#### LC Technical Note

**GL Sciences Inc.** 

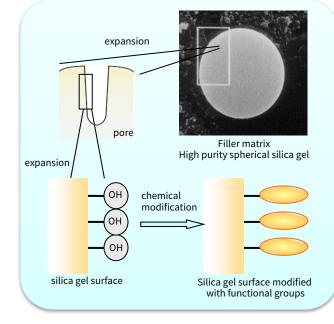
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### Introduction of InertSustain and Inertsil Series Reversed-Phase Columns

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)



functiona Column type Features l group This is the first choice reversed-phase column developed by pursuing inertness and InertSustain C18 chemical stability using our original synthesis technology. C18H37 This column is recommended for reversed-phase analysis of hydrophilic (highly polar) InertSustain AQ-C18 C18H37 compounds under eluent conditions close to 100% water. This column is suitable for high-speed analysis, designed to elute compounds faster InertSustainSwift C18 C18H37 than general ODS columns. This is a mixed-mode column chemically modified with C18 and tertiary amino groups. It is useful InertSustain AX-C18 C18H37 when you want to increase the retention of acidic highly polar compounds. NR<sub>2</sub> This column has a higher retention capacity for hydrophobic compounds because it Inertsil ODS-HL C18H37 contains more carbon than a typical ODS column. Overall, the elution is a little faster than InertSustain C18, and the planar recognition Inertsil ODS-4 C18H37 ability is slightly higher. Since its release in 1994, it has been highly evaluated for its retention strength and Inertsil ODS-3 C18H37 stable quality, and is still widely used around the world. A long-selling column that uses high-purity silica gel as the HPLC packing material for the first time. He is a Inertsil ODS-2 18H37 contributor who made the world know the goodness of Japanese fillers This column controls the amount of octadecyl (ODS, C18) groups introduced to speed Inertsil ODS-SP up the elution time of highly hydrophobic compounds. C18Hay This is an embedded column with a polar group introduced at the base of the octadecyl group. A separation Inertsil ODS-EP pattern different from that of ordinary ODS columns can be obtained. CisHar PG:揪性肉 Column modified with octadecyl groups at high density. It is particularly useful for separating Inertsil ODS-P C18H37 components with similar structures due to its high stereoscopic ability. Inertsil WP300 C18 Because of its large pores, this column can sharply elute large molecules. C18H37 This is a silica monolith column for HPLC that uses a columnar silica rod with a double MonoClad C18-HS pore structure of through pores and mesopores as a separation carrier. -C18H37

ODS/C18 columns

## Other reversed-phase columns

Column type	functional group	Features
InertSustain C8	SI -CaHir	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-CsH17	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-CsH17	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	Si 300-CsH17	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	Callo PG: REEM	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	G P	Various interactions such as hydrophobic interaction, dipole-dipole interaction, and $\pi$ -electron interaction work to exhibit unique separation behavior.
InertSustain Phenylhexyl		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	<u>(3)</u>	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		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 m <sup>2</sup> /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.

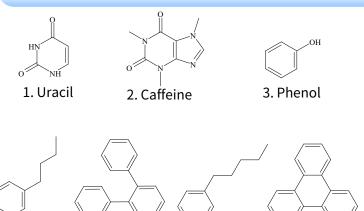
#### **HPLC Conditions**

Column	Reversed phase column				
	(5 μm, 250 × 4.6 mm I.D.)*				
Eluent	: A) $CH_3OH$ B) $H_2O$ 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.	: 5μL*				

 $^{*}$  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.

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.

#### Ingredients and structural formulas used for evaluation



4. Butylbenzene5. *o*-Terphenyl 6. Amylbenzene 7. Triphenylene

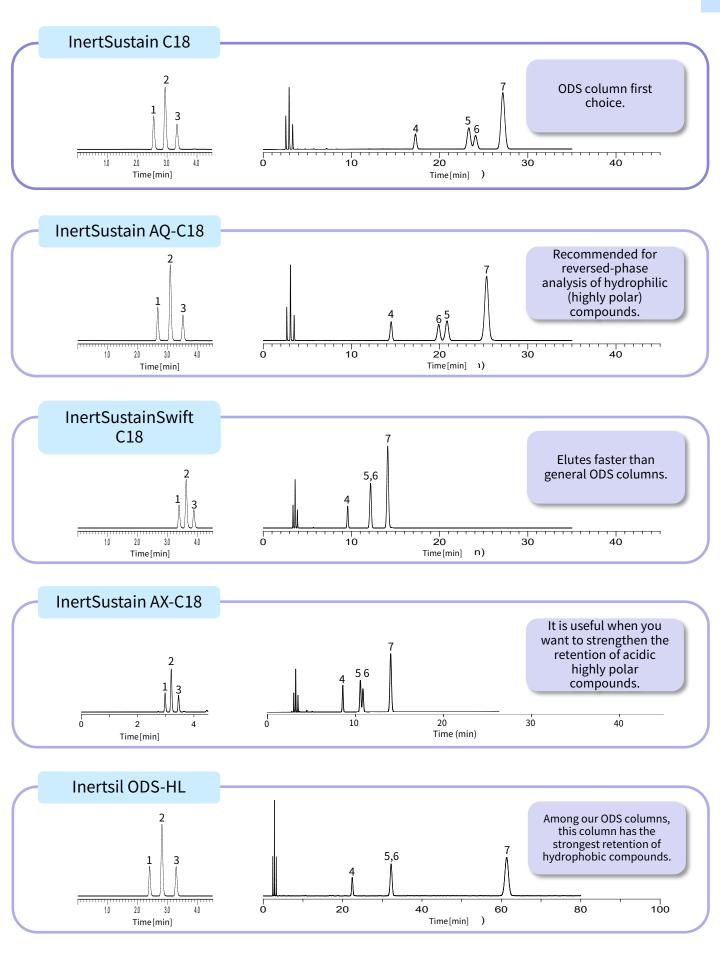
Structures are created using Chemistry 4-D Draw which is provided by ChemInnovation Software, Inc.

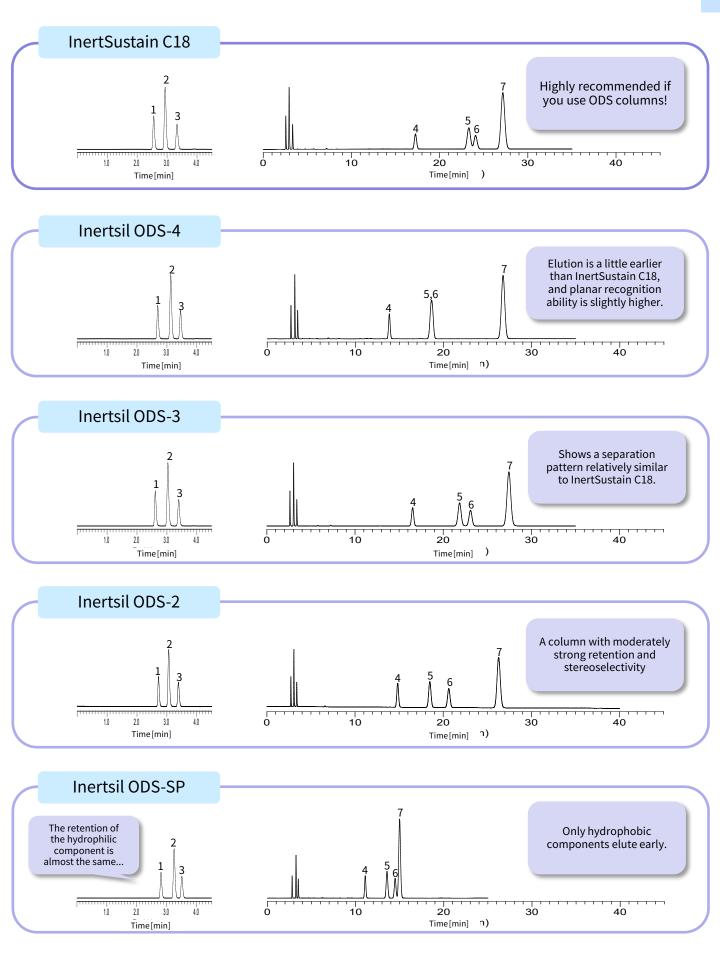
The structural formulas of the components used for evaluation are shown on the left. Differences in the separation of basic and acidic substances, alkylbenzenes and polycyclic aromatic compounds reveal various factors affecting the separation.

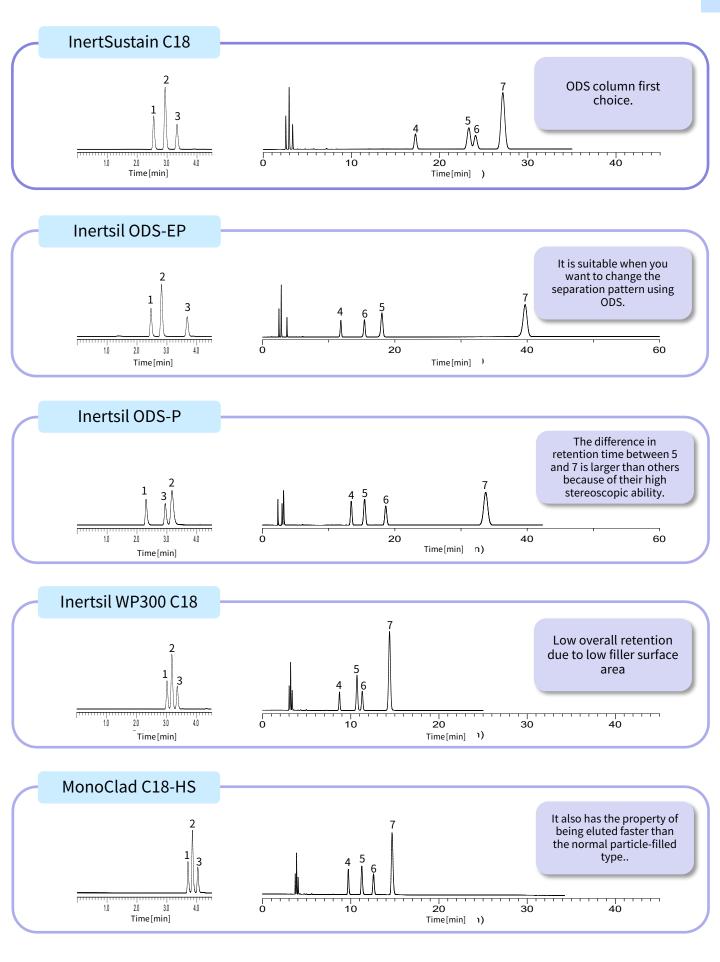
The more silanol, which is a weakly acidic functional group on the surface of the silica gel used as a packing material, the later caffeine (2) elutes than phenol (3)\*.

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.







Time[min]

# 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.

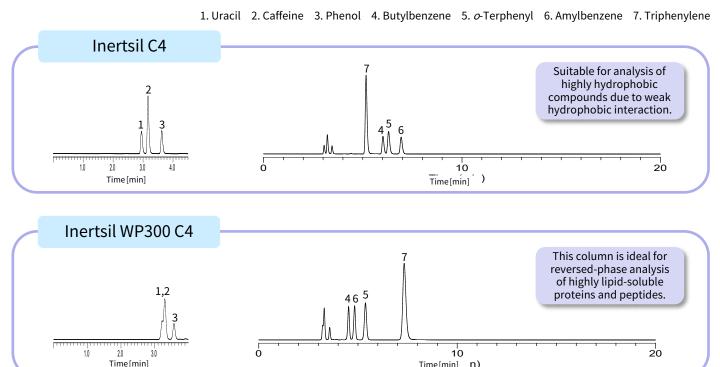
1	11 P				
column name chemic			arbon loading		
	ctyl group	Yes	8%	100 Å	350 m <sup>2</sup> /g
	ctyl group	Yes	6%	200 Å	200 m <sup>2</sup> /g
	ctyl group	Yes	5%	100 Å	450 m <sup>2</sup> /g
	ctyl group	Yes	9%	100 Å	450 m <sup>2</sup> /g
Inertsil WP300 C8 o	ctyl group	Yes	4%	300 Å	150 m²/g
	racil 2. Caffeine 3.	Phenol 4.	Butylbenzene 5.	<i>o</i> -Terphenyl	6. Amylbenzene 7. Triphenyle
InertSustain C8			,	1 2	, , , , , , , , , , , , , , , , , , , ,
	· · · · · · · · · · · · · · · · · · ·		4 6 5 10	7	C8 column first choice.
Time[min]			Time [min]	")	
InertSustainSwift C8			7		It is recommended when
2 1 3 10 20 30 40 Time[min]	, <u> </u>	4 6 <sup>5</sup>	10 Time[min]	יין און אין אין אין אין אין אין אין אין אין אי	h is recommended when you want to elute hydrophobic compounds quickly.
Inertsil C8-4					
mertsit Co-4			7		
2 1 3 1 10 20 30 40 Time[min]	; <u> </u>	5,6 4	10 Time[min]	່   20 າ)	Overall dissolution is fast, and stereoscopic recognition ability is also high.
Inertsil C8-3					
2 1 1 10 20 30 40 Time[min]	, o	<u></u>	4 5,6 10 Time[min]	7 	Retention strength is about the same as InertSustain C8, but the separation pattern is slightly different.
Inertsil WP300 C8					
	<u>_</u>	5,6			Similar to WP300 C18, retention is weaker overall due to less surface area
1.0 2.0 3.0 4.0 Time[min]	0		10 Time[min]	20 1)	30

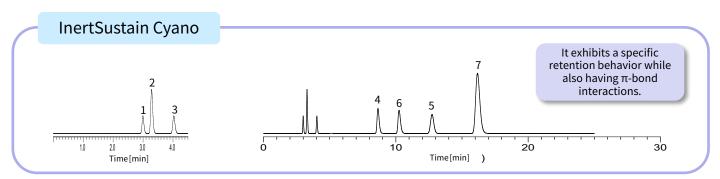
Time[min] n)

# 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.

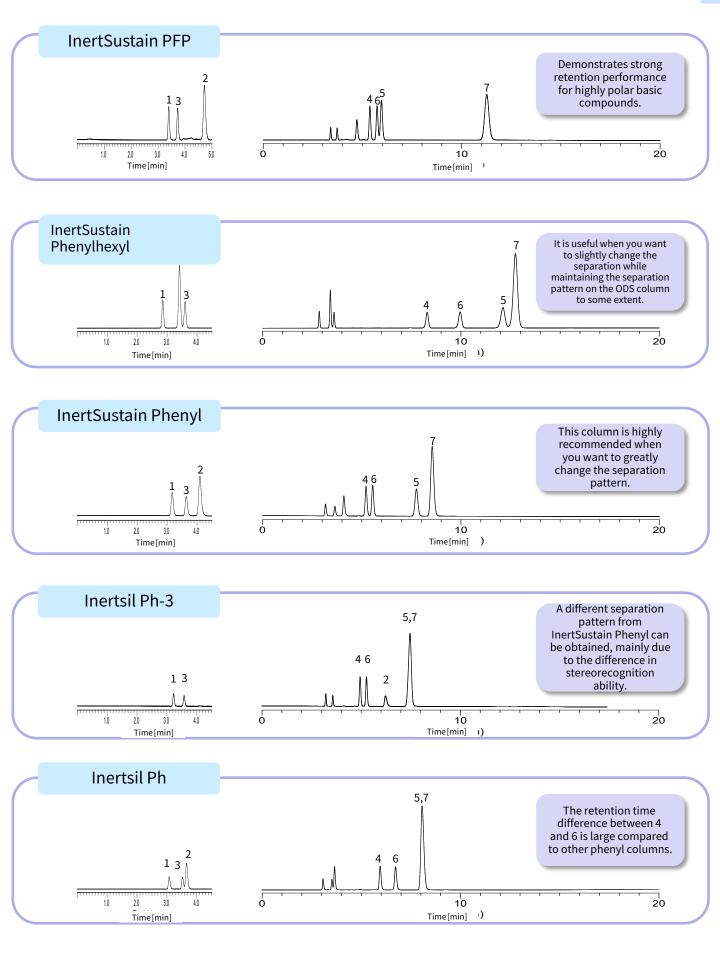
column name	chemical bonding group	E.C.	carbon loading		surface area
Inertsil C4	butyl group	Yes	7.5%	150 Å	320 m²/g
Inertsil WP300 C4	butyl group	No	3%	300 Å	150 m²/g
InertSustain Cyano	cyanopropyl group	Yes	8%	100 Å	350 m²/g
InertSustain PFP	Pentafluorophenylpropyl group	o Yes	10%	100 Å	350 m²/g
InertSustain Phenylhe	xyl 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





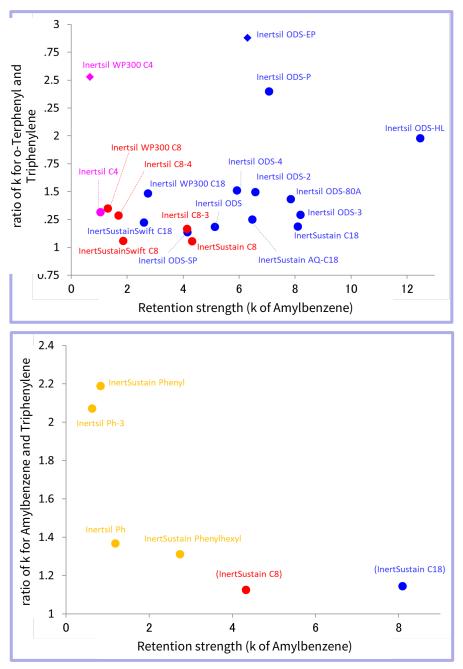
Time[min]

n)



### 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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