1997)



Column size

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About Shiseido

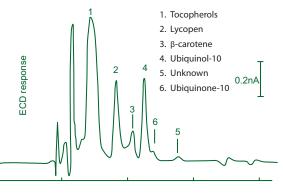
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#### For analysis of oxidative stress markers

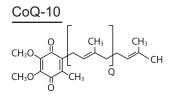
Oxidization stress by active oxygen and free radicals is considered to be closely related to the development of symptoms of various geriatric diseases and the progression of aging. A method of measuring stress accurately and easily is in great demand.

As an index used for diagnosis and treatment, coenzyme Q10, vitamin C, vitamin E, and glutathione that indicate anti-oxidization effect in the human body are receiving great attention.

Among them, reduced coenzyme Q10 is sensitive to oxidization by active oxygen and free radicals, and produces oxidized coenzyme Q10. Therefore, the ratio of oxidized coenzyme Q10 to total coenzyme Q10 may be a sensitive marker for oxidization stress.

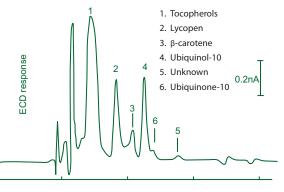
Shiseido, with its optimum catalytic column (reduction column) for high-sensitivity analysis of quinone derivatives, developed an analytical system for CoQ10 by combining an electrochemical detector and a reduction column.

1.S.Yamashita,Y.Yamamoto:Simultaneous Detection of Ubiquinol and Ubiquinone in Human Plasma as a Marker of Oxdative Stress,Anal.Biochem.,250,66-73(1997)



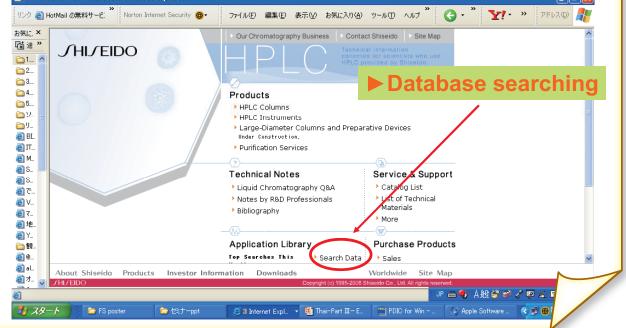
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