












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 (M.W. > 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.

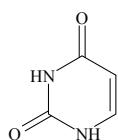
| Column name | Stationary phase | End-capping | Carbon load | Pore size | Surface area |
|--------------------|------------------|-------------|-------------|-----------|-----------------------|
| InertSustain C18 | ODS | Yes | 14% | 100 Å | 350 m ² /g |
| Inertsil ODS-4 | ODS | Yes | 11% | 100 Å | 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

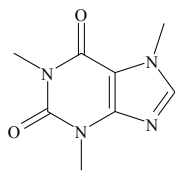
- Column : Columns for reversed-phase mode
(5 μm, 250 × 4.6 mm I.D.)
- Eluent : A) CH₃OH B) H₂O
A/B= 80/20, v/v , 1.0 mL/min
- Temperature : 40 °C
- Detection : UV 254 nm
- Injection Volume : 5 μL

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

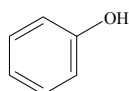
Compounds used for the comparison and their chemical structures



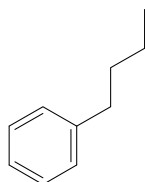
1. Uracil



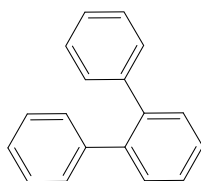
2. Caffeine



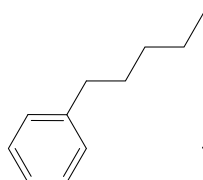
3. Phenol



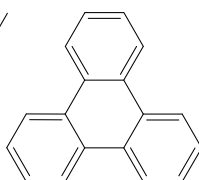
4. Butylbenzene



5. o-Terphenyl



6. Amylbenzene



7. Triphenylene

The sample solution consists of basic compounds, acidic compounds, alkylbenzenes, 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 elutes later against n-Butylbenzene when the hydrophobicity of the column is high.

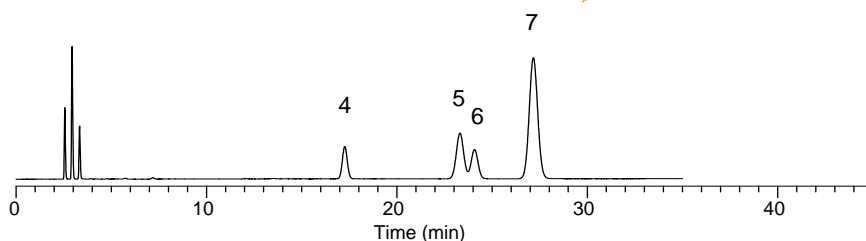
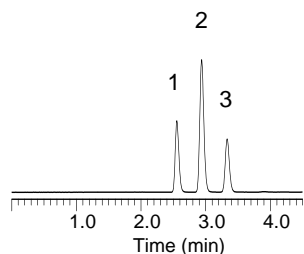
Stereoselectivity is indicated by Sample No.5, o-Terphenyl and Sample No.7, Triphenylene. o-Terphenyl has a twisted steric structure and Triphenylene has a planar structure. Triphenylene elutes later against o-Terphenyl when the stereoselectivity of the column is high.

* Reference;

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

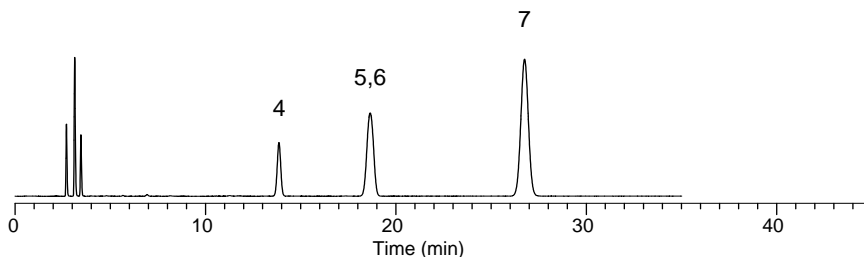
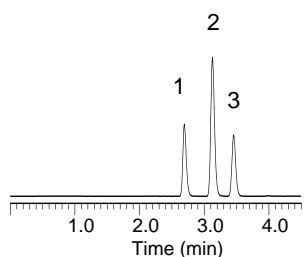
1. Uracil 2. Caffeine 3. Phenol 4. Butylbenzene 5. *o*-Terphenyl 6. Amylbenzene 7. Triphenylene

InertSustain C18



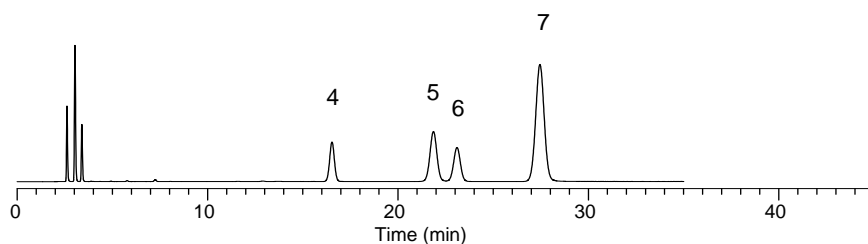
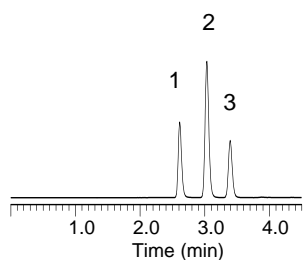
It is an excellent ODS column in various aspects.

Inertsil ODS-4



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

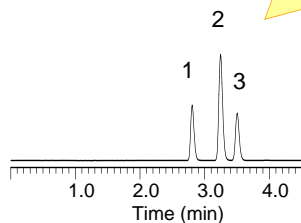
Inertsil ODS-3



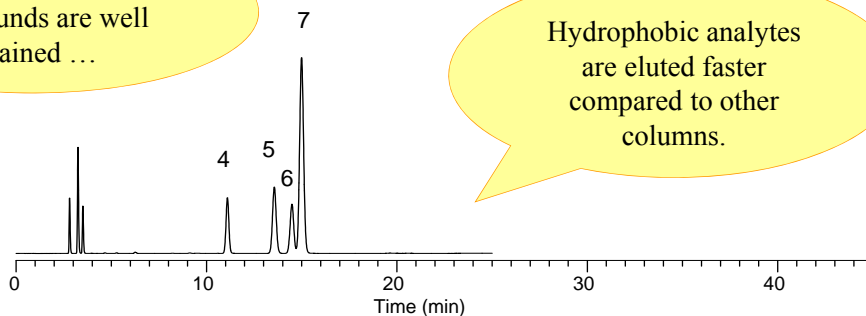
Selectivity of this column is relatively similar to that of InertSustain C18.

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

Inertsil ODS-SP

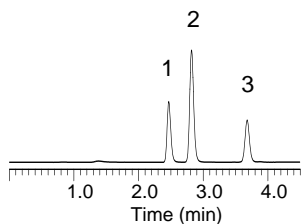


Although hydrophilic compounds are well retained ...

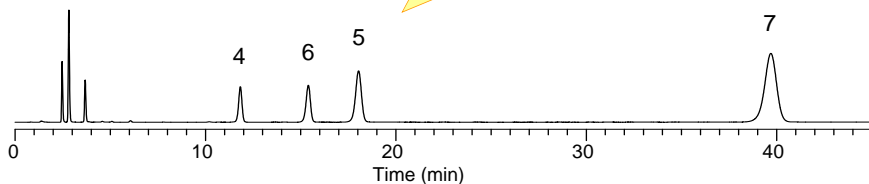


Hydrophobic analytes are eluted faster compared to other columns.

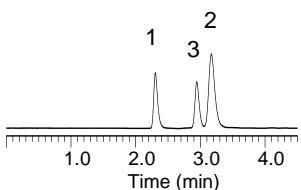
Inertsil ODS-EP



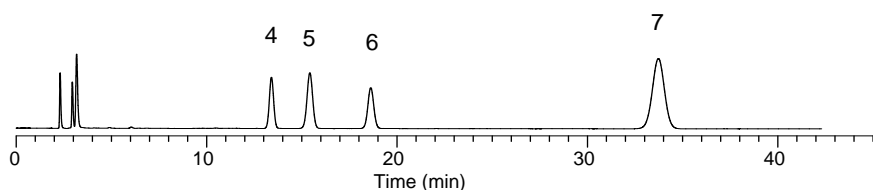
Selectivity of this column is significantly different from those of other ODS columns, while hydrophobic retention is moderate.



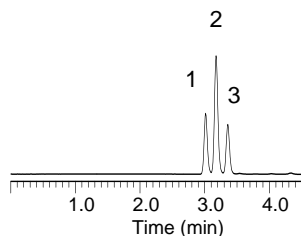
Inertsil ODS-P



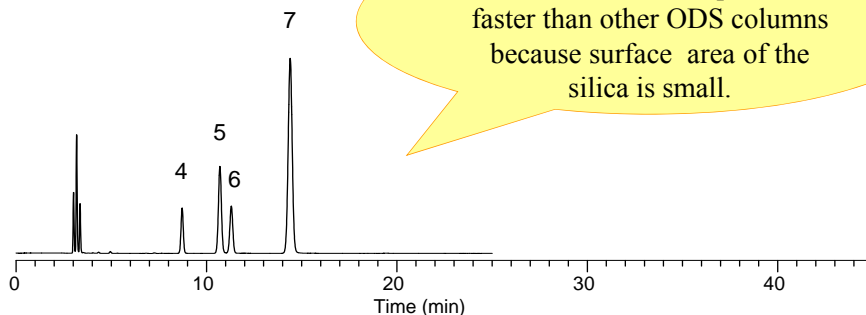
As steric selectivity is very high, the retention time ratio of 7 to 5 is larger than those of other columns.



Inertsil WP300 C18



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



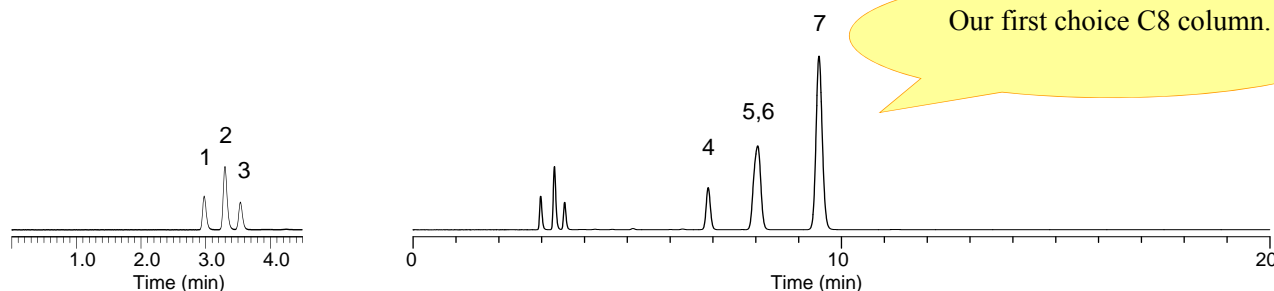
② Comparison of C8 and phenyl columns

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.

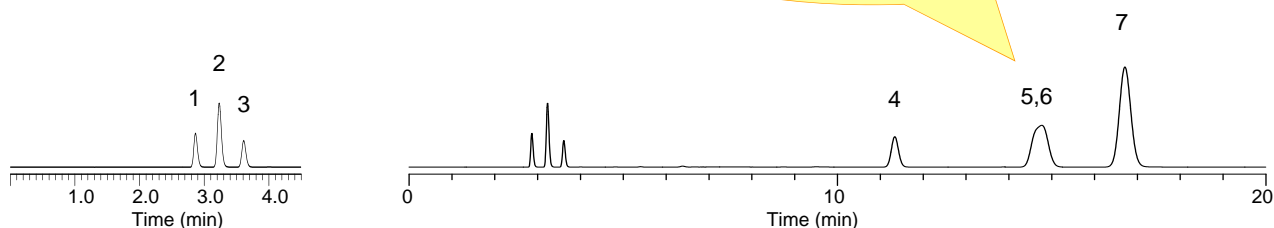
| Column name | Stationary phase | End-capping | Carbon load | Pore size | Surface area |
|-------------------|------------------|-------------|-------------|-----------|-----------------------|
| Inertsil C8-4 | C8 | Yes | 5% | 100 Å | 450 m ² /g |
| Inertsil C8-3 | C8 | Yes | 9% | 100 Å | 450 m ² /g |
| Inertsil WP300 C8 | C8 | Yes | 4% | 300 Å | 150 m ² /g |
| Inertsil Ph-3 | Ph | No | 9.5% | 100 Å | 450 m ² /g |

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

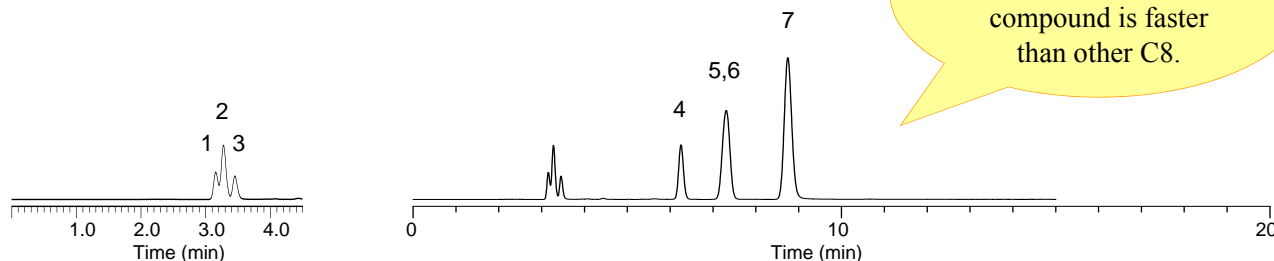
Inertsil C8-4



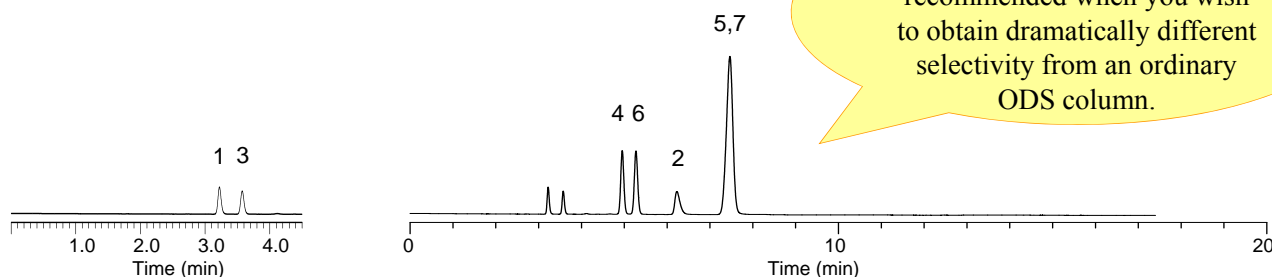
Inertsil C8-3



Inertsil WP300 C8



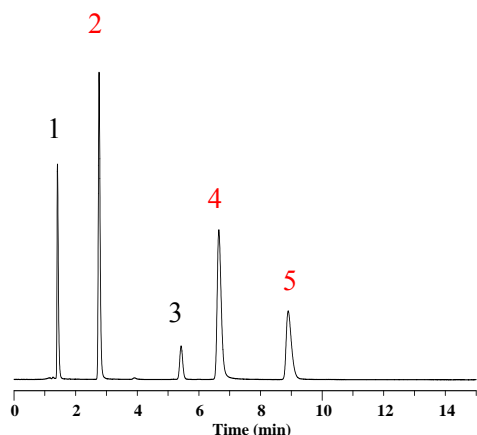
Inertsil Ph-3



In closing...

InertSustain C18 is the most recommended column because it not only offers strong hydrophobic retention but also has various advantages. Followings are the examples.

<Peak shape of basic compounds>



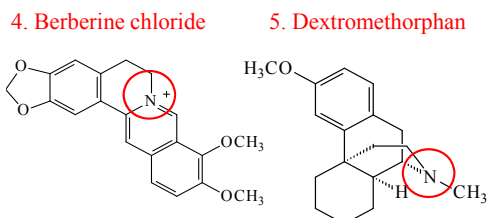
Peak 2, 4, and 5 are basic compounds. If silanol groups even slightly exist on surface of packed particles, the peak shapes, particularly 4 and 5, are likely to be adsorbed.

Conditions

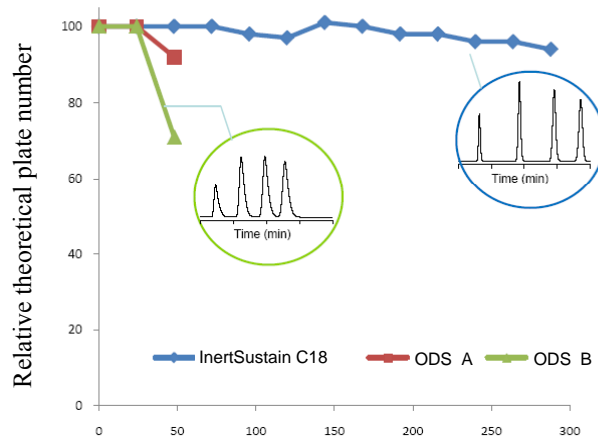
Column : InertSustain C18 (3 μ m, 2.1 x 150 mm)
 Eluent : A) CH₃CN
 B) 25 mM K₂HPO₄ (pH 7.0, KH₂PO₄)
 A/B = 30 / 70, v/v
 Col. Temp. : 40°C
 Detection : UV 230 nm
 Flow Rate : 0.2 mL/min

Sample;

- 1: Uracil
- 2: Pyridine
- 3: Phenol
- 4: Berberine chloride
- 5: Dextromethorphan



<Durability under an alkaline condition>



Hours of Purging of alkaline solution

Excellent durability of InertSustain C18 under alkaline conditions was achieved by combining improved Inertsil technology with newly-developed synthesis method for silica gel.

Conditions for purging alkaline solution

Eluent : 50 mM Triethylamine (pH10.0) /
 CH₃OH = 70/30, v/v
 Flow Rate : 1.0 mL/min
 Col. Temp. : 50°C

Conditions for obtaining the number of theoretical plates

Column : 5 μ m, 150 x 4.6 mm I.D.
 Eluent : CH₃CN/H₂O = 65/35, v/v
 Flow Rate : 1.0 mL/min
 Col. Temp. : 40°C
 Detection : UV 254 nm
 Sample : Naphthalene

Contact us

GL Sciences, Inc. Japan

22-1 Nishishinjuku 6-chome, Shinjuku-ku, Tokyo, 163-1130 Japan

TEL: +81-3 (5323)6620 FAX: +81-3 (5323)6621

GL Sciences, Inc. USA

4733 Torrance Blvd. Ste 255, Torrance, CA 90503

Tel: (310)265-4424 FAX (310)265-4425

Distributors Outside of Japan and USA

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<http://www.glsciences.com/products/contact.html>

E-MAIL: world@glsc.co.jp

