Inertsil HPLC Columns for Reversed Phase Separation (2)

HPLC has become the dominant analytical separation tools in all industries and laboratories. Numerous HPLC columns are commercially available in the market, but it is difficult to choose the correct HPLC column for your application needs. In the previous note (No. 50), we introduced various chromatograms comparing several reversed phase Inertsil HPLC columns, which was well-received.

Since then we released several new HPLC columns, such as InertSustain C18. Hence, this note is an updated version to the previous note which all reversed phase Inertsil columns are shown and compared again.

(C. Aoyama)

Columns compared in this note

Column name	Functional group	Features
InertSustain C18		InertSustain C18 inherits the advantages of all the current Inertsil HPLC columns, but now can be used for wide pH analysis with consistent performance.
Inertsil ODS-4		It is excellent at inertness. In general, elution time of Inertsil ODS-4 is shorter than InertSustain C18 and ODS-3.
Inertsil ODS-3		An ODS column released in 1994, but still is heavily used all over the world because of its strong hydrophobic property and excellent reproducibility.
Inertsil ODS-SP		The carbon load is controlled to elute highly hydrophobic analytes in shorter time.
Inertsil ODS-EP		Contains a polar group embedded between an ODS group and silica. Compared with ordinary ODS columns, this column often delivers unique selectivity.
Inertsil ODS-P		Bonding density of ODS group is very high. As its steric selectivity is quite high, this column is useful for separation of similar compounds.
Inertsil WP300 C18		It has larger pore on the surface of silica than ordinary columns. High-molecular-weight compounds can be eluted as sharp peaks.

Column name	Functional group	Features
Inertsil C8-4		Inertsil C8-4 is an octyl group (C8) bonded column providing the same separation pattern and extreme inertness to any type of compounds just like Inertsil ODS-4. Recommended as the first choice C8 column.
Inertsil C8-3		Bonding density of octyl group is higher than C8-4. Hydrophobic retention is strong among C8 columns.
Inertsil WP300 C8		We recommend it as the first choice for high-molecular-weight compounds (MW > 5000). Its pore size is the same as that of WP300 C18.
Inertsil Ph-3		Phenyl group is directly bonded to the silica. Unique selectivity is often delivered, which can be different from that of ODS columns because of the interaction caused by π electron.

① Comparison of ODS columns

Physical properties of our ODS columns are shown below. The comparison results are in the following pages, where enlarged chromatograms (between 0 min and 4.5 min) are shown on the left side and full scale chromatograms are on the right side.

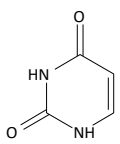
Column name	Stationary phase	End-capping	Carbon load	Pore size	Surface area
InertSustain C18	ODS	Yes	14 %	100 Å	350 m^2/g
Inertsil ODS-4	ODS	Yes	11 %	100 Å	450 m^2/g
Inertsil ODS-3	ODS	Yes	15 %	100 Å	450 m^2/g
Inerstil ODS-SP	ODS	Yes	8.5 %	100 Å	450 m^2/g
Inertsil ODS-EP	ODS	No	9 %	100 Å	450 m^2/g
Inertsil ODS-P	ODS	No	29 %	100 Å	450 m^2/g
Inertsil WP300 C18	ODS	Yes	9 %	300 Å	150 m^2/g

HPLC condition

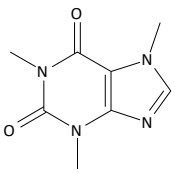
- Column : Columns for reversed-phase mode (5 µm, 250 × 4.6 mm I.D.)
- Eluent : A) CH_3OH B) H_2O A/B= 80/20, v/v , 1.0 mL/min
- Temperature : 40 ℃
- Detection : UV 254 nm
- Injection Volume : 5 µL

The mobile phase consists of water and methanol. Column dimension and particle size are the same for the all column used in this examination.

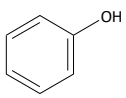
Compounds used for the comparison and their chemical structures



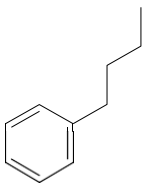
1. Uracil



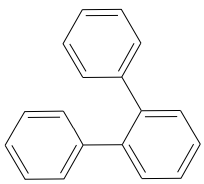
2. Caffeine



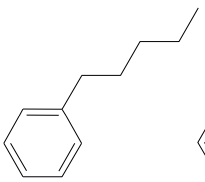
3. Phenol



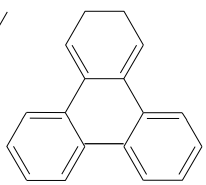
4. Butylbenzene



5. *o*-Terphenyl



6. Amylbenzene



7. Triphenylene

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

The sample solution consists of basic compounds, acidic compounds, alkylbenzens, and polycyclic aromatic hydrocarbons. The retention times and the elution order clarify features of ODS columns.

Sample No.1, Uracil cannot be retained in a reversed phase mode when using the mobile phase condition described on the right. Therefore, Uracil was used to determine the t0 position.
t0: Void Volume

Sample No.2, 3, Caffeine and Phenol are used to confirm the amount of residual silanol on the surface of the silica gel. Caffeine elutes later against Phenol when there is a lot of residual silanol on the surface of the silica gel. *

Sample No.4, *n*-Butylbenzene and Sample No.6, *n*-Amylbenzene were used to determine the hydrophobic property of the column. n-Amylbenzene elu later against *n*-Butylbenzene when the hydrophobicity of the column is high.

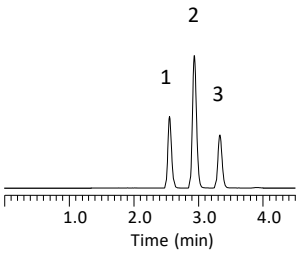
Stereoselectivity is indicated by Sample No.5, *o*-Terphenyl and Sample No.7, Triphenylene. *o*-Terphenyl has a twisted steric structure and Triphenylene has a planar structure.
Triphenylene elutes later against *o*-Terphenyl when the stereoselectivity of the column is high.

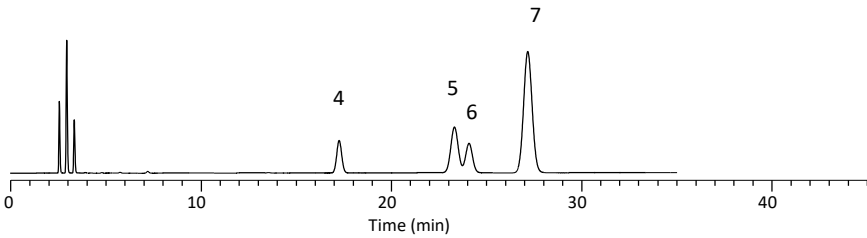
* Reference;
K. Kimata, K. Iwaguchi, S. Onishi, K. Jinno, R. Eksteen, K. Hosoya, M. Araki, N.Tanaka, J. Chromatogr. Sci. 1989, 27, 721-728.

1. Uracil 2. Caffeine 3. Phenol 4. Butylbenzene 5. *o*-Terphenyl 6. Amylbenzene 7. Triphenylene

InertSustain C18

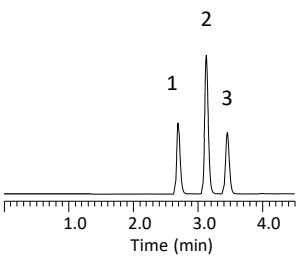
It is an excellent ODS column in various aspects.

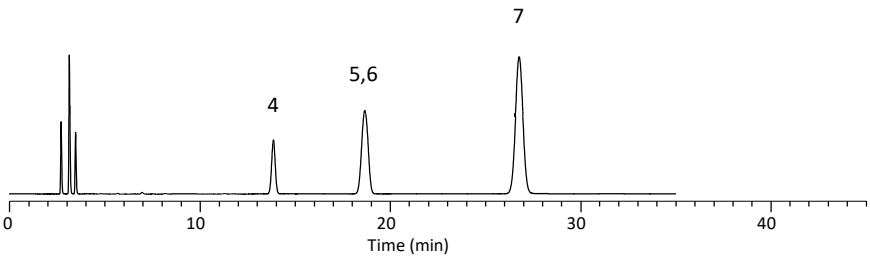




Inertsil ODS-4

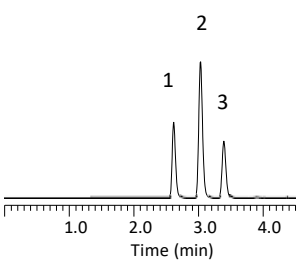
Compared to InertSustain C18, retentivity is a little weaker and steric selectivity is higher.

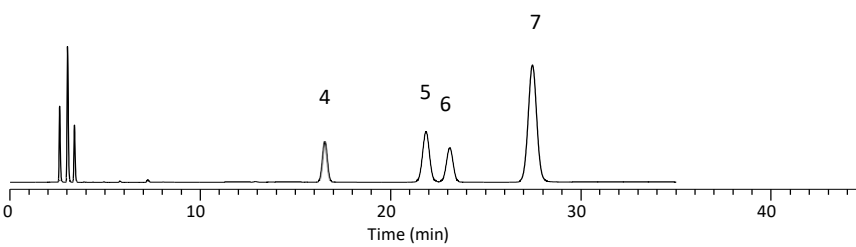




Inertsil ODS-3

Selectivity of this column is relatively similar to that of InertSustain C18.



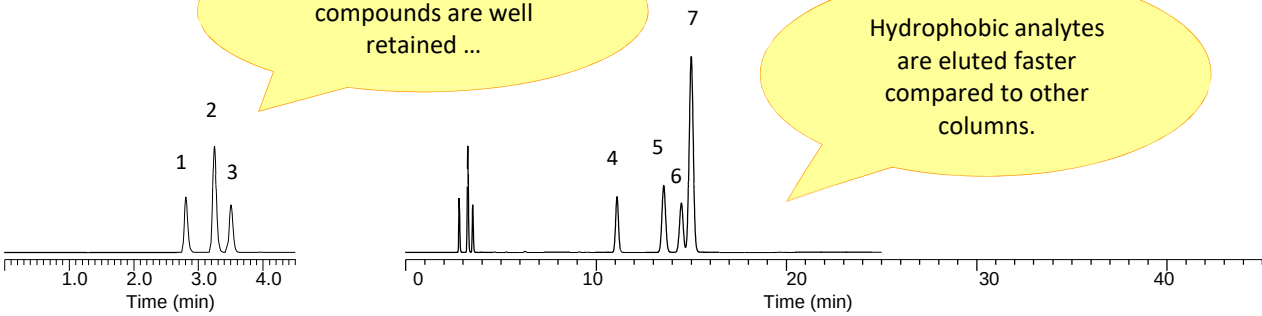


1. Uracil 2. Caffeine 3. Phenol 4. Butylbenzene 5. *o*-Terphenyl 6. Amylbenzene 7. Triphenylene

Inertsil ODS-SP

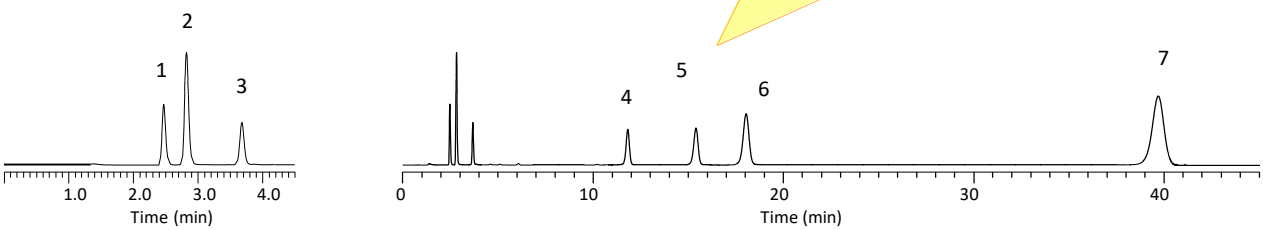
Although hydrophilic compounds are well retained …

Hydrophobic analytes are eluted faster compared to other columns.



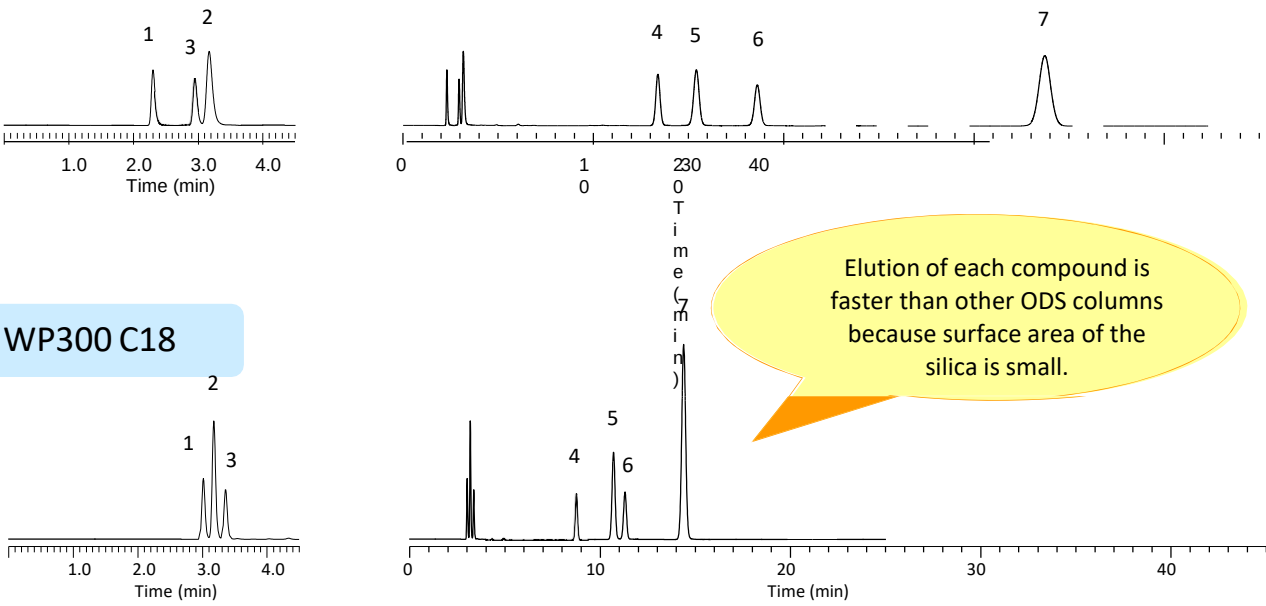
Inertsil ODS-EP

Selectivity of this column is significantly different from those of other ODS columns, while hydrophobic retention is moderate.



Inertsil ODS-P

As steric selectivity is very high, the retention time ratio of 7 to 5 is larger than those of other columns.



Inertsil WP300 C18

Elution of each compound is faster than other ODS columns because surface area of the silica is small.

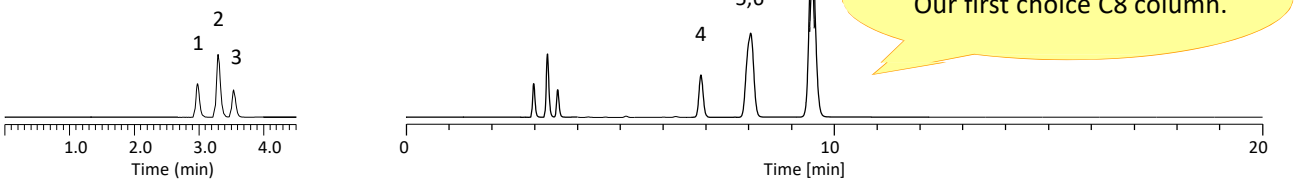
② Comparison of C8 and phenyl columns

Physical properties of our C8 and phenyl columns are shown below. The comparison results are also obtained under the same condition as the ODS columns.

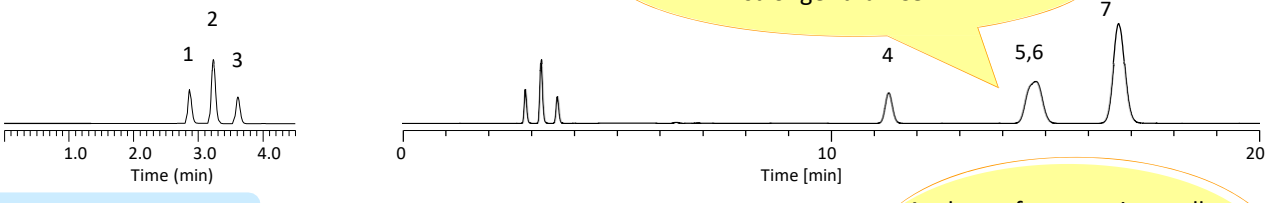
Inertsil C8-4 Inertsil	C 8	Y e s	5 %	100 Å	450 m²/g
C8-3 Inertsil	C 8	Y e s	9 %	100 Å	450 m²/g
WP300 C8 Inertsil	C8	Y e s	4 %	300 Å	150 m²/g
Ph-3	Ph	No	9.5 %	100 Å	450 m²/g

1. Uracil 2. Caffeine 3. Phenol 4. Butylbenzene 5. *o*-Terphenyl 6. Amylbenzene 7. Triphenylene

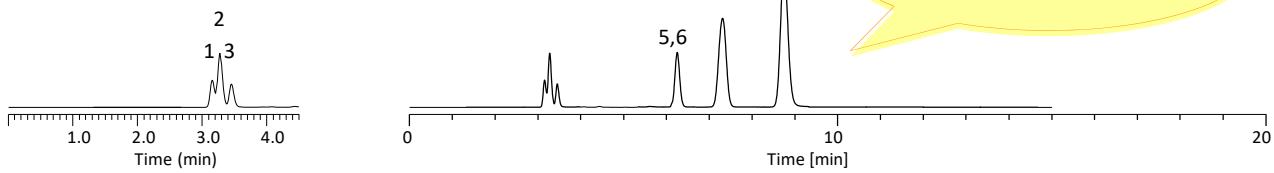
Inertsil C8-4



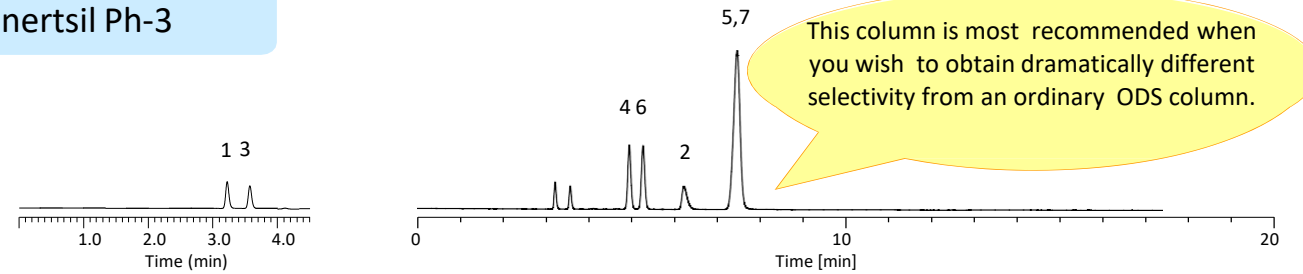
Inertsil C8-3



Inertsil WP300 C8



Inertsil Ph-3



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