

# Column catalog

High performance columns for HPLC



## Introduction

CERI serves in creating harmony between human beings, chemicals, and environments.

Chemicals Evaluation and Research Institute, Japan (CERI) engages in a wide scope of activities related to chemicals, including testing, analysis, evaluations, and research and development. Our ultimate objective is to further the sound development of industry and enhance people's lifestyles by upgrading the quality of chemicals and assuring their safety while at the same time emphasizing environmental preservation and the protection of health.

As an independent, unbiased organization that is committed to keep chemicals and the environment in balance, CERI plays a supporting role in the research and development projects of clients utilizing its services. Working in state-of-the-art facilities, our highly qualified professionals conduct tests, analysis, research, and studies in the field of chemicals.

Chromatography plays an important role in the analysis field. CERI has been developing convenient and high performance columns based upon our experiences as users of chromatographs since 1984. During this time we have developed and supplied products for HPLC and GC including G-column for gas chromatography, wide-bore, open tubular columns, and L-column and L-column2 for high performance liquid chromatography. We respond rapidly to new technology and help your test, research and development.



CERI Tokyo Laboratory

## Website Guide

You may browse a wealth of information including technical product information, applications data and seminar schedules on our website. You may also order columns directly from the website.

<http://www.cerij.or.jp>



## Quality control

Our quality management system is assessed and verified to be in accordance with ISO 9001:2000 for the design, development, production and supply of columns for gas chromatography and high performance liquid chromatography. We maintain probing inspection systems of raw materials and production processes at each step. All finished products are thoroughly inspected. These steps ensure a tight control of quality.

We are continuously refining our operations to supply products for the satisfaction of our customers.



Certificate of registration

## Lineup

		Particle size (μm)	Pore size (Å)	Surface area (m <sup>2</sup> /g)	Carbon conten (%)	USP category
<b>L-column2</b>	<b>L-column2 ODS</b>	5, 3	120	340	17	L 1
<b>L-column</b>	<b>L-column ODS</b>	5, 3	120	340	17	L 1
	<b>L-column ODS-V</b>	5	120	340	17	L 1
	<b>L-column ODS-P</b>	5	300	150	9	L 1
	<b>L-column C8</b>	5	120	340	10	L 7

# L-column series

The column which pioneered rugged high-performance and convenience

## Media development concept

Hydrophobic interaction is the primary mechanism of solute retention on ODS columns. A correlation exists between the hydrophobicity of the solute, expressed as log Pow, and the retention factor, k. Therefore elution behavior should be easily understood and predicted; however, secondary interactions between residual silanol groups on the base silica and trace metal impurities complicate elution behavior. Much skill is often required for mobile phase selection and method development.

**L-column** ODS has been designed with a highly predictable separation mechanism by improving the packing material and excluding secondary interactions.

## L-column

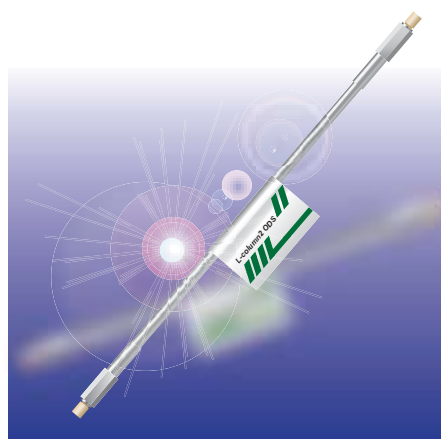
We developed a high temperature vapor-phase end-capping technique in 1990 which was patented in Japan, the United States and Europe (JP 2611545, USP 5,134,110 and EP 0 443 860 B1, respectively). This method dramatically improved the reaction efficiency of end-capping so that very few residual silanols and metallic impurities remain to effect the separation. The **L-column ODS** demonstrates near ideal ODS performance and was placed as a pioneer among commercial HPLC columns.

## L-column2

Recently, analytical sensitivity has increased with the wide use of LC/MS/MS and improvement of analytical precision is demanded. A very small level of silanol that was not previously an issue, now is detectable in its impact on the separation of trace components. Again CERI developed an improved end-capping technique to address this need and the **L-column2 ODS** was introduced in 2007. This new material demonstrates excellent results under demanding conditions.

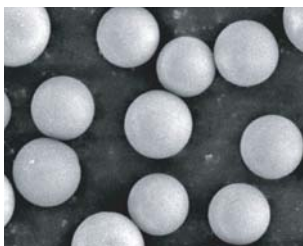
- Lowest adsorption  
Basic compounds do not adsorb to **L-column2 ODS** so their peaks are sharper.
- Highest reproducibility  
The very low concentration of metal impurities provides good quantitative results, even with trace amounts of chelating compounds.
- Excellent durability  
**L-column2 ODS** can be used in a wide range of mobile phases and temperatures maintaining performance over an extended lifetime.

Strict standards are set for each packing material lot. Thorough quality control procedures check each variable to maintain highly uniform lots. The lot transcript as well as the individual column test results are attached to each **L-column2 ODS**.

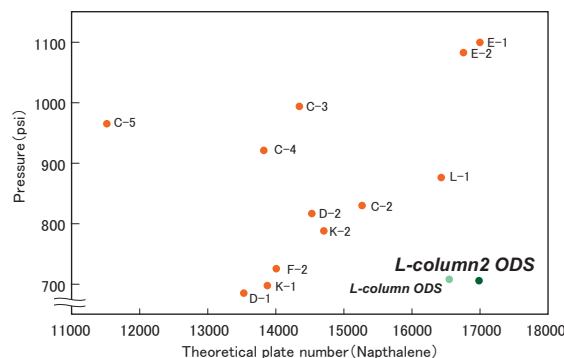


## Base silica gel

High purity silica gel, in which metal impurities are reduced to the absolute limit, is used as the starting material, facilitating analysis of chelating compounds. The material has a narrow particle size distribution and a perfect spherical shape which results in lower column pressure drop and higher theoretical plate number (Fig. 1).



Specifications silica particles



[Analytical conditions]  
Column: 4.6 × 150 mm (C18, 5 μm)  
Mobile phase: CH<sub>3</sub>CN/H<sub>2</sub>O (60/40); Flow rate: 1 mL/min; Temp.: 40°C

Fig. 1 Relationship between column pressure and theoretical plate number

Note: 1 psi = 6.9 × 10<sup>-3</sup> MPa

# L-column2 ODS

Next generation high performance silica-based ODS column

Average particle size 3  $\mu$ m, 5  $\mu$ m  
Average pore size 120 Å  
Range of pH 2~9  
USP category L 1

**L-column2 ODS** exceeds even the high performance of **L-column ODS** by virtue of its advanced new end-capping method. It accommodates the analysis of acidic, basic and chelating compounds. It exceeds expectations as the column of first choice for new method development.

## Characteristics of L-column2 ODS

- Sharper peaks for acidic, basic and chelating compounds due to extremely low silanol adsorption.
- Economical due to high durability in a wide range of pH and temperature.
- Uniform lot to lot reproducibility of analyses due to extensive quality control measures.
- Excellent peak shapes in both acetonitrile/water and methanol/water mobile phases makes **L-column2 ODS** convenient to use.
- A wide range of column sizes (0.075~10.0 mm I.D.) accommodates any instrument and application requirements.

## Residual silanol groups

The level of residual silanol groups is measured by FT-IR spectrum. The spectra of C18 without end-capping and the fully end-capped L-column2 ODS are shown. The spectrum region for C-H and O-H provides quantitative information as well as qualitative identification. FT-IR spectra show virtually no presence of silanol groups on L-column2 ODS. In addition, the spectrum region for O-H (the right spectra) shows that L-column2 ODS has the least residual silanol groups of any column tested.

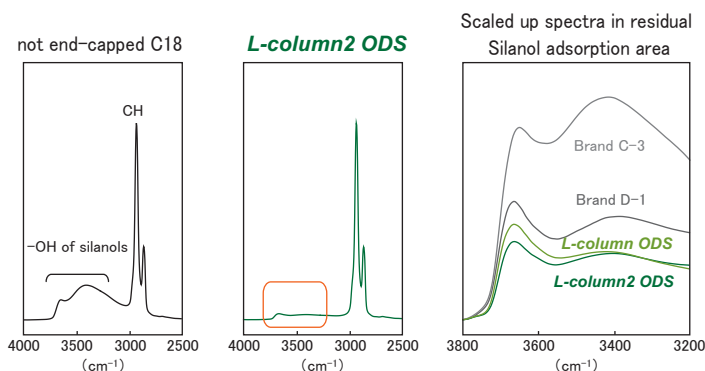
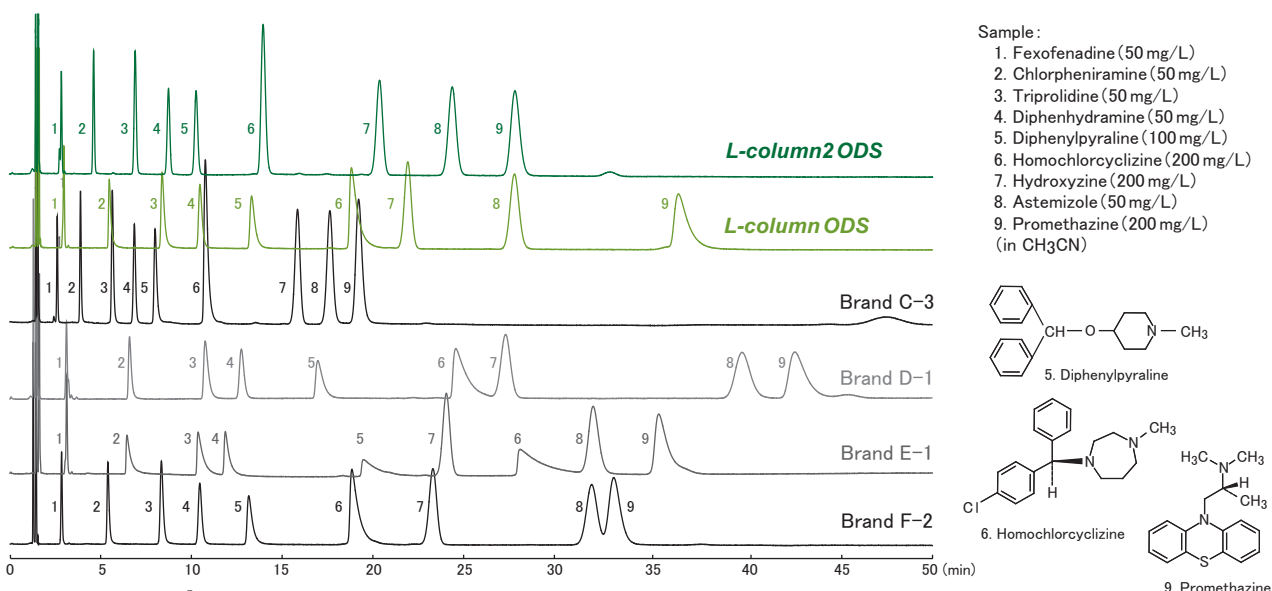


Fig. 2 FT-IR spectra for **L-column2 ODS** and other ODS columns

## Improved peak shape

Peaks of basic compounds may show tailing caused by adsorption to residual silanol groups. When this interaction is eliminated through perfect end-capping, the essential ODS partition behavior appears. Peaks are sharp and good, and repeatable data can be obtained, even for trace components.

All ODS columns are not created equal. The column that provides sharp peaks for basic compounds (Fig. 3) as well as acidic compounds (Fig. 4) and accommodates any analysis should be evaluated first!



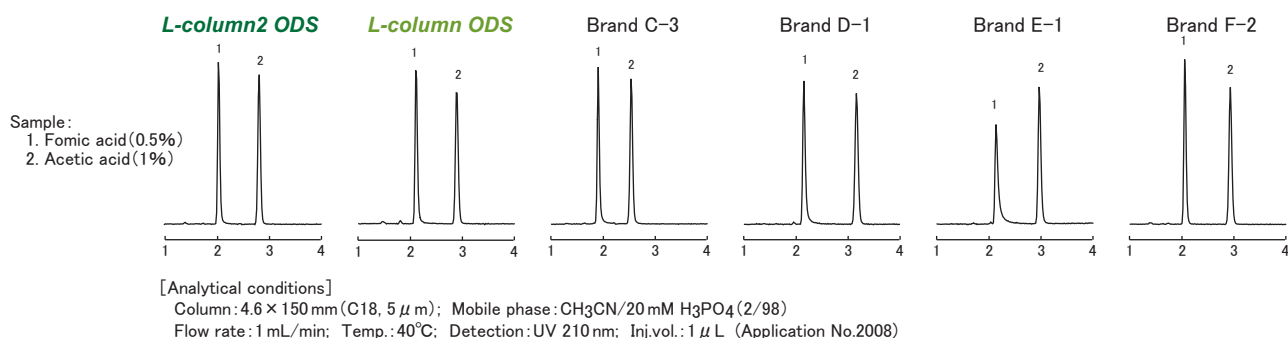
[Analytical conditions]

Column: 4.6 × 150 mm (C18, 5  $\mu$ m); Mobile phase: CH<sub>3</sub>CN/25 mM Phosphate buffer pH7 (40/60)  
Flow rate: 1 mL/min; Temp.: 40°C; Detection: UV 220 nm; Inj.vol.: 1  $\mu$ L (Application No. L2006)

※USP tailing factor

Sample	L-column2 ODS		L-column ODS		Brand C-3		Brand D-1		Brand E-1		Brand F-2	
	tR	T.F.*	tR	T.F.*	tR	T.F.*	tR	T.F.*	tR	T.F.*	tR	T.F.*
5. Diphenylpyraline	10.107	1.080	13.104	2.037	7.876	1.631	16.702	2.319	19.148	2.579	12.938	2.106
6. Homochlorcyclizine	13.737	1.155	18.513	3.082	10.601	1.969	24.182	3.355	27.642	N.D.	18.533	2.928
9. Promethazine	27.382	1.119	36.225	1.995	18.912	1.227	42.561	1.828	35.157	1.958	32.734	1.267

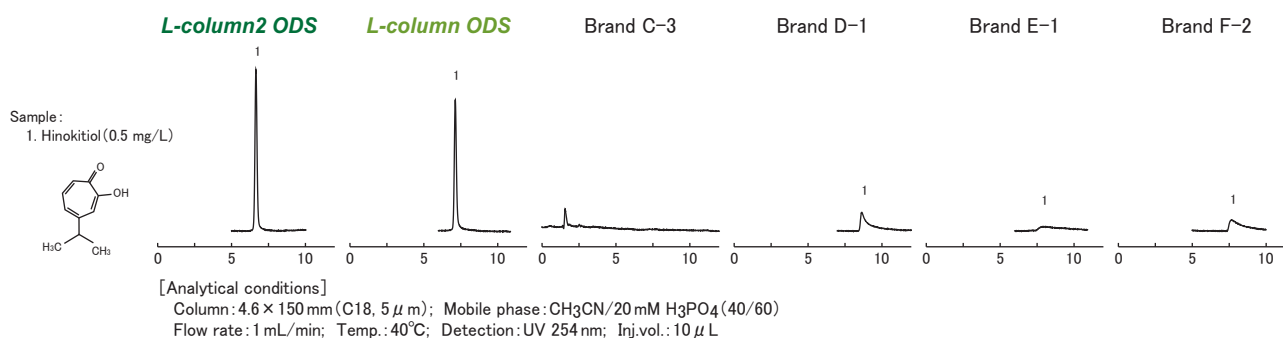
Fig. 3 Basic compounds (Antiallergy drugs)



※USP tailing factor

Sample	t <sub>R</sub>	N	T.F. <sup>※</sup>	t <sub>R</sub>	N	T.F. <sup>※</sup>	t <sub>R</sub>	N	T.F. <sup>※</sup>	t <sub>R</sub>	N	T.F. <sup>※</sup>	t <sub>R</sub>	N	T.F. <sup>※</sup>	t <sub>R</sub>	N	T.F. <sup>※</sup>
1. Formic acid	2.047	11647	1.158	2.107	9950	1.249	1.900	9450	1.205	2.149	8273	1.364	2.137	5285	2.034	2.054	10768	1.164
2. Acetic acid	2.813	15087	1.061	2.892	12987	1.134	2.537	11693	1.098	3.169	12320	1.091	2.970	13621	1.239	2.932	12855	1.091

Fig. 4 Acidic compound (Formic acid, Acetic acid)



※USP tailing factor

Sample	t <sub>R</sub>	N	T.F. <sup>※</sup>	t <sub>R</sub>	N	T.F. <sup>※</sup>	t <sub>R</sub>	N	T.F. <sup>※</sup>	t <sub>R</sub>	N	T.F. <sup>※</sup>	t <sub>R</sub>	N	T.F. <sup>※</sup>	t <sub>R</sub>	N	T.F. <sup>※</sup>
1. Hinokitiol	6.640	9858	1.158	7.167	10497	1.277	N.D.	N.D.	N.D.	8.647	2150	6.638	7.910	287	3.299	7.686	511	3.661

Fig. 5 Chelating compound (Hinokitiol)

## Improved durability

A durability test was carried out under high temperature conditions which accelerate deterioration of columns. **L-column2 ODS** was stable for the longest time. Although it is silica-based, it shows excellent durability even under alkaline conditions due to the extremely dense end-capping. Temperature of 40~50°C may be used routinely and temperatures as high as 80~90°C have been tested with superior results.

### ■ Accelerated acidic mobile phase lifetime test (Fig. 6)

Under acidic conditions, below pH 1, both the end-capping group and the ODS group are hydrolyzed. Retention time decreases with the decrease of ODS groups. Resolution decreases with the progression of the hydrolysis.

**L-column2 ODS** resists hydrolysis even under these harsh conditions to maintain retention and resolution for an extended lifetime.

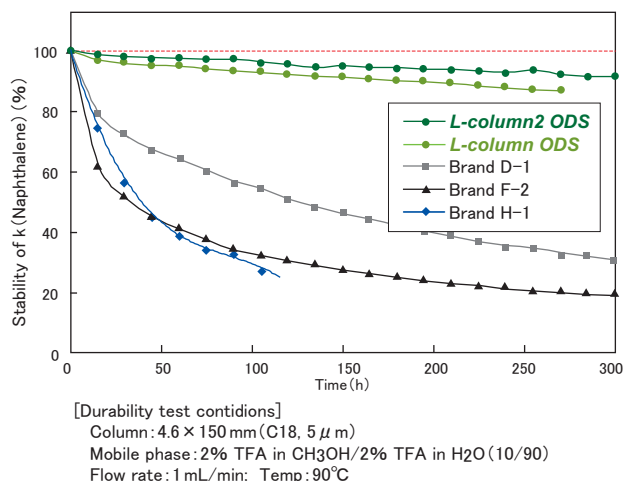


Fig. 6 Accelerated durability test with acidic mobile phase (under pH1)

### ■ Accelerated alkaline mobile phase lifetime test (Fig. 7)

Dissolution of the base silica is accelerated in alkaline mobile phase. Efficiency (theoretical plate number) drops suddenly in these conditions. **L-column2 ODS** has excellent durability under high pH conditions (pH 10) due to the protection of the silica surface afforded by the advanced end-capping process.

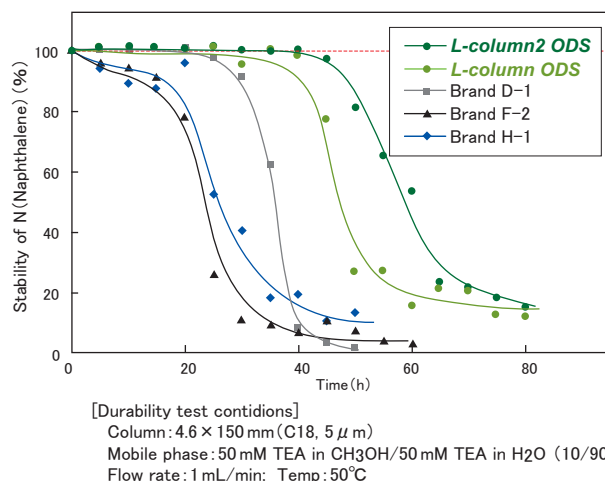


Fig. 7 Accelerated durability test with alkaline mobile phase (pH10)



Excellent reproducibility

Variation between product lots due to residual silanol groups is prevented by extensive end-capping and tested through measuring the retention time of difficult compounds (Fig. 8 and Fig. 9). This delivers high analytical precision. Comprehensive quality control of **L-column2 ODS** product lots results in columns you may depend upon for your analyses.

■ Certification

Specifications and test results of each product lot as well as test results for each column are supplied with the column. In addition we support method validation by supplying columns from three different media lots.

Items of quality assurance

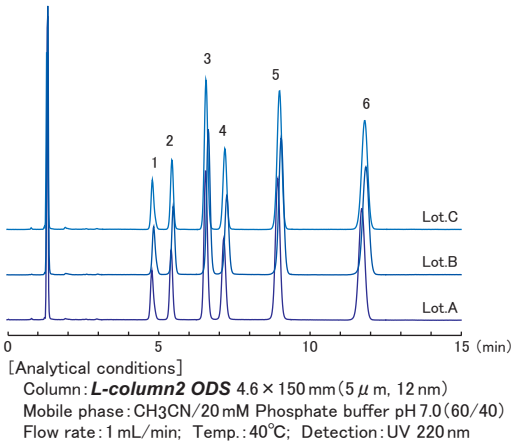
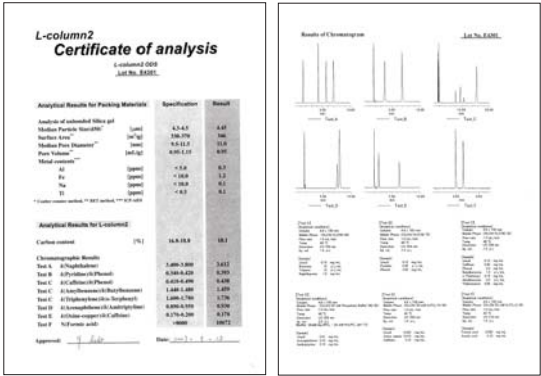
Physical properties of the base silica gel:

- Median particle size (d50)
- Surface area
- Median pore diameter
- Pore volume
- Metal content

Properties of the media:

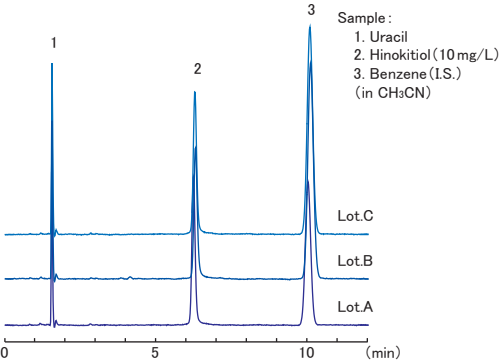
- Capacity factor of a standard
- Adsorptive property for basic compounds
- Adsorptive property for acidic compounds
- Adsorptive property for chelating compounds
- Surface hydrophobicity
- Planar and non-planar compounds separation performance, etc.

Quality of packing as measured by theoretical plate number



Sample	Retention time (min)			CV (%)
	Lot.A	Lot.B	Lot.C	
1. Doxepin	5.115	5.180	5.134	0.647
2. Imipramine	5.796	5.868	5.823	0.626
3. Amitriptyline	7.008	7.103	7.022	0.725
4. Mianserin	7.647	7.755	7.687	0.707
5. Chlorpheniramine	9.546	9.669	9.609	0.640
6. Trimipramine	12.50	12.64	12.60	0.575

Fig. 8 Reproducibility between product lots (Basic drugs, antidepressants)



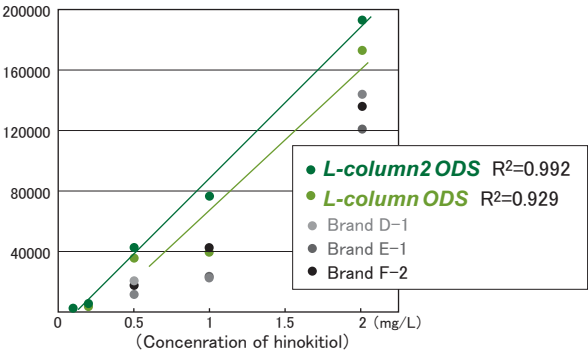
[Analytical conditions]  
Column: **L-column2 ODS** 4.6 × 150 mm (5 μm, 12 nm)  
Mobile phase: CH<sub>3</sub>CN/20 mM H<sub>3</sub>PO<sub>4</sub> (40/60)  
Flow rate: 1 mL/min; Temp.: 40°C; Detection: UV 210 nm; Inj.Vol.: 1 μL

Sample	Retention time (min)			CV (%)
	Lot.A	Lot.B	Lot.C	
2. Hinokitiol	6.277	6.294	6.265	0.549

Fig. 9 Reproducibility between product lots (Coordinate compound, Hinokitiol)

■ Improved quantitative sensitivity

Detection limits improve with improved end-capping. At low analyte concentration, the peak may be lost to adsorption on the column. On some columns (Fig. 10), the peak is not detected under 0.2 mg/mL due to severe tailing.



[Analytical conditions]  
Column: 4.6 × 150 mm (C18, 5 μm)  
Mobile phase: CH<sub>3</sub>CN/20 mM H<sub>3</sub>PO<sub>4</sub> (40/60)  
Flow rate: 1 mL/min; Temp.: 40°C; Detection: UV 210 nm  
Inj.Vol.: 10 μL; Sample: Hinokitiol (10 mg/L in CH<sub>3</sub>CN)

Fig. 10 Quantitative analysis (Hinokitiol)

## Separation differences by mobile phase

Methanol is often preferred as a mobile phase when separating basic drugs and other analytes with adsorptive tendencies because they tend to show less tailing in methanol than in acetonitrile (Fig. 11 and Fig. 12). However, the high viscosity and resulting high back pressure of methanol-water solutions, as well as the higher uv absorbance of methanol, may make acetonitrile the organic solvent of choice. **L-column2 ODS** shows good peak shape and separation in both methanol/water and acetonitrile/water mobile phases due to the advanced end-capping.

It must be noted that elution order may be impacted by choice of organic modifier and buffer.

**Methanol** Mobile phase: CH<sub>3</sub>OH/10 mM Ammonium acetate (65/35)

**Acetonitrile** Mobile phase: CH<sub>3</sub>CN/10 mM Ammonium acetate (40/60)

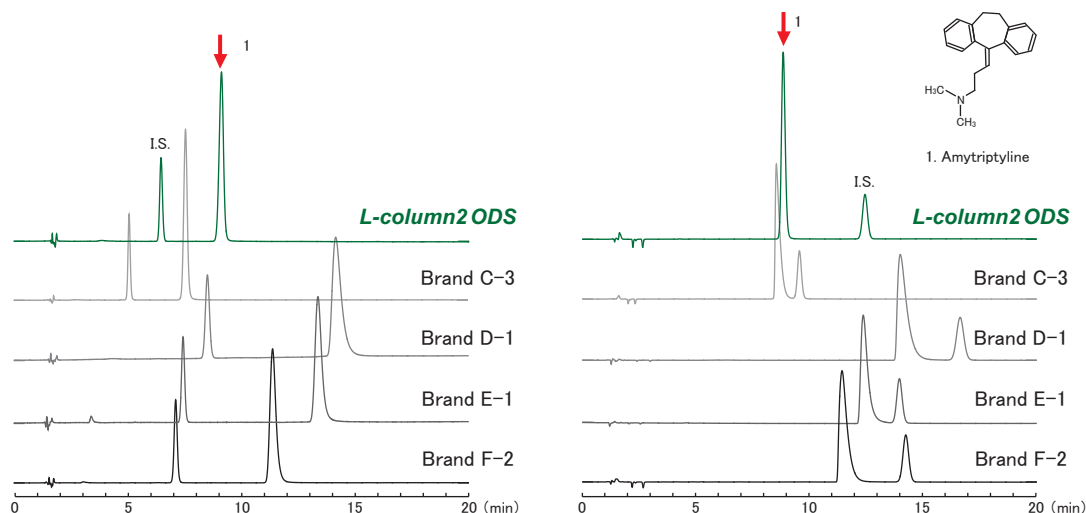


Fig. 11 Separation differences by mobile phase (Amytriptyline)

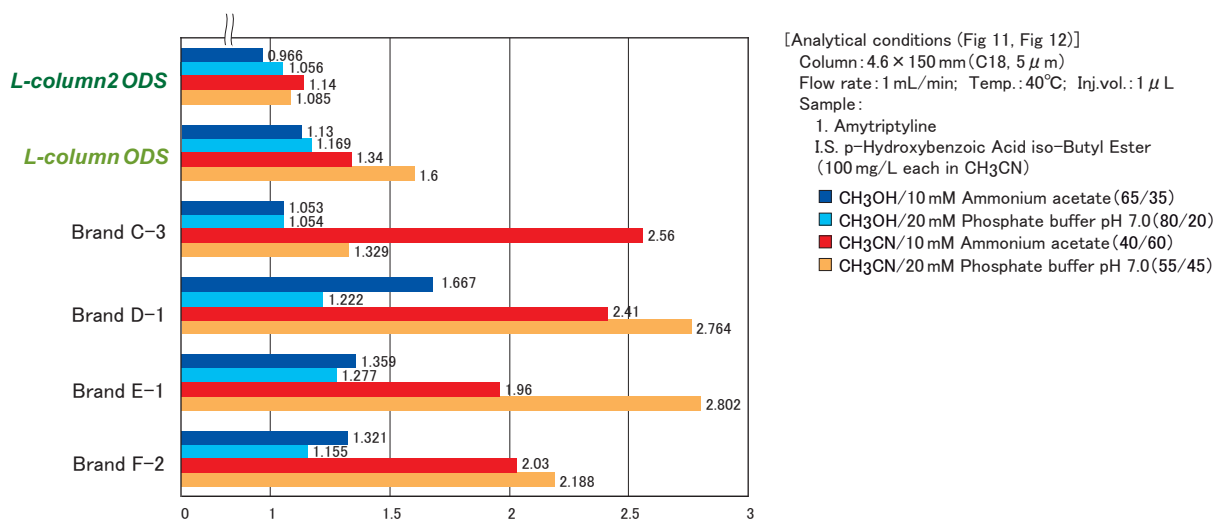
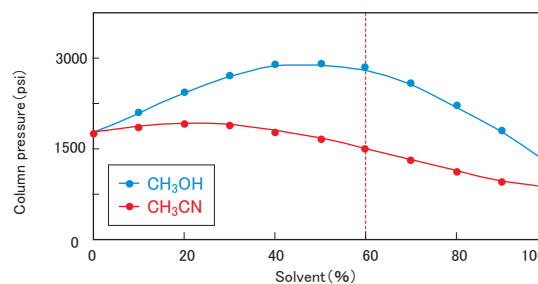


Fig 12. Difference of tailing factor by kind of solvent (Amytriptyline)

## Organic solvents and column pressure

Column pressure depends upon the selection of organic solvent in the mobile phase. Column pressure using acetonitrile is half that of methanol at 40°C and a ratio of 60% (Fig. 13). In fact, acetonitrile in the mobile phase creates a low back pressure in a wide range of ratios with water, which coupled with a low absorbance at low UV wavelength makes it a most useful HPLC solvent. **L-column2 ODS** can use this popular solvent without any tailing issues as described above.



[Analytical conditions]

Column: **L-column2 ODS** 4.6 × 150 mm (3 μm, 12 nm)  
Flow rate: 1 mL/min; Temp.: 40°C

Fig. 13 Difference of column pressure by kind of solvent

## Characteristics of 3 $\mu$ m particle

Although the most popular particle size for reversed phase HPLC remains 5  $\mu$  m, many manufacturers have introduced 3  $\mu$  m and sub 2  $\mu$  m particles into the market for their ability to improve separation and shorten analysis time through use of shorter column length made possible by higher efficiencies and higher optimum flow rates.

### ■ Efficiency and resolution of 3 $\mu$ m particle

Since the theoretical plate number is in inverse proportion to particle diameter, the theoretical plate number of 3  $\mu$  m particle is one point seven times as high as that of 5  $\mu$  m particle when both column sizes are the same (Table 1 and Fig. 14).

Table 1 Relationship of particle size, theoretical plate number and column pressure

Particle size ( $\mu$ m)	Theoretical plate number	Column pressure
2	$\frac{5}{2}$	$\frac{25}{4}$
3	$\frac{5}{3}$	$\frac{25}{9}$
5	1	1
10	$\frac{1}{2}$	$\frac{1}{4}$

In the analysis of an enzyme digest of BSA, 47 peaks were detected using a 5  $\mu$  m particle size while 67 peaks (1.4  $\times$  as many peaks) were detected using a 3  $\mu$  m particle. This is due to the increased efficiency of 3  $\mu$  m particles. Since peaks become sharper and higher, sensitivity increases (Fig. 15). Changing the particle size from 5  $\mu$  m to 3  $\mu$  m is the most effective way to improve resolution using the same conditions and analysis time.

### ■ Column pressure

Columns must be used below their maximum rated pressure to prolong the life of the column. The maximum pressure depends upon the packing pressure and the physical strength of the silica gel (Table 2). CERI employs high pressure packing technology to increase the pressure rating for **L-column2 ODS**. The spherical silica exhibits excellent particle strength. Pressure ratings for different column dimensions are listed below.

Table 2 Maximum withstand pressure  
(**L-column2 ODS** and **L-column ODS**)

Diameter	Length	Max pressure rating	
		3 $\mu$ m	5 $\mu$ m
$\leq 6$ mm	$\leq 50$ mm	3000 psi	1500 psi
$\leq 6$ mm	100 ~ 150 mm	2200 psi	3600 psi
$\leq 6$ mm	250 mm	4500 psi	3000 psi
$\geq 10$ mm	—	—	1500 psi

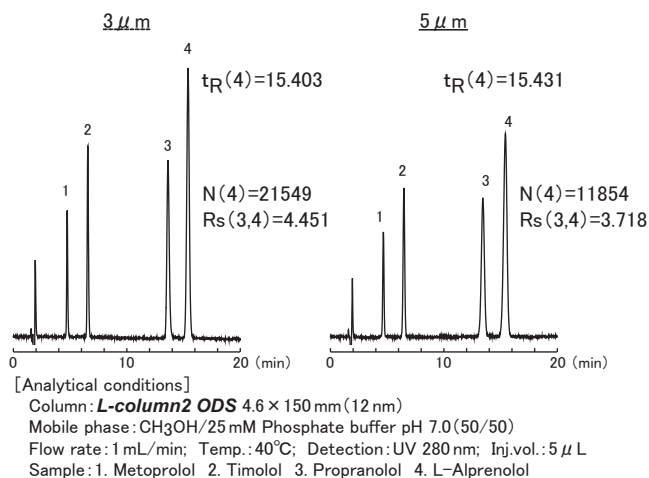


Fig. 14  $\beta$  blockers (3  $\mu$  m vs 5  $\mu$  m)

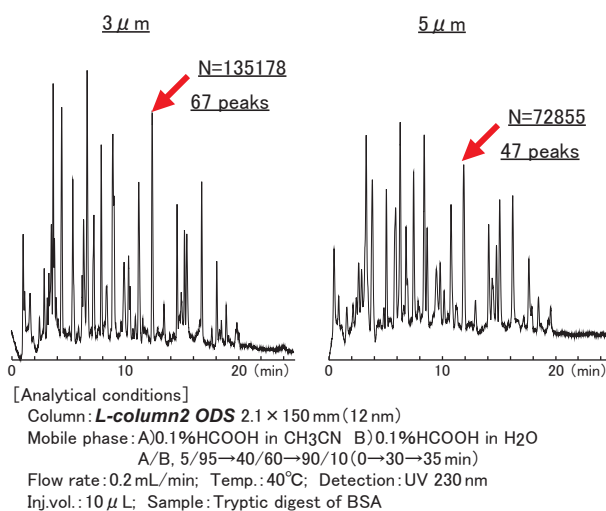


Fig. 15 Tryptic digest of BSA

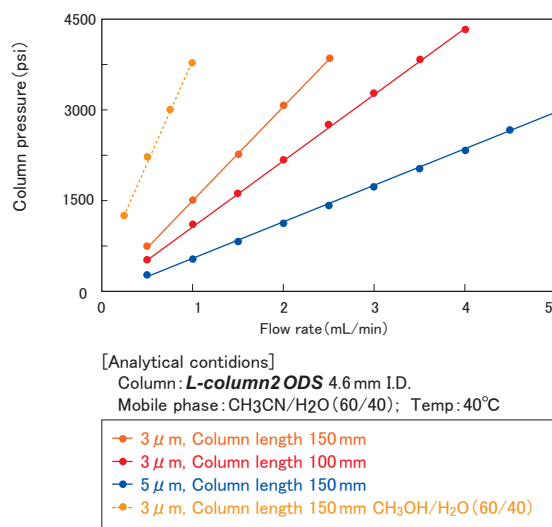


Fig. 16 Relation between column pressure and flow rate



### Theoretical plate number and flow rate

A plot of HETP vs flow rate shows the optimum flow rate for a column packed with 5  $\mu\text{m}$  particles to be about 1.5 mL/min while that for a column packed with 3  $\mu\text{m}$  to be 1.5 mL/min to 2.5 mL/min when the inner diameter is 4.6 mm (Fig. 17). A 3  $\mu\text{m}$  column benefits from both a higher flow rate and a wide range of optimum flow rates in achieving high efficiency separations in the shortest amount of time, when operating at higher pressure is convenient.

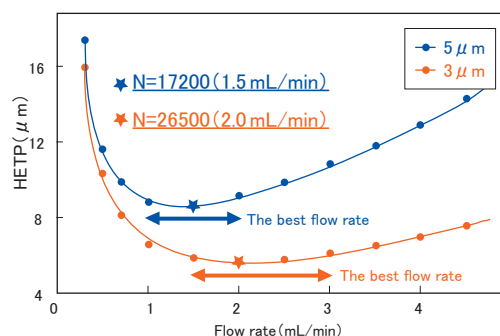


Fig. 17 Relation between Height Equivalent to a Theoretical Plate and flow rate

### Reduction of analysis time (high throughput analysis)

Theoretical plate number (efficiency) is proportional to column length. The process to adapt a method from 5  $\mu\text{m}$  to 3  $\mu\text{m}$  and obtain the same resolution is as follows:

5  $\mu\text{m}$  particle size and 250 mm length  $\rightarrow$  3  $\mu\text{m}$  particle size and 150 mm length

5  $\mu\text{m}$  particle size and 150 mm length  $\rightarrow$  3  $\mu\text{m}$  particle size and 100 mm length

These changes shorten analysis time and decrease solvent consumption. In addition, because the internal volume is decreased, solvent changeover in gradient analysis and column washing is more rapid. The fact that very little efficiency is lost at higher flow rates with 3  $\mu\text{m}$  columns makes this high throughput possible.

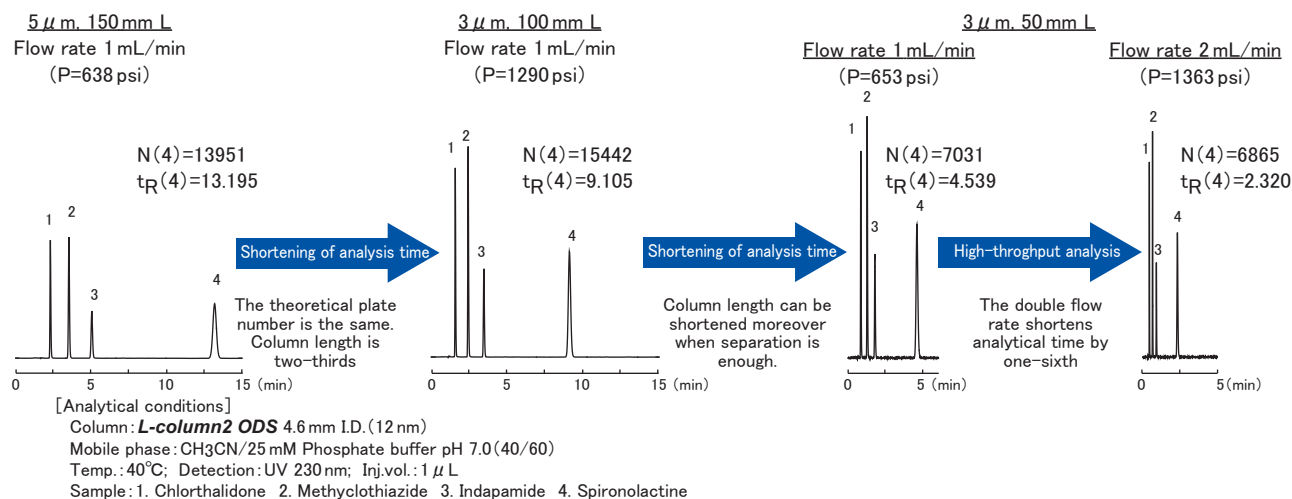


Fig. 18 High-throughput analysis (Diuretics)

## [Lineup]

### L-column2 ODS

Particle ( $\mu\text{m}$ )	Length (mm)	I.D. (mm)							
		1.5	2.1	3.0	4.0	4.6	6.0	10.0	20.0
3		Cat.No.	Cat.No.	Cat.No.	Cat.No.	Cat.No.	Cat.No.	Cat.No.	Cat.No.
	35		711240			721250			
	50	711130	711140	712490		721150			
	100	711160	711170	721330		721180			
	150	711010	711020	721260		721070			
5	250			721320		721080			
	35		712240			722250			
	50	712130	712140			722150			
	100	712160	712170	722330		722180			
	150	712010	712020	722260	722040	722070	722090		
	250		712220	722320	722310	722080		742100	

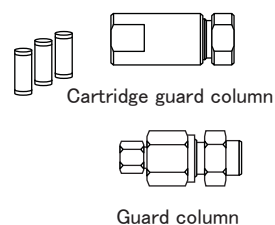
◆ Please contact us for dimensions not listed here.

◆ Micro and nano size columns are described in the **L-column Micro** section (p.14)

Connection type: 1/16" Waters (W)

### Guard column (Particle size 5 $\mu\text{m}$ )

	Column size (mm)	Specification	Cat.No.
Cartridge guard column	2.0 × 5	Cartridge x 3	752330
		Set (3 × cartridges plus holder)	752331
		Holder	752332
	4.6 × 10	Cartridge x 3	752050
		Set (3 × cartridges plus holder)	752051
Guard column	4.0 × 10	—	752030
	10.0 × 20	—	752110



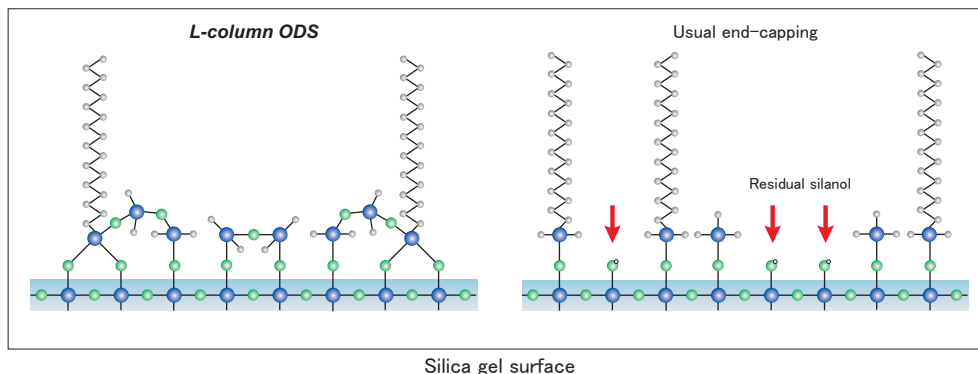
Connection type: 1/16" Waters (W)

# L-column ODS

Established, high performance standard column

Average particle size	3 $\mu$ m, 5 $\mu$ m
Average pore size	120 Å
Range of pH	2~9
USP category	L 1

**L-column ODS** has been supplied since 1990. Many applications have been compiled on this popular column and it continues to deliver a top level of performance in many validated methods.



## Performance with basic compounds

**L-column ODS** exhibits very little peak tailing because very few silanol groups remain accessible to samples.

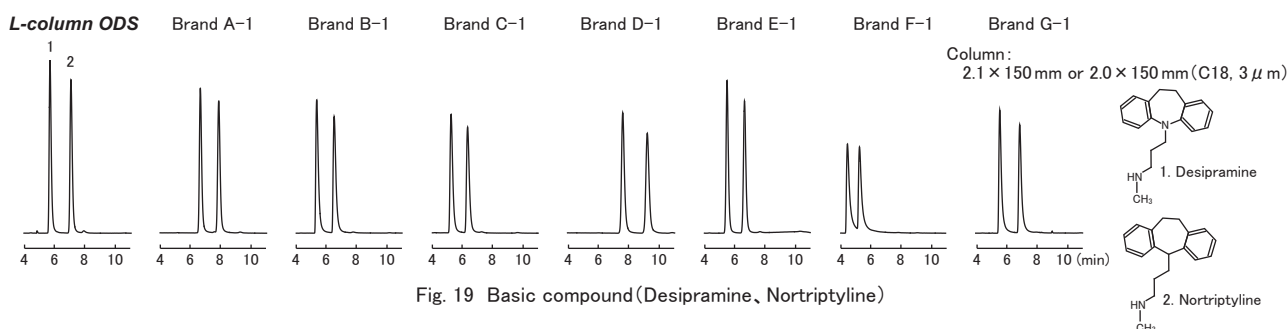


Fig. 19 Basic compound (Desipramine, Nortriptyline)

## Performance with chelating compounds

There is virtually no interaction between chelating compounds and metal impurities in **L-column ODS** because of the use of highly pure silica gel and extensive surface coverage using the patented end-capping technique.

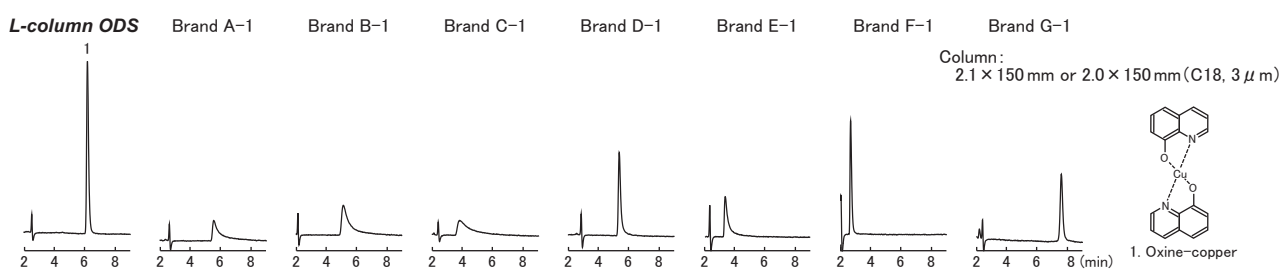


Fig. 20 Coordination compound (Oxine-copper)

## Chemical stability

**L-column ODS** maintains retention under TFA mobile phase conditions due to the well protected silica surface by the patented end-capping. This decreases running cost of HPLC analysis

## High efficiency and low column pressure

Well established column preparation technology and uniform, strong, spherical particles produce a column that is both efficient and easy to operate with a low back pressure (Fig. 1).

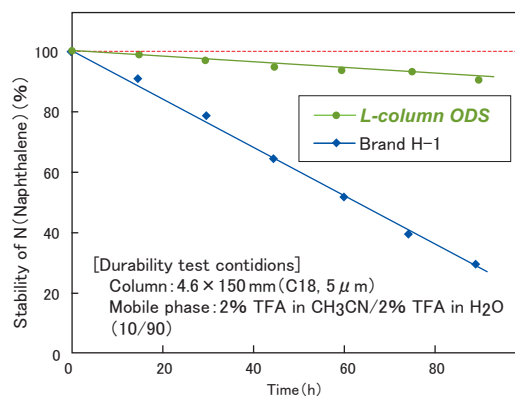


Fig. 21 Accelerated durability test with acidic mobile phase

# L-column ODS-V

## L-column ODS designed for validated methods

Average particle size 5  $\mu$ m  
 Average pore size 120 Å  
 Range of pH 2~9  
 USP category L 1

GMP and GLP for drugs and pesticides demand validation of analytical methods and high reproducibility of HPLC columns.

**L-column ODS-V** is the popular **L-column ODS** with additional supporting certificates of quality testing. Test results for the media and each product lot, as well as an individual quality report for each column packed are included.

### Items of quality assurance

Physical properties of the base silica gel:

Median particle size (d50)  
 Surface area  
 Median pore diameter  
 Pore volume  
 Metal content

Properties of the media:

Capacity factor of a standard  
 Adsorptive property for basic compounds  
 Adsorptive property for acidic compounds  
 Adsorptive property for chelating compounds  
 Surface hydrophobicity  
 Planar and non-planar compounds separation performance,  
 etc.

Quality of packing as measured by theoretical plate number

### Validation support

Set of three columns prepared from different media lots.

### [Lineup]

#### L-column ODS

Particle ( $\mu$ m)	Length (mm)	I.D.(mm)							
		1.5	2.1	3.0	4.0	4.6	6.0	10.0	20.0
		Cat.No.	Cat.No.	Cat.No.	Cat.No.	Cat.No.	Cat.No.	Cat.No.	Cat.No.
3	35		611240			621250			
	50	611130	611140	612490		621150			
	100	611160	611170	621330		621180			
	150	611010	611020	621260		621070			
	250			621320		621080			
5	35		612240			622250			
	50	612130	612140			622150			
	100	612160	612170	622330		622180			
	150	612010	612020	622260	622040	622070	622090		
	250		612220	622320	622310	622080		642100	

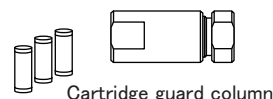
◆ Please contact us for dimensions not listed here.

◆ Micro and nano size columns are described in the **L-column Micro** section (p.14)

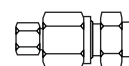
Connection type; 1/16" Waters(W)

#### Guard column (Particle size 5 $\mu$ m)

	Column size(mm)	Specification	Cat.No.
Cartridge guard column	2.0 × 5	Cartridge x 3	652330
		Set (3 × cartridges plus holder)	652331
		Holder	652332
	4.6 × 10	Cartridge x 3	652050
		Set (3 × cartridges plus holder)	652051
		Holder	652052
Guard column	4.0 × 10	—	652030
	10.0 × 20	—	652110



Cartridge guard column



Guard column

Connection type; 1/16" Waters(W)

#### L-column ODS-V

Column size(mm)	Specification	Cat.No.
4.6 × 150	1	622078
	3 lots set	622208
4.6 × 250	1	622088
	3 lots set	622218

Connection type; 1/16" Waters(W)

# L-column ODS-P

Wide pore C18 column for analysis of protein and peptide

Average particle size	5 $\mu$ m
Average pore size	300 Å
Range of pH	2~9
USP category	L 1

**L-column ODS-P** is ideal for the analysis of proteins and peptides. The base silica has a pore diameter of 300 Å. Adsorption is minimized and proteins and peptides elute with sharp peaks. Biological samples are often analyzed using 0.1% TFA in the mobile phase and **L-column ODS-P** is exceptionally stable in strongly acidic mobile phase.

## Role of Pore diameter in protein and peptide analysis

Insulin B chain with a molecular weight of 3,500 does not show different peak shape between 120 Å pore diameter and 300 Å pore diameter. Retention is determined by carbon load. On the other hand, myoglobin with a molecular weight of 17,400 shows a broad peak when analyzed on the 120 Å **L-column ODS** and the main component is not separated from the impurities. Using 300 Å **L-column ODS-P** with 300 Å pore diameter, the main component is separated from the impurities with good peak shape (Fig. 22). Analytes of molecular weight of approximately 5,000 to 20,000 are suitable for this column.

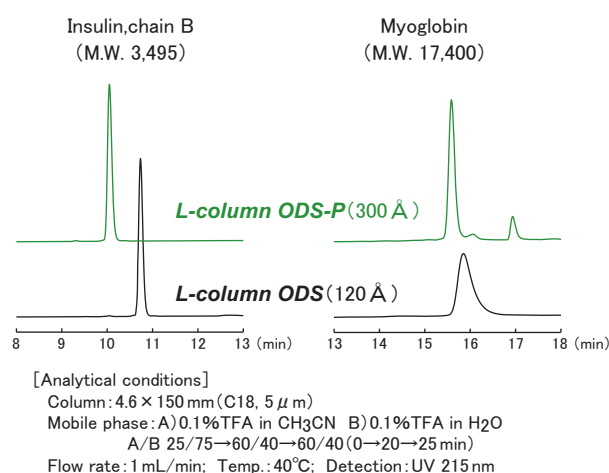
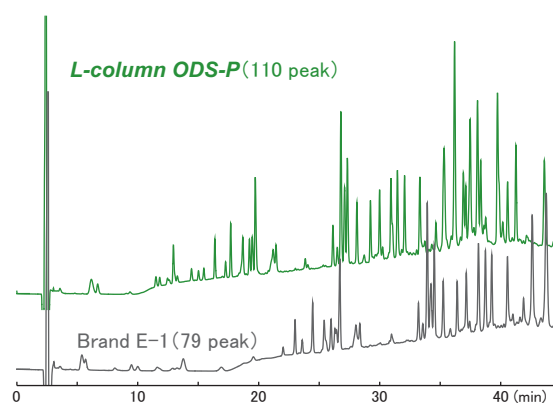


Fig. 22 Peptide and protein analysis using packing materials with different pore diameters

## Low adsorption and high resolution

Peptide mapping, identifying original peptides by simultaneous analysis of the peptides obtained by enzyme degradation of protein, demands sharp peaks and large peak capacity. The column must exhibit very low adsorptive properties and provide very high resolution. The 300 Å **L-column ODS-P** provides better separation than the 120 Å **L-column ODS** and is an ideal column for peptide mapping.

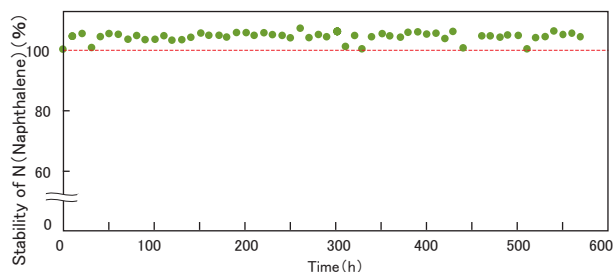


[Analytical conditions]  
 Column: 4.6 × 150 mm (C18, 5  $\mu$ m, 30 nm)  
 Mobile phase: A) 0.1% TFA in CH<sub>3</sub>CN B) 0.1% TFA in H<sub>2</sub>O  
 A/B 10/90 → 60/40 → 60/40 (0 → 40 → 45 min)  
 Flow rate: 1 mL/min; Temp.: 40°C; Detection: UV 215 nm  
 Inj. vol.: 20  $\mu$ L; Sample: BSA (2 mg/mL)

Fig. 23 Comparison between **L-column ODS-P** and another column for a digest of BSA

## High durability

**L-column ODS-P** can be used in a pH range from 2 to 9. It demonstrates long lifetimes and stable performance in mobile phases containing 0.1% TFA (Fig. 24).



[Durability test conditions]  
 Column: **L-column ODS-P** 4.6 × 150 mm (5  $\mu$ m)  
 Mobile phase: 1% TFA in CH<sub>3</sub>CN/1% TFA in H<sub>2</sub>O (50/50)  
 Flow rate: 1 mL/min Temp: 60°C

Fig. 24. Stability of **L-column ODS-P**

## [Lineup]

Particle ( $\mu$ m)	Length (mm)	I.D. (mm)	
		2.1	4.6
5	50	612147	622157
	150	612027	622077

◆ Please contact us for dimensions not listed here.

Connection type: 1/16" Waters (W)

# L-column C8

## High performance, stable octyl column

Average particle size	5 $\mu$ m
Average pore size	120 Å
Range of pH	2~7.5
USP category	L 7

**L-column C8** employs the same high purity silica with a C8 bonded phase. It is believed that the silica surface is more open to adsorption for some compounds with a C8 than with a C18 bonded phase due to lessened steric hindrance. The advanced end-capping procedures have produced a particularly stable and durable C8 making it the optimum column for highly hydrophobic compounds and a time and solvent saving option for many applications.

### Reduction of analysis time

**L-column C8** reduces the elution time for hydrophobic compounds and shows good peak shape even for basic samples such as antihistamines (Fig. 25).

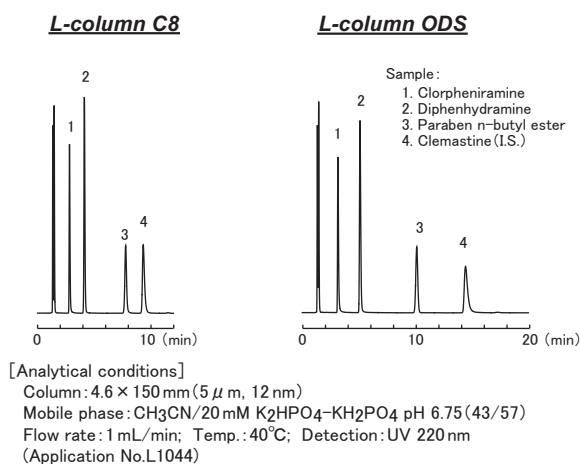


Fig. 25 Chromatogram of antihistamine

### Improved separation

In general C8 columns show similar separation behavior to C18 columns. However, there are separations that are enhanced by a slightly different selectivity for a few components on the shorter chain of a C8 column (Fig. 26).

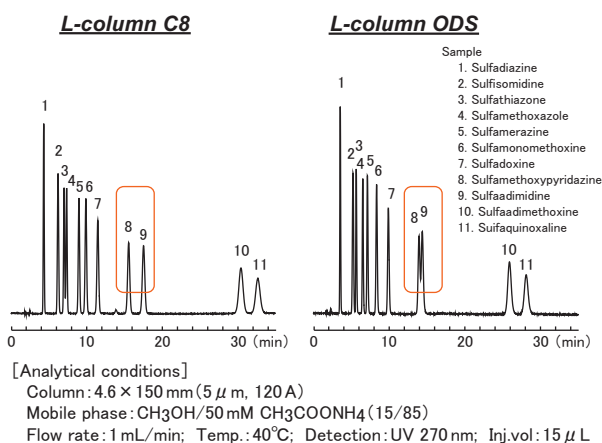


Fig. 26 Chromatogram of sulfa drugs

### High durability

Although some C8 columns deteriorate more rapidly, **L-column C8** is chemically stable, producing a long useable column life (Fig. 27).

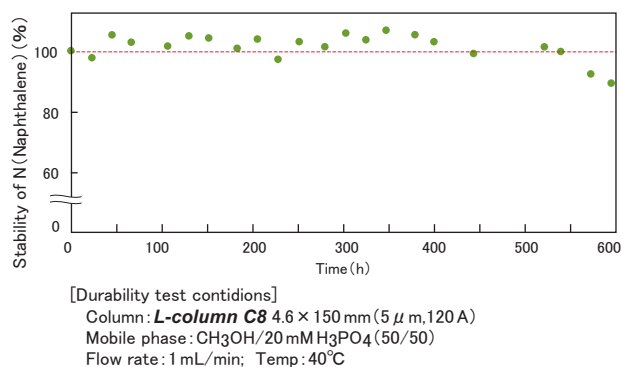


Fig 27. Stability of **L-column C8**

### 【Lineup】

Particle ( $\mu$ m)	Length (mm)	I.D. (mm)		
		1.5 Cat.No.	2.1 Cat.No.	4.6 Cat.No.
5	35		612241	622251
	50	612131	612141	622151
	100	612161	612171	622181
	150	612011	612021	622071
	250		612221	622081

◆Please contact us for dimensions not listed here.

Connection type; 1/16" Waters (W)



# L-column Micro

## Next generation high performance silica-based micro column

**L-column Micro** is a high performance column for proteome analysis. It combines the low adsorptive packing media with a proprietary column packing technology and a low dead volume column hardware design. It is expected that **L-column Micro** will make a sensitivity improvement similar to an upgrade to LC/MS/MS performance.

### Column structure

The column tubing is inert fused silica, available in two formats.

#### ■ PEEK-sleeved type

The outside of the fused silica is sleeved with PEEK resin. The column is easy to handle and to connect to the MS. The end fitting is 1/16 inch stainless steel. PEEK-sleeved **L-column Micro** is available in 0.075, 0.1, 0.2 and 0.3 mm I.D.

#### ■ Non-sleeved type

The outside of the fused silica is not sleeved and has no external end fitting. This allows direct coupling to the MS for minimal dead volume (Fig. 28). The non-sleeved format is available in 0.075 and 0.1 mm I.D.

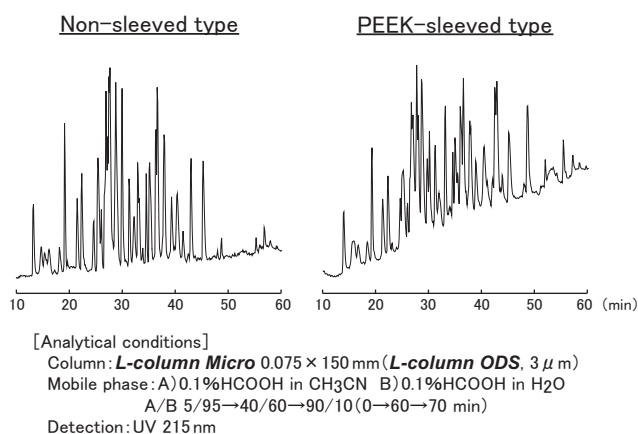
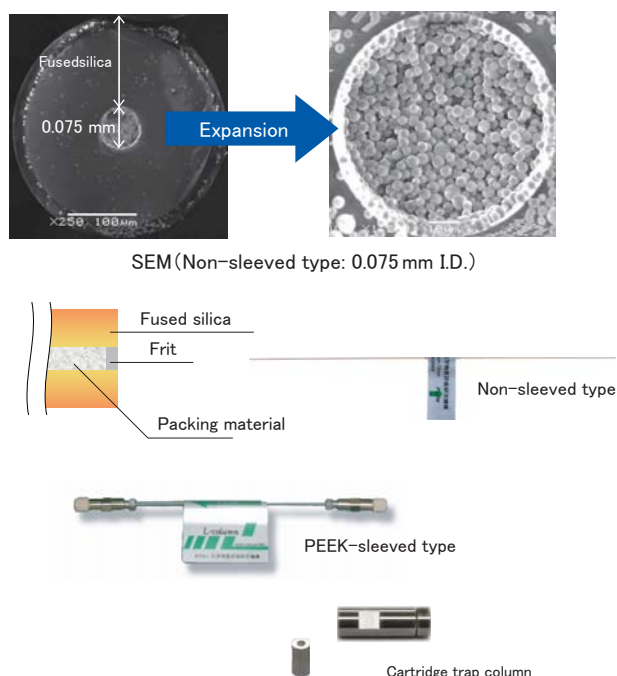


Fig. 28 Non-sleeved type vs. PEEK-sleeved type

### Trap column

A trap column is necessary for a large injection volume because the flow rate of nano and micro columns are very small. The ideal trap column must show low adsorption, very small dead volume and high retention. Performance was compared between our trap column and one from another manufacturer using an enzyme digest of a protein which is a mixture of low polarity peptides and high polarity peptides (Fig. 29). Our trap column retains them fully. There is very little irreversible adsorption to the packing material.

There is no loss of theoretical plate number because of the minimal additional dead volume. The cartridge format is economical sample concentration and protection for the **L-column Micro**.

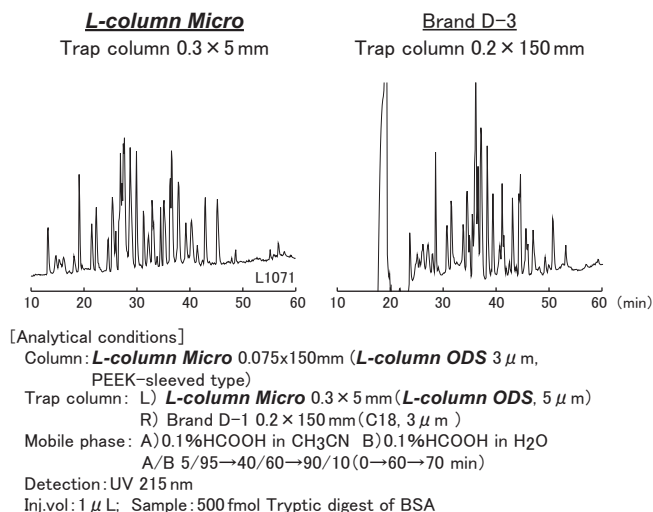
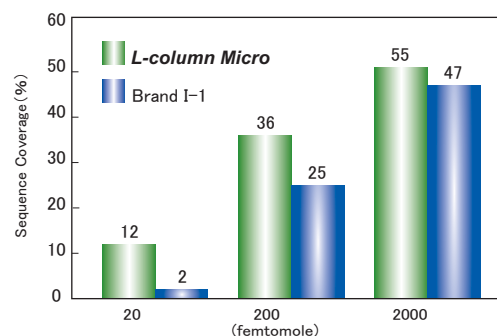


Fig. 29 Comparison of trap columns

## Proteome analysis

A tryptic digest of BSA is analyzed using LC/MS/MS and the sample concentration and cover ratio is determined using an **L-column Micro** and another brand (Fig. 30). A higher cover ratio means that more amino acid sequences are read.

**L-column Micro** shows advantage at all concentration levels, but excels at the lower levels. With extreme end-capping and very high theoretical plate numbers per column, **L-column Micro** permits identification of many proteins and is the optimum choice for proteome analysis.



[Analytical conditions]

Column: L) **L-column Micro** 0.075 × 150 mm (**L-column ODS**, 3 μm, PEEK-sleeved type)

R) Brand I-1 0.075 × 150 mm (C18, 3 μm)

Mobile phase: A) 0.1% HCOOH in CH<sub>3</sub>CN B) H<sub>2</sub>O  
A/B 5/95 → 40/60 → 90/10 (0 → 60 → 70 min)

Temp.: Ambient

Sample: Tryptic digest of BSA

System: Cap LC (Waters Co.) Q-TOF micro (Waters Co.)

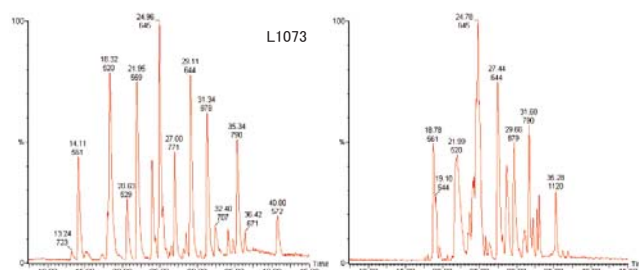
Fig. 30. Sequence coverage (Tryptic digest BSA)

## Low adsorption and high efficiency

**L-column Micro** is able to separate many peptides because of the extremely low adsorption media and the high efficiency of the proprietary packing technology. This is effective for the identification of proteins (Fig. 31) and the high efficiency of the column enables good quantitative results at trace levels. It is an optimum column for microanalysis, and methods scale-up easily to preparative column dimensions.

**L-column Micro**

Brand I-1



[Analytical conditions]

Column: L) **L-column Micro** 0.075 × 150 mm (**L-column ODS**, 3 μm, PEEK-sleeved type)

R) Brand I-1 0.075 × 150 mm (C18, 3 μm)

Mobile phase: 0.1% HCOOH in CH<sub>3</sub>CN/H<sub>2</sub>O

Flow rate: 2.5 μL/min; Temp.: Ambient

System: Cap LC (Waters Co.) Q-TOF micro (Waters Co.)

(Application No.1073)

Fig. 31. TIC of tryptic digest of Enolase

## [Lineup]

### L-column2 ODS

Particle (μm)	Length (mm)	I.D. (mm)					
		0.075		0.1		0.2	0.3
		non-sleeved Cat.No.	PEEK-sleeved Cat.No.	non-sleeved Cat.No.	PEEK-sleeved Cat.No.	PEEK-sleeved Cat.No.	PEEK-sleeved Cat.No.
3	50	711370	711410	711350	711390	711290	711270
	150	711380	711420	711360	711400	711300	711280
5	50	712370	712410	712350	712390	712290	712270
	150	712380	712420	712360	712400	712300	712280

Outside diameter: 0.375 mm (Coated type)

Connection: 1/16" Waters (W) (PEEK-sleeved type)

### L-column ODS

Particle (μm)	Length (mm)	I.D. (mm)					
		0.075		0.1		0.2	0.3
		non-sleeved Cat.No.	PEEK-sleeved Cat.No.	non-sleeved Cat.No.	PEEK-sleeved Cat.No.	PEEK-sleeved Cat.No.	PEEK-sleeved Cat.No.
3	50	611370	611410	611350	611390	611290	611270
	150	611380	611420	611360	611400	611300	611280
5	50	612370	612410	612350	612390	612290	612270
	150	612380	612420	612360	612400	612300	612280

Outside diameter: 0.375 mm (Coated type)

Connection: 1/16" Waters (W) (PEEK-sleeved type)

Cartridge trap column (Column size 0.3 mm I.D. × 5 mm L, Particle size 5 μm)

Bulking agent's kind	Specification	Cat.No.
<b>L-column2 ODS</b>	Cartridge × 3	752450
<b>L-column ODS</b>	Cartridge × 3	652450
—	Holder	652452

Connection type; 1/16" Waters (W)

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Design, Development, Production  
and supply of Column  
for GC and HPLC