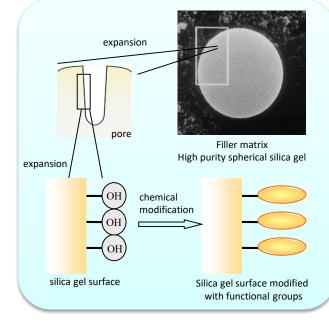
GL Sciences Inc.

### Introduction of InertSustain and Inertsil Series Reversed-Phase Columns

Many manufacturers have released various types of columns for HPLC. In LC Technical Notes No.50 and No.100, in order to respond to the customer's voice that "there are too many types and I don't know which one to choose", we provide reversed-phase HPLC columns that we offer. made a comparison.

Since the previous technical note was published, we have also expanded our column lineup. Therefore, as No.200, we will introduce the comparison data of the reversed-phase column again.

(K. Tanaka)



## ODS/C18 columns

Column type	functional group	Features
InertSustain C18	Si-C18H37	This is the first choice reversed-phase column developed by pursuing inertness and chemical stability using our original synthesis technology.
InertSustain AQ-C18	SI-CasH37	This column is recommended for reversed-phase analysis of hydrophilic (highly polar) compounds under eluent conditions close to 100% water.
InertSustainSwift C18	Si -C18H37	This column is suitable for high-speed analysis, designed to elute compounds faster than general ODS columns.
InertSustain AX-C18	Si -CisHar -NR2	This is a mixed-mode column chemically modified with C18 and tertiary amino groups. It is useful when you want to increase the retention of acidic highly polar compounds.
Inertsil ODS-HL	Si -CısHər	This column has a higher retention capacity for hydrophobic compounds because it contains more carbon than a typical ODS column.
Inertsil ODS-4	Si-C18H37	Overall, the elution is a little faster than InertSustain C18, and the planar recognition ability is slightly higher.
Inertsil ODS-3	Si-C18H37	Since its release in 1994, it has been highly evaluated for its retention strength and stable quality, and is still widely used around the world.
Inertsil ODS-2	SI-C18H37	A long-selling column that uses high-purity silica gel as the HPLC packing material for the first time. He is a contributor who made the world know the goodness of Japanese fillers
Inertsil ODS-SP	Si-C18H37	This column controls the amount of octadecyl (ODS, C18) groups introduced to speed up the elution time of highly hydrophobic compounds.
Inertsil ODS-EP	CaeHar	This is an embedded column with a polar group introduced at the base of the octadecyl group. A separation pattern different from that of ordinary ODS columns can be obtained.
Inertsil ODS-P	Si C18H37	Column modified with octadecyl groups at high density. It is particularly useful for separating components with similar structures due to its high stereoscopic ability.
Inertsil WP300 C18	Si 300-C18H37	Because of its large pores, this column can sharply elute large molecules.
MonoClad C18-HS	-C18H37	This is a silica monolith column for HPLC that uses a columnar silica rod with a double pore structure of through pores and mesopores as a separation carrier.

# Other reversed-phase columns

Column type	functional group	Features
InertSustain C8	Si – CsHir	First choice column for C8 columns. It also has excellent inertness, durability, and lot-to-lot reproducibility.
InertSustainSwift C8	Si –CaH17	Designed to elute compounds quickly, this C8 column is suitable for high- speed and high-sensitivity analysis.
Inertsil C8-4	Si-CaH17	It is also a C8 column with excellent inertness. Elution is faster than InertSustain C8, and it shows high stereoscopic recognition ability.
Inertsil C8-3	Si-CaH17	As a C8 column, it is designed to have stronger retention, and a separation pattern slightly different from that of InertSustain C8 can be obtained.
Inertsil WP300 C8	51 300 - CaH17	Due to its large pores, it is ideal for rapid analysis of macromolecular compounds such as proteins and peptides.
Inertsil C4	Si-C4H9	This is a column with butyl group introduced, which has the weakest hydrophobic interaction among Inertsil's alkyl group-bonded columns.
Inertsil WP300 C4	SOD PG-CaHa PG : MIRES	This column is ideal for reversed-phase analysis of highly lipid-soluble proteins and peptides, with its unique embedding treatment to suppress adsorption.
InertSustain Cyano	Si ∕~∕ C≡N	It is known as a column that has interaction by $\pi$ bond like Ph column and shows more characteristic behavior.
InertSustain PFP		Various interactions such as hydrophobic interaction, dipole-dipole interaction, and $\pi$ -electron interaction work to exhibit unique separation behavior.
InertSustain Phenylhexyl	(si) · · · · · · · · · · · · · · · · · · ·	This is an alkylphenyl group bonding type column. It is useful when you want to slightly change the separation pattern on the ODS column.
InertSustain Phenyl	si)-	This column contains phenyl groups. Since $\pi$ electron interaction works, it is used when you want to greatly change the separation pattern.
Inertsil Ph-3	(Si)-{\bar}	Although the $\pi$ -electron interaction also works, since the stereorecognition ability is different from that of InertSustain Phenyl, a different separation pattern can be obtained.
Inertsil Ph		Unlike other phenyl columns, this column has an ethyl group (phenethyl group) introduced between the phenyl group and the packing material.



### 1. Pattern comparison on ODS column

The specifications and comparison results of our ODS columns are shown below. From the next page onwards, the entire chromatogram is shown on the right, and the 4-minute zoom is shown on the left.

column name	chemical bonding group	E.C.	carbon loading	pore size	surface area
InertSustain C18	Octadecyl group	Yes	14%	100 Å	350 m²/g
InertSustain AQ-C18	Octadecyl group	Yes	13%	100 Å	350 m²/g
InertSustainSwift C18	Octadecyl group	Yes	9%	200 Å	200 m²/g
InertSustain AX-C18	Octadecyl + Tertiary amino	Yes	8%	200 Å	200 m²/g
Inertsil ODS-HL	Octadecyl group	Yes	23%	100 Å	450 m²/g
Inertsil ODS-4	Octadecyl group	Yes	11%	100 Å	450 m²/g
Inertsil ODS-3	Octadecyl group	Yes	15%	100 Å	450 m²/g
Inertsil ODS-2	Octadecyl group	Yes	18.5%	150 Å	320 m²/g
Inerstil ODS-SP	Octadecyl group	Yes	8.5%	100 Å	450 m²/g
Inertsil ODS-EP	Octadecyl group	No	9%	100 Å	450 m²/g
Inertsil ODS-P	Octadecyl group	No	29%	100 Å	450 m²/g
Inertsil WP300 C18	Octadecyl group	Yes	9%	300 Å	150 m²/g
MonoClad C18-HS	Octadecyl group	Yes	14%	180 Å*	200 m²/g
	, , ,				

\* Since MonoClad C18-HS is based on silica monolith, the mesopore diameter corresponding to the pore diameter of the packed particles is listed.

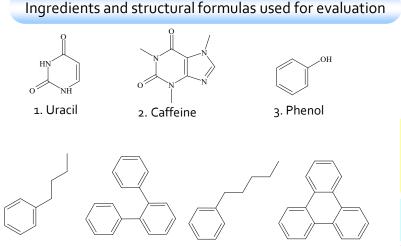
#### **HPLC** Conditions

4. Butylbenzene 5. *o*-Terphenyl

Column	: Reversed phase column				
	(5 μm, 250 × 4.6 mm I.D.)*				
Eluent	: A) CH <sub>3</sub> OH B) H <sub>2</sub> O A/B= 80/20, v/v				
	(InertSustain Cyano only A/B= 60/40, v/v )				
Flow rate	: 1.0 mL/min*				
Col. Temp.	: 40 °C				
Detection	: UV 254 nm				
Injection Vol.	: 5 μL*				

Conditions for comparing reversed-phase column selectivity and hydrophobic interaction are shown on the left. The mobile phase used was a methanol-water system, and all columns were of the same size and had the same particle size.

\* For MonoClad C18-HS, the flow rate was set at 0.4 mL/min and the injection volume was set at 2  $\mu$ L, because the measurement was performed using a 3 mm inner diameter column.



The structural formulas of the components used for evaluation are shown on the left. Differences in the separation of basic and acidic substances, alkylbenzenes and polycyclic aromatic compounds reveal various factors affecting the separation.

The more silanol, which is a weakly acidic functional group on the surface of the silica gel used as a packing material, the later caffeine (2) elutes than phenol (3)\*.

Amylbenzene (6) elutes later than butylbenzene (4) in columns with stronger hydrophobicity.

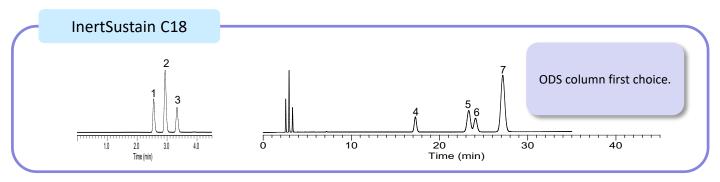
Triphenylene (7) elutes later than o-terphenyl (5) in columns with higher planar recognition ability.

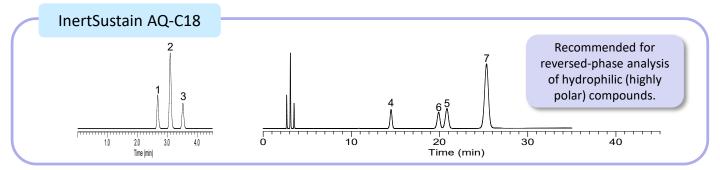
Structures are created using Chemistry 4-D Draw which is provided by ChemInnovation Software, Inc.

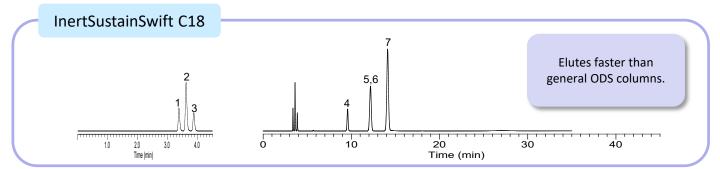
7. Triphenylene

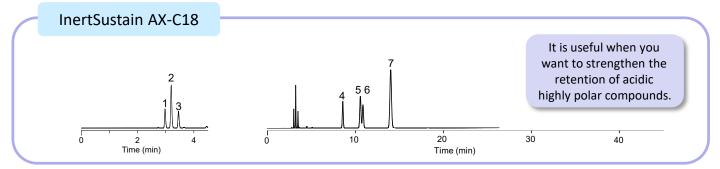
6. Amylbenzene

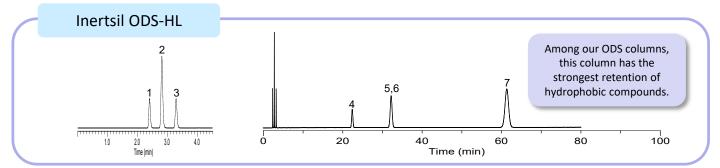
\*References
K. Kimata, K. Iwaguchi, S. Onishi, K. Jinno, R. Eksteen, K. Hosoya, M. Araki, N.Tanaka, J. Chromatogr. Sci. 1989, 27, 721-728.

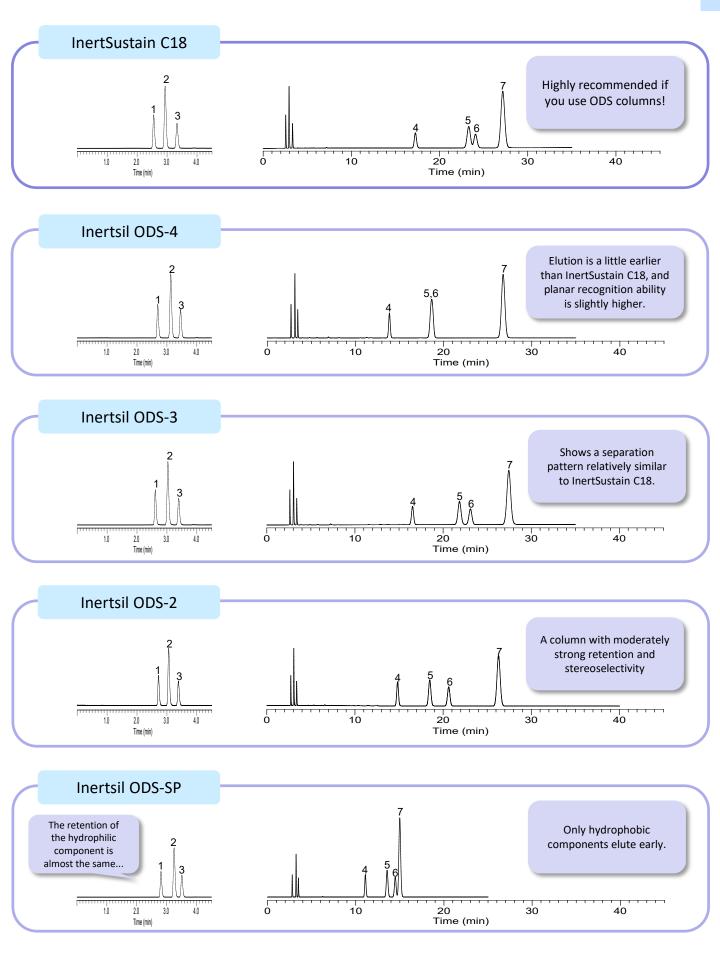


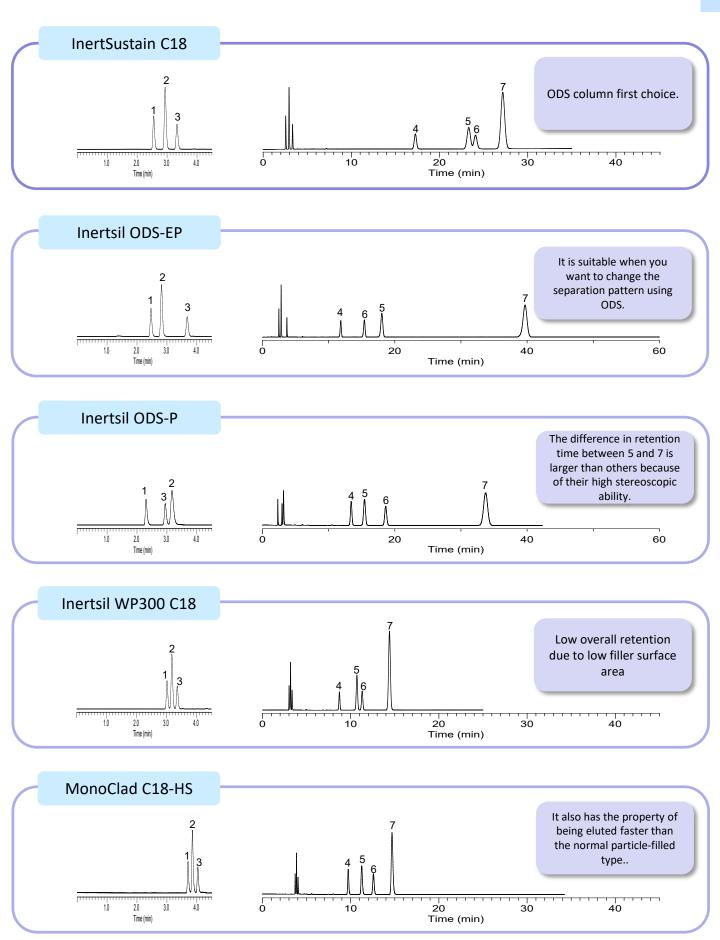








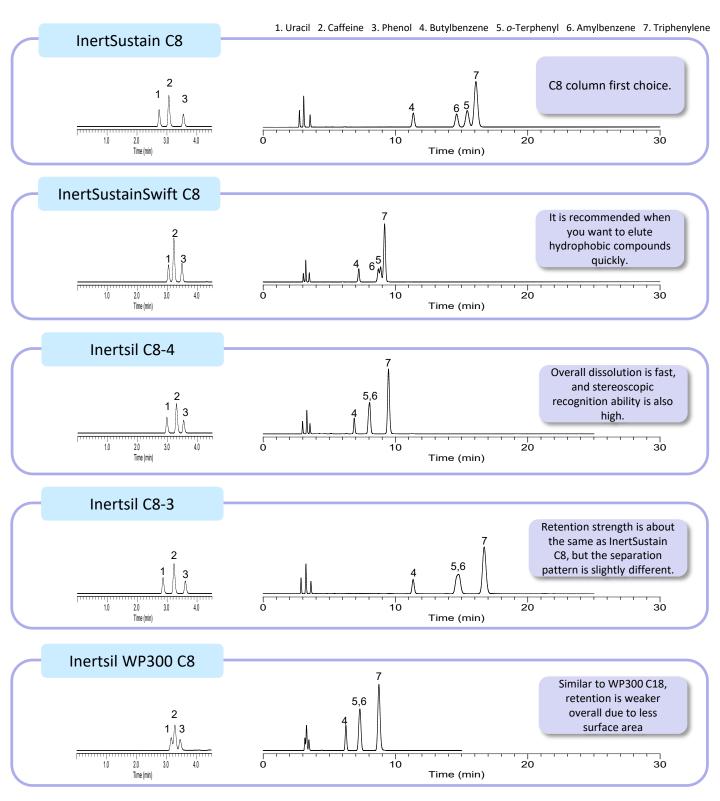




# 2. Pattern comparison on C8 column

The specifications and comparison results of our C8 columns are shown below. The measurement conditions are the same as in (1).

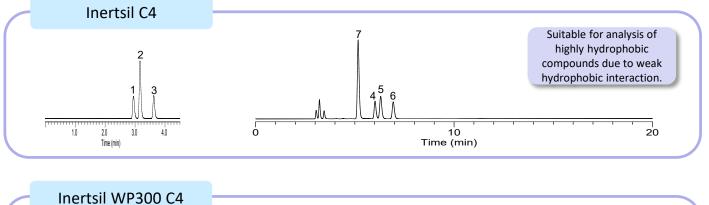
InertSustain C8 octyl group Yes 8% 100 Å 350 m <sup>2</sup> /g	column name	chemical bonding group	E.C.	carbon loading	pore size	surface area
InertSustainSwift C8 octvl group Ves 6% 200 Å 200 m <sup>2</sup> /g	InertSustain C8	octyl group	Yes	8%	100 Å	350 m²/g
	InertSustainSwift (	28 octyl group	Yes	6%	200 Å	200 m²/g
Inertsil C8-4 octyl group Yes 5% 100 Å 450 m <sup>2</sup> /g	Inertsil C8-4	octyl group	Yes	5%	100 Å	450 m²/g
Inertsil C8-3 octyl group Yes 9% 100 Å 450 m <sup>2</sup> /g	Inertsil C8-3	octyl group	Yes	9%	100 Å	450 m²/g
Inertsil WP300 C8 octyl group Yes 4% 300 Å 150 m <sup>2</sup> /g	Inertsil WP300 C8	octyl group	Yes	4%	300 Å	150 m²/g

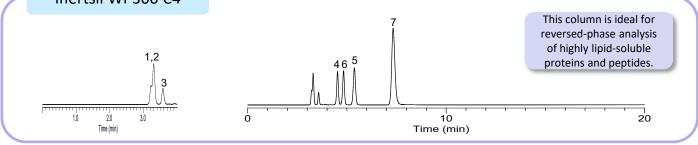


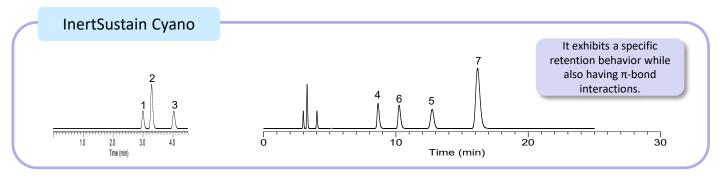
# 3. Pattern comparison on C4/cyano/phenyl column

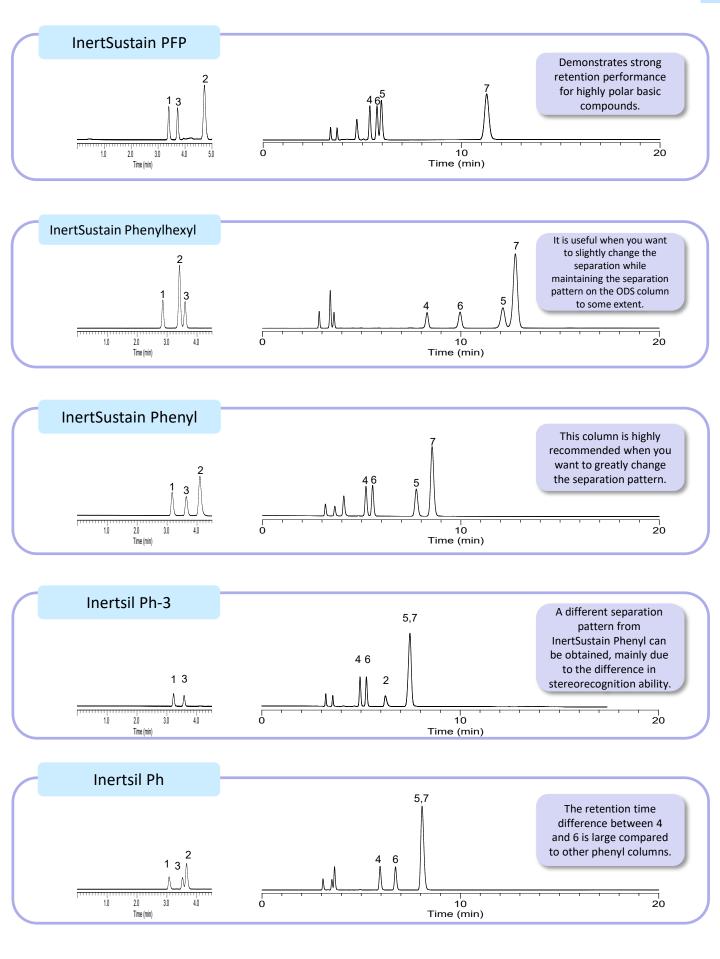
Similar to 1 and 2, we show the specifications and comparison results of our C4/cyano/phenyl column. The measurement conditions are the same as 1 and 2.

column name	chemical bonding group	E.C.	carbon loading	pore size	surface area
Inertsil C4	butyl group	Yes	7.5%	150 Å	320 m²/g
Inertsil WP300 C4	butyl group	No	3%	300 Å	150 m²/g
InertSustain Cyano	cyanopropyl group	Yes	8%	100 Å	350 m²/g
InertSustain PFP	Pentafluorophenylpropyl group	Yes	10%	100 Å	350 m²/g
InertSustain Phenylhexyl	phenylhexyl group	Yes	9%	100 Å	350 m²/g
InertSustain Phenyl	phenyl group	No	10%	100 Å	350 m²/g
Inertsil Ph-3	phenyl group	No	9.5%	100 Å	450 m²/g
Inertsil Ph	phenethyl group	Yes	10%	150 Å	320 m²/g



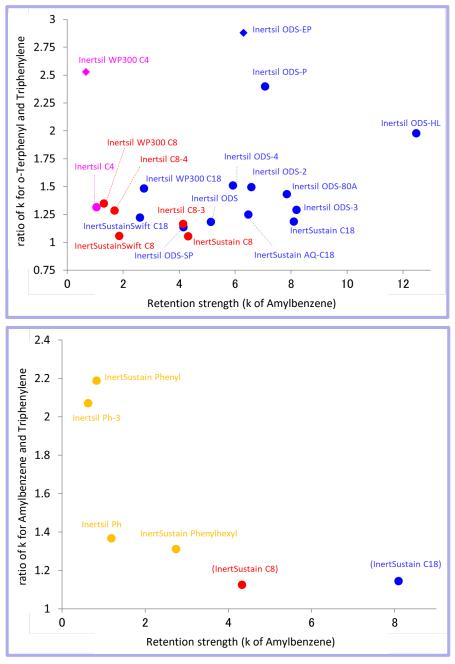






#### InertSustain, Inertsil series distribution model

The figure below shows the stereoselectivity and the strength of the  $\pi$ -electron interaction with respect to the retention strength of the reversed-phase column. "Retention strength" is the retention coefficient of Amylbenzene, "Stereoselectivity" is the retention ratio of o-Terphenyl and Triphenylene, and "Strength of  $\pi$ -electron interaction" is the retention ratio of Amylbenzene and Triphenylene.



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