TOSOH

SEPARATION REPORT NO. 102

TSKgel ODS-100V AND ODS-100Z REVERSED PHASE HPLC COLUMNS

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1. Introduction

Reversed phase chromatography (RPC) is the most frequently utilized mode of HPLC, because:

(1) the combination of high column efficiency and selectivity allows rapid separations of complex mixtures,
(2) compounds that can be analyzed with the technique ranging from very hydrophobic to hydrophilic.
(3) the technique is reproducible in terms of retention, selectivity and column efficiency, and
(4) RPC is a robust technique that's easy to use.

Octadecylsilyl (ODS or C18) columns are most often utilized in RPC. ODS columns are packed with spherical silica gel particles that have been derivatized with octadecylsilane reagent. Only about half of the available silanol groups on the silica surface can react due to steric hindrance. When the mobile phase pH is above 4, unreacted silanol groups on the C18-bonded surface can dissociate, allowing for secondary interactions with positively charged compounds. This can lead to peak tailing and loss of accuracy in quantification. Various endcapping techniques to cover accessible silanol groups are available to prevent or reduce secondary interactions with residual silanol groups.

Tosoh scientists have recently developed two new ODS-type columns with different surface properties utilizing highly efficient bonding and endcapping procedures. In this Separation Report, the properties of the two novel ODS packing materials and their chromatographic performance are shown along with extensive application data.

2. Specifications and characteristics

Table 1 shows the available TSKgel ODS-100V and TSKgel ODS-100Z columns packed with three or five micron diameter particles. Additional three micron columns will be available later this year.

The 4.6mm I.D. columns are most suitable for general analysis, while the 2mm I.D. columns are intended for use in LC-MS or when faced with limited sample mass.

The characteristics of the TSKgel ODS-100V and TSKgel ODS-100Z columns are discussed below.

Characteristics

- Spherical, porous, silica particles with nominal particle size of 3µm or 5µm.
- Two different surface chemistries for the bonding and endcapping reactions,
- Similar basic separation properties, but different selectivity for all but non-polar compounds,
- Superior peak shapes for basic, acidic and chelating compounds,
- Minimal lot-to-lot variability,
- Columns from three different bonding lots are available for method development support,
- A gel-batch test report (Certificate of Analysis) is provided with each column, showing the results of sensitive test solutes under five different mobile phase conditions.
- All columns are USP L1 compliant and are available worldwide.

Characteristics of TSKgel ODS-100V

- Medium carbon content (15%) and high surface polarity, which promotes relatively strong retention of hydrophilic compounds.

Characteristics of TSKgel ODS-100Z

- High carbon content (20%) and low surface polarity, which favors strong retention of moderately and highly hydrophobic compounds.

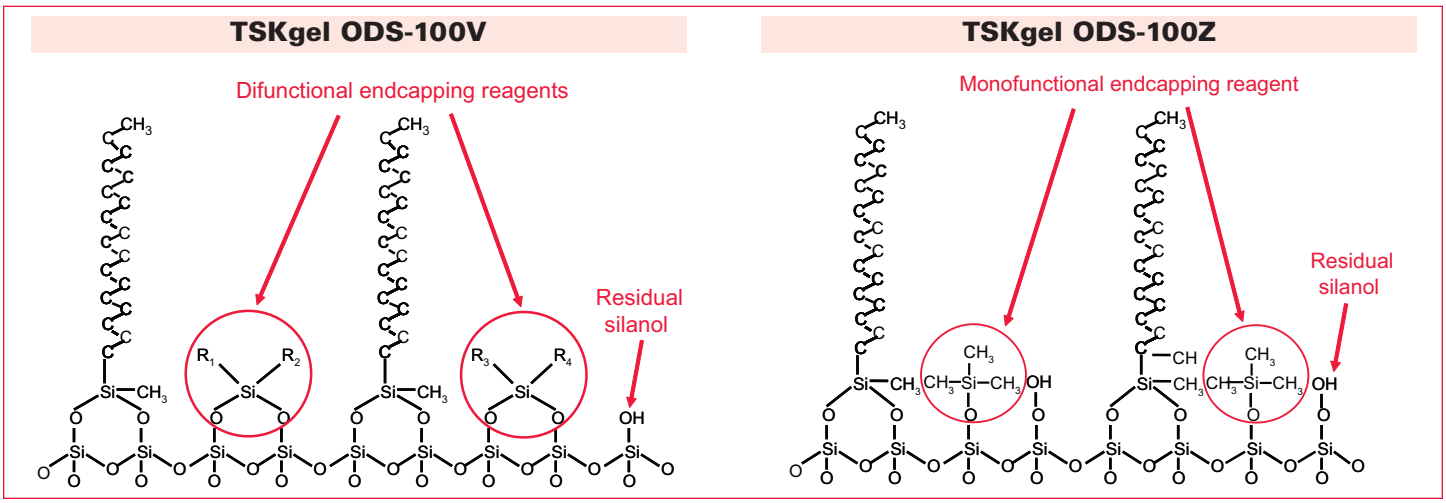
Table 1: TSKgel ODS-100V and TSKgel ODS-100Z Product Line

Part Number	Product / Product Name	Particle Size, µm	Column Dimensions
21810	TSKgel ODS-100V	3	2.0mm x 15cm
21811	TSKgel ODS-100V	3	2.0mm x 7.5cm
21812	TSKgel ODS-100V	3	2.0mm x 5cm
21813	TSKgel ODS-100V	3	2.0mm x 3.5cm
21814	TSKgel ODS-100V, 3pk	3	2.0mm x 1.0cm
19308	TSKgel Holder for P/N 21814		
21455	TSKgel ODS-100V	5	4.6mm x 15cm
21456	TSKgel ODS-100V	5	4.6mm x 25cm
21457	TSKgel ODS-100V	5	2.0mm x 5cm
21458	TSKgel ODS-100V	5	2.0mm x 15cm
21453	TSKgel ODS-100V Guard Cartridge, 3pk	5	3.2mm x 1.5cm
19018	TSKgel Guard Cartridge Holder for P/N 21453		
21461	TSKgel ODS-100Z	5	4.6mm x 15cm
21462	TSKgel ODS-100Z	5	4.6mm x 25cm
21460	TSKgel ODS-100Z	5	2.0mm x 5cm
21459	TSKgel ODS-100Z	5	2.0mm x 15cm
21454	TSKgel ODS-100Z Guard Cartridge, 3pk	5	3.2mm x 1.5cm
19018	TSKgel Guard Cartridge Holder for P/N 21454		

Table 2: Physical Properties

Packing Material	Particle size (micron)	Pore size (nm)	Pore Vol. (mL/g)	Surface Area (m^2/g)	Functional Group	Carbon Content (%)	Phase Structure	Endcapped
TSKgel ODS-100V	3, 5	10	1.10	450	C18	15	Monolayer	Yes
TSKgel ODS-100Z	5	10	1.10	450	C18	20	Monolayer	Yes

Figure 1: Bonded phase structures



3. Properties of the packing materials

Table 2 shows the properties of the TSKgel ODS-100V and TSKgel ODS-100Z columns. Both product lines contain reversed phase packing materials with a nominal particle diameter of 3µm or 5µm and 100Å pores. The specific surface area of the base silica, 450 m^2/g, is larger than the surface area of most commercially available reversed phase columns, which are usually in the range of 175-350m^2/g. The high surface area coupled with a relatively low particle density translates into a high carbon content (%C/g silica), resulting in beneficial retention properties.

The novel bonding chemistries employed in the preparation of TSKgel ODS-100V and TSKgel ODS-100Z are depicted in *Figure 1*.

TSKgel ODS-100Z is prepared by bonding the surface with a difunctional octadecylsilane reagent, followed by repeated endcapping with monofunctional trimethylsilane reagent. TSKgel ODS-100V is prepared by an incomplete first reaction with a difunctional octadecylsilane reagent, which is followed by endcapping with a mixture of two difunctional dialkylsilane reagents. Both materials are made under conditions that promote the formation of a monomeric bonded phase layer.

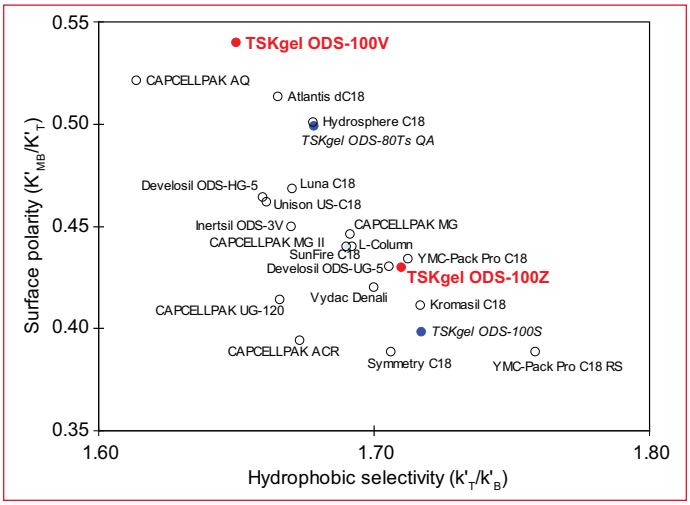
The carbon content of TSKgel ODS-100Z is 20%. The ODS-100Z stationary phase is very hydrophobic and retains low molecular weight organic compounds very strongly. In contrast, the carbon content of TSKgel ODS-100V is 15%. For a reversed phase packing material the polarity of the ODS-100V stationary phase is high, which favors the retention of polar low molecular weight organic compounds.

Chromatographic properties, such as the theoretical plate number, asymmetry factor and pressure-flow characteristics, are related to the base silica, which is the same for ODS-100V and ODS-100Z. Retention and selectivity properties (hydrophobic, polar, and steric), which are related to the chemical composition of the stationary phase, vary between the two columns. The ion exchange activity, an indicator for the activity of remaining silanol groups, is comparable between the ODS-100V and ODS-100Z columns because of the effectiveness of the endcapping procedures.

Figure 2 shows the relationship between hydrophobicity and surface polarity parameters for TSKgel ODS-100V, TSKgel ODS-100Z and other commercial ODS columns.

The hydrophobic selectivity of ODS-100Z is relatively high, while surface polarity is moderate. On the other hand, the hydrophobicity of ODS-100V is low, and its surface polarity is high.

Figure 2: Hydrophobic selectivity and surface polarity of commercial ODS columns



4. Chromatographic properties

4-1. Column efficiency

Figure 3 compares the theoretical plate number (N) for naphthalene calculated from its retention time and peak width on the various ODS columns. The theoretical plate numbers for TSKgel ODS-100V and ODS-100Z are among the highest of all columns studied.

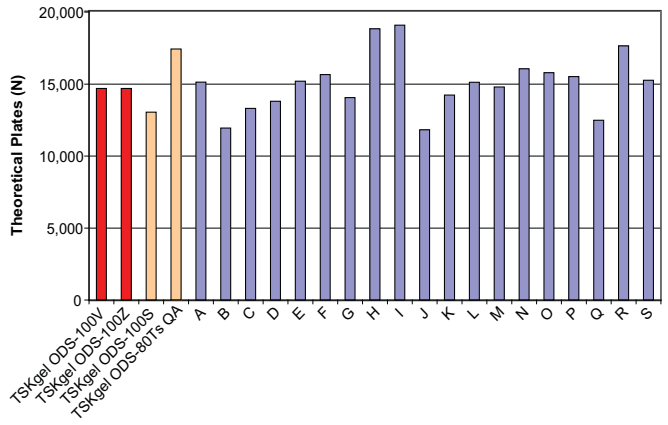
4-2. Retention

Figure 4 compares naphthalene retention among the various ODS columns under the conditions of *Figure 3*. The retention of a neutral hydrophobic compound such as naphthalene depends mainly on carbon content. Since the carbon content of ODS-100Z is approximately 20%, naphthalene is strongly retained. On the other hand, the carbon content of ODS-100V is approximately 15%, and its naphthalene retention is average among the ODS columns tested.

4-3. Column pressure drop

Figure 5 compares column back pressure in 30% water/70% methanol. The pressure drop of ODS-100V and ODS-100Z is moderate compared to other commercial ODS columns. The fact that ODS-100V and ODS-100Z exhibit a high number of theoretical plates, as shown in *Figure 3*, but low back pressure compared to most ODS columns, suggests a narrow particle size distribution, which results in a homogenous packed bed with excellent pressure-flow characteristics.

Figure 3. Comparison of theoretical plate numbers



Columns:	(all 4.6mmID x 15cm) TSKgel ODS-100V, TSKgel ODS-100Z, TSKgel ODS-100S, TSKgel ODS-80Ts QA, A) L-Column, B) Inertsil ODS-3V, C) CAPCELLPAK UG-120, D) CAPCELLPAK ACR, E) CAPCELLPAK MG, F) CAPCELLPAK MGII, G) CAPCELLPAK AQ, H) YMC-Pack Pro C18, I) YMC-Pack Pro C18 RS, J) Luna C18, K) Develosil ODS-UG-5, L) Develosil ODS-HG-5, M) Symmetry C18, N) SunFire C18, O) Kromasil C18, P) Vydac Denali, Q) Atlantis dC18, R) Hydrosphere C18, S) Unison US-C18
Mobile phase:	H_2O/MeOH (30/70)
Flow rate:	1.0mL/min
Temp:	40°C
Inj. Volume:	10µL
Sample:	Naphthalene

4-4. H - u curve (van Deemter curve)

Figure 6 shows the relationship between linear velocity and the height equivalent of a theoretical plate (HETP, or, H) on a 15cm x 4.6mm TSKgel ODS-100Z column.

When using a methanol-containing mobile phase (o), column efficiency has a broad optimum (minimum HETP value) at a linear velocity of about 5cm/min or a flow rate of about 0.5mL/min. On the other hand, when using a less viscous acetonitrile-containing mobile phase, (Δ), optimum column efficiency shifts to a linear velocity of about 10cm/min or a flow rate of about 1.0mL/min). Furthermore, the H - u curve was shallower in the acetonitrile-containing mobile phase. Thus, to obtain the highest column efficiency it is best to use an aqueous-acetonitrile mobile phase rather than an aqueous-methanol mobile phase. In practice, the flow rate is usually selected based on the column back pressure and the recommended flow rate range as stated in the Operating Conditions and Specifications (OCS) sheet that accompanies the column.

Footnote: Linear velocity (u, cm/min) is the linear distance an unretained solute travels through the column per unit time. It is calculated by dividing the column length by the retention time of the unretained solute.

Figure 4. Comparison of retention

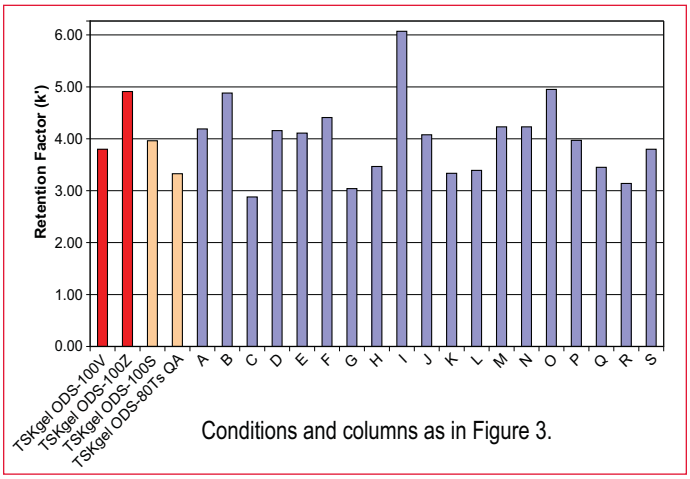


Figure 5. Comparison of column pressure drop

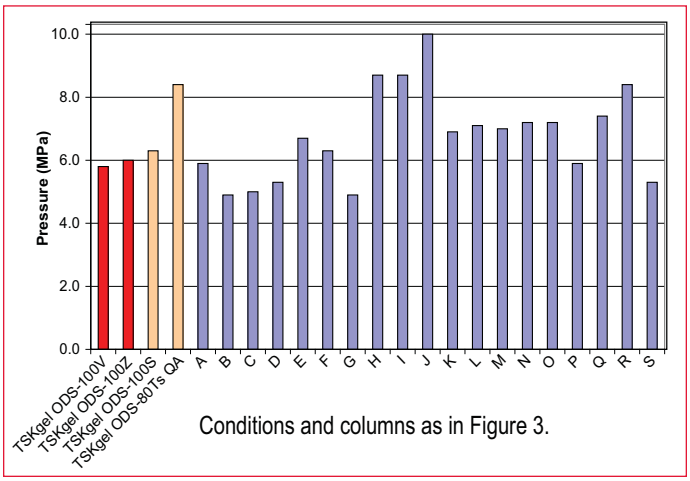
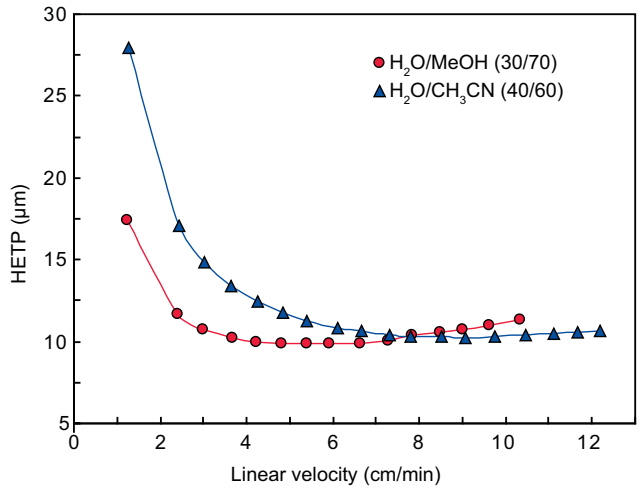
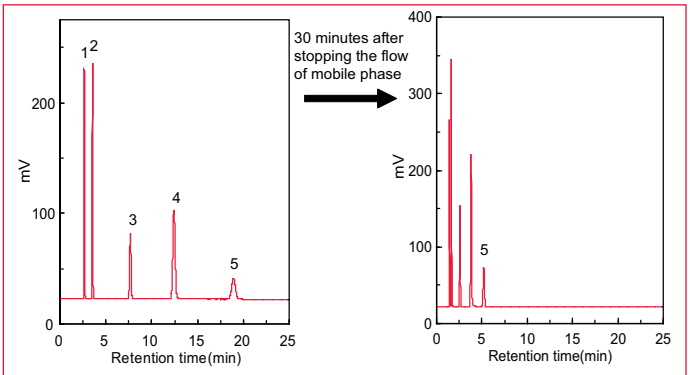


Figure 6. Relationship between HETP and linear velocity



Column:	TSKgel ODS-100Z (4.6mmID x 15cm)
Mobile phase:	H_2O/MeOH (30/70)
	H_2O/CH_3CN (40/60)
Temp:	40°C
Inj. Volume:	10μL
Sample:	Naphthalene

Figure 7. Retention decreases when using a buffer without organic solvent as mobile phase (typical ODS column)



Column:	ODS (pore size: 100Å)
Size:	4.6mmID x 15cm
Mobile phase:	10mmol/L phosphate buffer, pH 6.8
Flow rate:	1.0mL/min
Temp:	40°C
Detection:	UV 260 nm
Inj. Volume:	10μL
Samples:	1. Cytosine, 2. Uracil, 3. Uridine, 4. Guanosine, 5. Adenine

Figure 8. Schematic diagrams to illustrate loss of retention in mobile phase without organic solvent

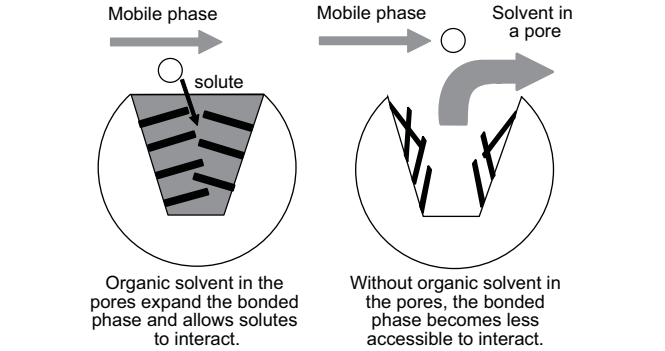
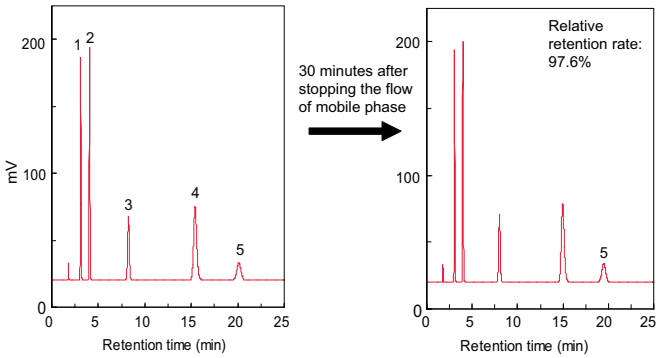


Figure 9. Minimal retention loss when using a mobile phase without an organic modifier (ODS-100V)



Column:	TSKgel ODS-100V, 5μm
Size:	4.6mmID ×15cm
Mobile phase:	10mmol/L Phosphate buffer, pH 6.8
Flow rate:	1.0mL/min
Temp.	40°C
Detection:	UV(260nm)
Injection:	10μL
Sample:	1.Cytocine, 2.Uracil, 3.Uridine, 4. Adenine, 5. Guanosine

4-5. Retention in 100% aqueous mobile phase

In reversed phase chromatography using a porous packing material, sample components are partitioned between the stationary and mobile phase and are retained in the pores as a function of their hydrophobic properties. However, when the concentration of organic solvent in the mobile phase is very low or zero, sample retention is not always stable, as is shown in *Figure 7* for a standard C18 bonded phase column. At first the solutes were retained as expected (left panel), but after the flow was stopped for 30 minutes, solute retention could not be restored to their original values (right panel).

Footnote: Loss of retention can also be observed in 100% aqueous mobile phase without stopping (and restarting) the flow, although it requires a longer time to see a marked loss of retention.

This phenomenon has been explained as follows. When the concentration of organic solvent in the mobile phase is low (or when solvent polarity is high), the stationary phase cannot easily disperse into the mobile phase, and the C18 alkyl chains are more likely to adsorb onto each other by hydrophobic interaction, similar to the formation of oil droplets on a water surface. The net effect is that in the absence of organic solvent, water or buffer is pushed out of the pores, inhibiting sample molecules to enter the pores and be retained on the column. This is shown schematically in *Figure 8*.

When using a TSKgel ODS-100V column, rather than a standard C18 column, in the above experiment, sample retention is hardly affected after stopping the flow for 30 minutes, as is shown in *Figure 9*. This demonstrates that ODS-100V columns can be used with mobile phases that do not contain organic solvent, making this column type very suitable for analysis of very hydrophilic compounds.

4-6. Residual ion exchange activity

ODS phases are generally synthesized by chemically bonding C18 alkyl chains to silica gel. After the reaction has been completed, a relatively large number of silanol groups remain unreacted on the silica gel surface due to steric hindrance. These residual silanol groups tend to affect sample retention and peak shape. This section will describe how residual ion-exchange activity influences solute retention for basic and acidic compounds.

1) Effect on basic compounds

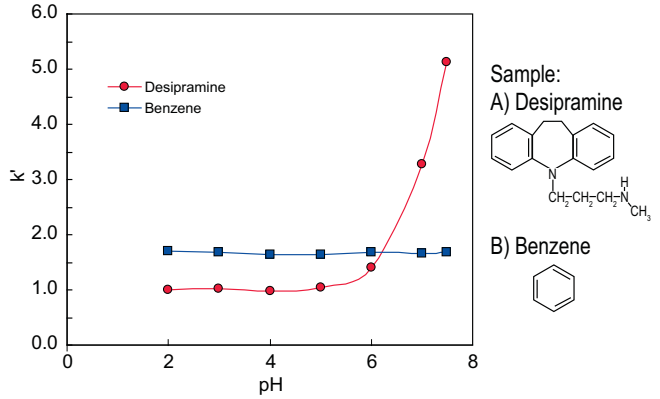
Figure 10 shows the changes in the retention of a neutral compound, benzene, and a basic compound, desipramine, on TSKgel ODS-100V at various mobile phase pH values. It is evident that the retention of benzene is not affected because its chemical properties do not change as a function of mobile phase pH. However, the retention of desipramine is affected, because the hydrophobicity of this compound increases at higher pH as fewer amino groups are protonated.

Using three TSKgel ODS columns differing in their endcapping procedures, changes in the retention and peak shape of desipramine were compared at various mobile phase pH values. The results are shown in *Figures 11 and 12*.

Figure 11 shows that as the pH of the mobile phase increases so does the retention time of desipramine on all TSKgel ODS columns. At pH ≥ 5, retention gradually increased for all three columns, but increased most for TSKgel ODS-80Ts QA. In comparison with TSKgel ODS-100V and TSKgel ODS-100Z, the endcapping efficiency of TSKgel ODS-80Ts QA is poor and the number of residual silanol groups is relatively high. Thus, when the pH of the mobile phase increases, the electrostatic interaction between the dissociated silanol groups and the amino groups of desipramine becomes more pronounced.

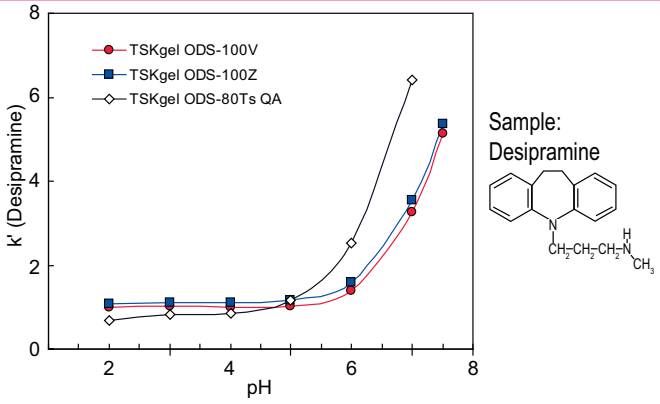
Figure 12 shows how the asymmetry factor of desipramine changes as a function of mobile phase pH. With ODS-80Ts QA, the higher the mobile phase pH, the greater the asymmetry factor for the desipramine peak. However, with TSKgel ODS-100V and to a lesser extent with TSKgel ODS-100Z, there were no distinct changes in asymmetry factor, and, irrespective of mobile phase pH, there was minimal peak tailing. The reason for this is that the endcapping efficiency of TSKgel ODS-100V and TSKgel ODS-100Z is very high, which reduces the number of accessible, residual silanol groups. Because there is very little peak tailing for basic compounds with both TSKgel ODS-100V and TSKgel ODS-100Z when using a neutral mobile phase, it is possible to analyze basic compounds even when they are strongly retained.

Figure 10. Retention as a function of mobile phase pH for a neutral and a basic compound



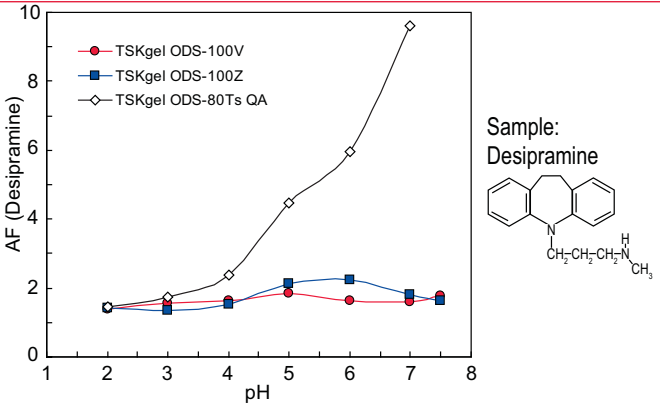
Column:	TSKgel ODS-100V (4.6mmID x 15cm)
Mobile phase:	50mmol/L phosphate buffer/MeOH (30/70)
Flow rate:	1.0mL/min
Temp:	40°C
Inj. Volume:	10µL
Sample:	A) Desipramine, B) Benzene

Figure 11. Retention as a function of pH for a basic compound on three TSKgel ODS columns



Column:	TSKgel ODS-100V, TSKgel ODS-100Z and TSKgel ODS-80Ts QA
Column size:	4.6mmID x 15cm
Mobile phase:	50mmol/L phosphate buffer/MeOH (30/70)
Flow rate:	1.0mL/min
Temp:	40°C
Inj. Volume:	10µL
Sample:	Desipramine

Figure 12. Asymmetry factor of a basic compound as a function of mobile phase pH



Conditions:	Same as in Figure 11

Figure 13 compares the asymmetry factor of basic compounds using a neutral or moderately acidic mobile phase among a large selection of commercial ODS columns. In comparison with other ODS columns, the asymmetry factor of TSKgel ODS-100V and TSKgel ODS-100Z demonstrates minimal peak tailing.

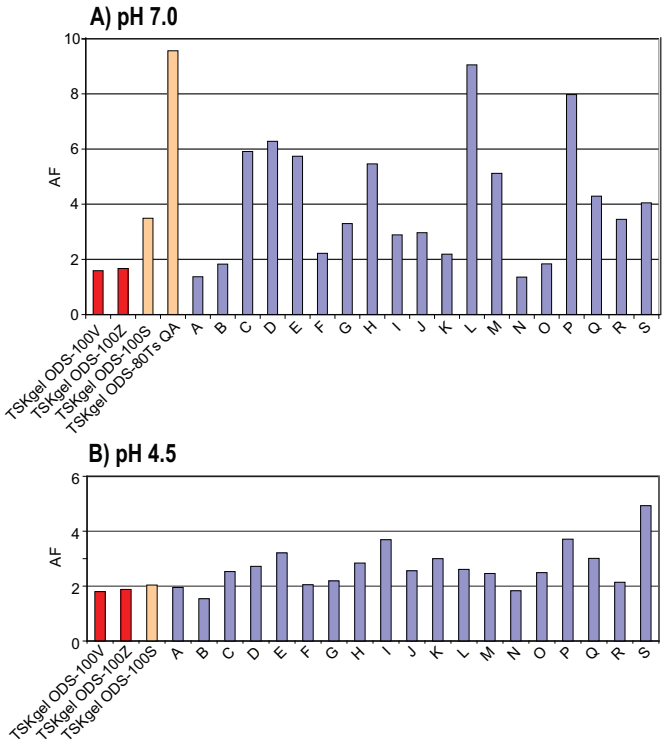
From *Figures 10-13* it is clear that the TSKgel ODS-100V and ODS-100Z columns are among the best performing C18 columns for the analysis of basic compounds at low and neutral pH values.

Figure 14 compares chromatograms of basic compounds on TSKgel ODS-100V with that of several commercial AQ-type (AQueous compatible) ODS columns. In contrast to AQ-type ODS columns, the TSKgel ODS-100V column showed minimal peak tailing for both desipramine and imipramine.

2) Effect on acidic compounds

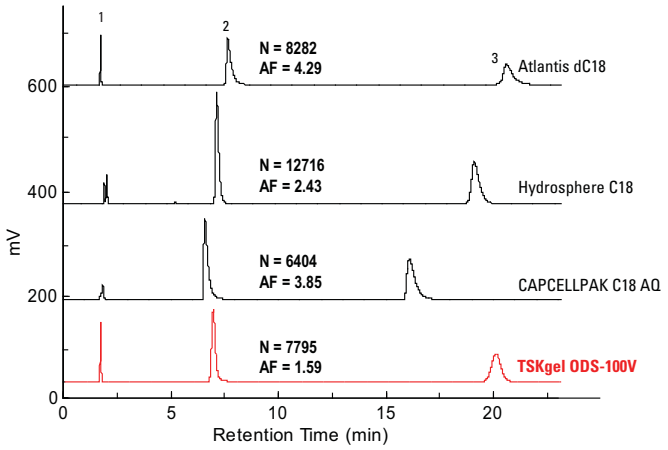
Figure 15 compares the asymmetry factor of formic acid, using an acidic mobile phase, for the same group of commercial columns. The peak shape of acidic compounds is generally symmetrical in an acidic mobile phase, but as shown in *Figure 15*, peak tailing was found on several commercial ODS columns. The asymmetry factor of formic acid on TSKgel ODS-100V and TSKgel ODS-100Z were close to one, indicating a symmetrical peak shape. *Figure 16* compares chromatograms of acidic compounds on TSKgel ODS-100V and three other commercially available AQ-type ODS columns. Symmetrical peaks were observed for formic acid and acetic acid on TSKgel ODS-100V.

Figure 13. Comparing the asymmetry factor of basic compounds on commercial ODS columns



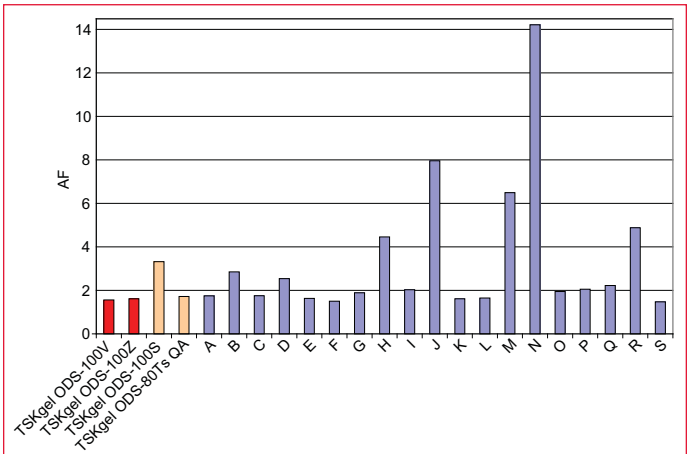
Columns: (all 4.6mmID x 15cm) same as Figure 3
Mobile phases: A) 50mmol/L phosphate buffer (pH 7.0)/MeOH (30/70)
B) 50mmol/L NaH_2PO_4/MeOH (50/50)
Flow rate: 1.0mL/min
Temp: 40°C
Inj. Volume: 10µL
Sample: A) Desipramine, B) Nortriptyline

Figure 14. Efficiency and peak shape of basic compounds on ODS-100V and other commercial AQ-type ODS columns



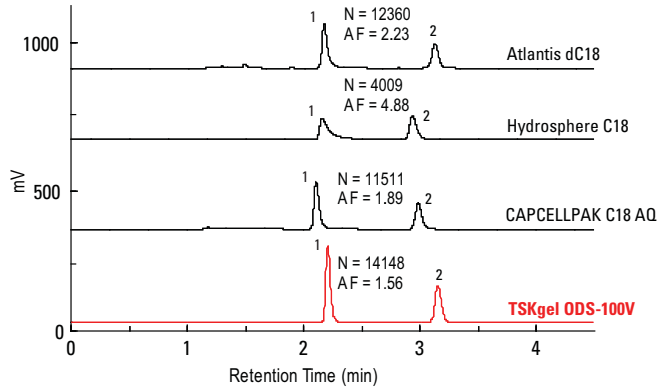
Column size: 4.6mm x 15cm
Eluent: 50mmol/L phosphate buffer, pH 7.0/MeOH (30/70)
Flow Rate: 1.0mL/min
Temp.: 40°C
Detection: UV @ 254nm
Inj. volume: 10µL
Sample: 1. Uracil, 2. Desipramine, 3. Imipramine

Figure 15. Comparing the asymmetry factor of an acidic compound on commercial ODS columns



Columns: (all 4.6mmID x 15cm) same as Figure 3
Mobile phase: H_2O/CH_3CN (98/2) + 0.1% H_3PO_4
Flow rate: 1.0mL/min
Temp: 40°C
Inj. Volume: 10µL
Sample: Formic acid

Figure 16. Chromatograms of acidic compounds on commercial ODS columns

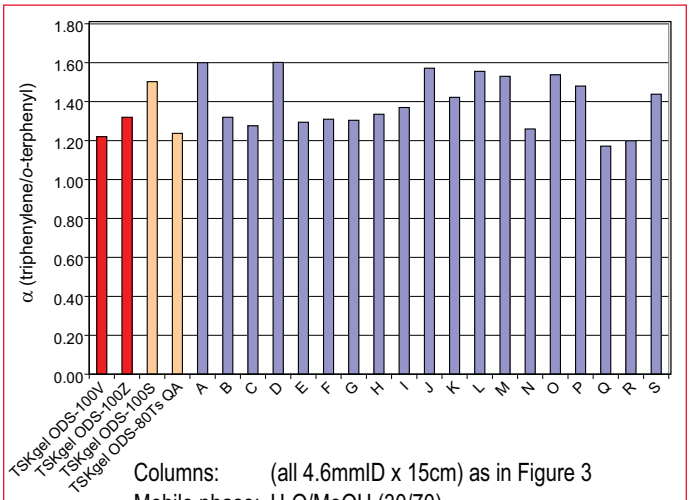


Column size: 4.6mm x 15cm
Mobile phase: H_2O/CH_3CN (98/2) +0.1% H_3PO_4
Flow rate: 1.0mL/min
Detection: UV @ 210nm
Temp: 40°C
Inj. volume: 10µL
Sample: 1. Formic acid, 2. Acetic acid

4-7. Steric selectivity

Figure 17 compares the steric selectivity of various commercial ODS columns. In general, the steric selectivity of polymeric stationary phases having a high surface density of alkyl chains is superior to that of monomeric stationary phases. Columns for which the selectivity or separation factor (α) for triphenylene and o-terphenyl is 1.5 or higher are expected to have a polymeric stationary phase structure. Both TSKgel ODS-100V and TSKgel ODS-100Z columns are monomeric stationary phases, and because the carbon content of TSKgel ODS-100Z is higher than that of TSKgel ODS-100V (approximately 20% vs. 15%, respectively), the steric selectivity of ODS-100Z is expected to be higher, as is shown in *Figure 18*). Thus, TSKgel ODS-100Z is better suited for the analysis of heterocyclic compounds.

Figure 17. Comparison of steric selectivity

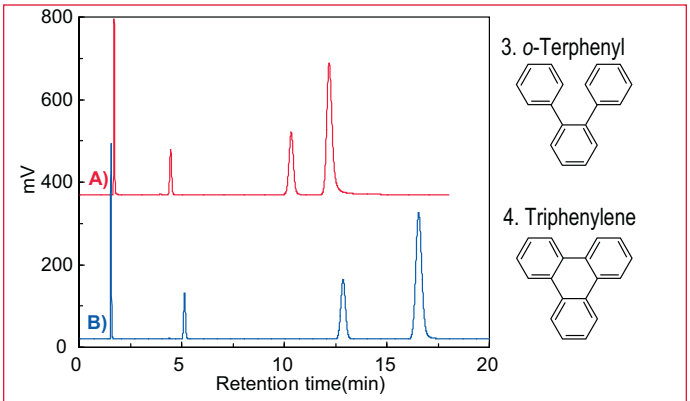


Columns: (all 4.6mmID x 15cm) as in Figure 3
Mobile phase: H_2O/MeOH (30/70)
Flow rate: 1.0mL/min
Temp: 40°C
Inj. Volume: 10µL
Sample: Triphenylene, o-Terphenyl

4-8. Durability

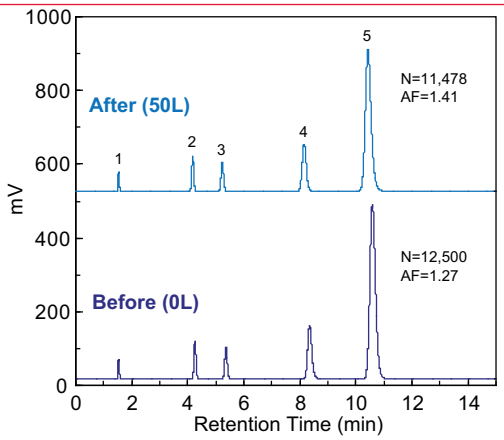
In general, ODS columns show a gradual decline of retention with prolonged use of a low pH (< 2.0) mobile phase. To test this effect, a commonly utilized acidic mobile phase (50% methanol solution containing 0.1% TFA) was flushed through a TSKgel ODS-100Z column over a long period of time. The chromatograms taken before and after this experiment are compared in *Figure 19*. After flushing the column with 50L mobile phase we observed minimal changes in retention time, theoretical plate number, and asymmetry factor for amitriptyline, a strong basic compound.

Figure 18. Comparison of steric selectivity between ODS-100V and ODS-100Z



Columns: A) TSKgel ODS-100V (4.6 mm I.D. x 15 cm)
B) TSKgel ODS-100Z (4.6 mm I.D. x 15 cm)
Mobile phase: H_2O/MeOH (20/80)
Flow rate: 1.0mL/min
Temp: 40°C
Inj. Volume: 10µL
Sample: 1. Uracil, 2. Benzene, 3. o-Terphenyl, 4. Triphenylene

Figure 19. Comparison of chromatograms before and after flushing the column with an acidic mobile phase



Column: TSKgel ODS-100Z (4.6mmID x 15cm)
Mobile phase: 20 mmol/L phosphate buffer (pH 7.0)/MeOH (20/80)
Flow rate: 1.0mL/min
Detection: 254nm
Temp: 40°C
Inj. Volume: 2µL
Sample: 1. Uracil, 2. Toluene, 3. Ethyl benzene, 4. Quinizarin, 5. Amitriptyline
Flushing conditions:
Mobile phase: H_2O/MeOH (50/50) + 0.1% TFA
Flow rate: 1.0mL/min
Flushing vol.: 50L

4-9. Effect of organic solvent concentration in sample solution

In reversed phase chromatography, the lower the polarity of the mobile phase (the higher the concentration of the organic solvent), the greater the proportion of sample that is present at any time in the mobile phase, and the shorter the retention time.

When the concentration of an organic solvent in the sample solution is higher than that in the mobile phase, the partitioning of the sample components between mobile phase and stationary phase is affected, resulting in a peak broadening for the most polar compounds in the sample. Using p-hydroxybenzoate esters, we investigated the effect of the concentration of organic solvent in the sample solution on peak width (HETP). The results are shown in *Figure 20*. Using 40% acetonitrile in the mobile phase and methyl p-hydroxybenzoate as solute, peak width broadened when the concentration of organic solvent in the sample increased above 50%. Also, as the hydrophobicity of the sample components increased, the organic solvent concentration at which a solute peak started to broaden increased as well. The more hydrophilic the sample, the more pronounced the effect of organic solvent concentration in the sample solution. To avoid this loss of efficiency, it is desirable to lower the organic solvent concentration in the sample solution as much as possible (i.e., by diluting with a high polarity solvent such as water) or to lower the injection volume.

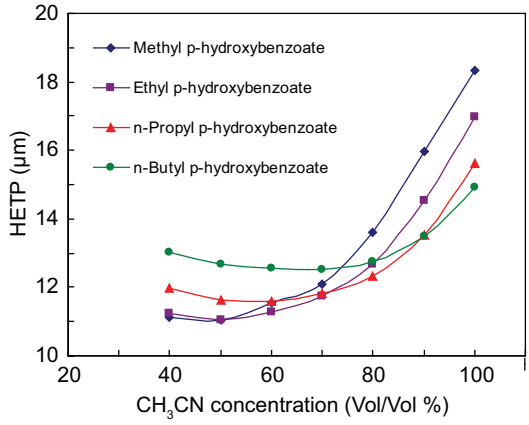
4-10. Lot-to-lot variability and column characterization

Figure 21 shows the chromatograms for the SRM 870 test mixture on TSKgel ODS-100Z columns prepared from various base silica lots. The results show no marked differences among the chromatograms confirming that minimal lot-to-lot variability and high consistency of the manufactured packing material.

Note the good peak shape for the metal-chelating compound quinizarine (peak 4), and the symmetrical peak shape for the organic base amitriptyline (peak 5). These results indicate low activity towards chelating compounds and very low activity towards organic bases, respectively.

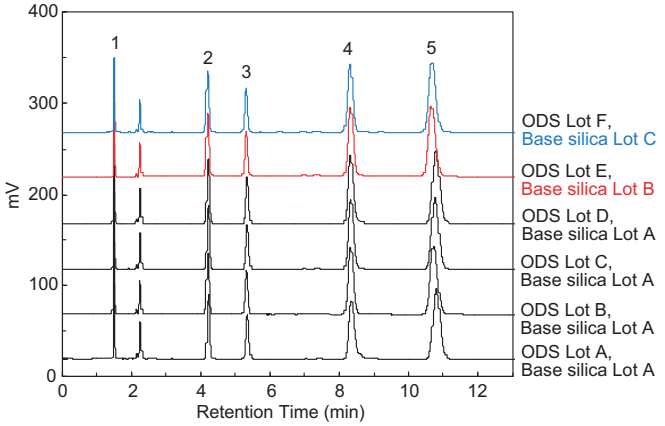
Footnote: SRM 870 is a column test mixture available from NIST (National Institute of Standards and Technology, Gaithersburg, MD). The mixture is used to characterize C18 columns.

Figure 20. Effect of organic solvent in the sample on peak shape



Column:	TSKgel ODS-100V (4.6mmID × 15cm)
Mobile phase:	H_2O/CH_3CN (60/40)
Flow rate:	1.0mL/min
Temp:	40°C
Inj. Volume:	10µL
Samples:	(Methyl, Ethyl, n-Propyl and n-Butyl)-p-hydroxybenzoic acid esters

Figure 21. Lot-to-lot variability for gel and base silica (ODS-100Z)



Column:	TSKgel ODS-100Z, 5µm (4.6mmID x 15cm)
Mobile phase:	20mmol/L phosphate buffer (pH 7.0)/MeOH = 20/80
Flow Rate:	1.0mL/min
Temp:	40°C
Detection:	UV (254nm)
Inj. Volume:	10µL
Sample:	SRM870 -- 1. Uracil, 2. Toluene, 3. Ethyl benzene, 4. Quinizarin, 5. Amitriptyline

5. Application data

Figures 22-26 show several applications of TSKgel ODS-100V and ODS-100Z columns.

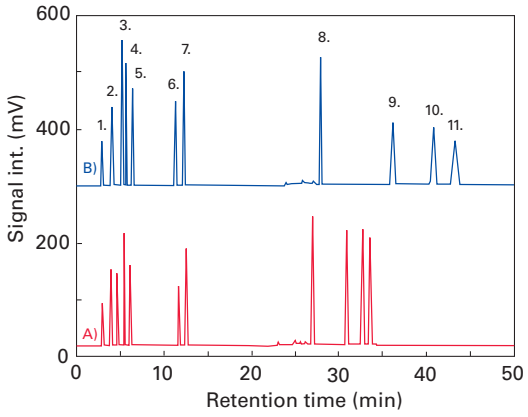
The analysis of vitamins is shown in *Figure 22*. The standard sample is a mixture of vitamins ranging from the very polar water-soluble vitamin ascorbic acid to the very hydrophobic tocopherol derivatives.

A linear gradient was run from 0% to 40% acetonitrile over 20 minutes. The polar vitamins elute in the beginning of the chromatogram under aqueous or low organic mobile phase conditions. A steep gradient from 40% ACN to 100% ACN is initiated from 20 to 22 minutes to elute retinol and the tocopherols. Clearly, the TSKgel ODS-100V column provides better overall resolution for the polar compounds in the mixture, while much shorter analysis time is obtained on TSKgel ODS-100V for the late eluting non-polar compounds.

The analysis of catechins is shown in *Figure 23*. Catechins are flavonoid phytochemical compounds that are predominantly found in green tea. Catechins, which are claimed to have many health benefits, are being investigated for their ability to prevent cancer and heart disease. The standard mixture of catechins used in *Figure 23* is clearly better separated on TSKgel ODS-100Z than on TSKgel ODS-100V.

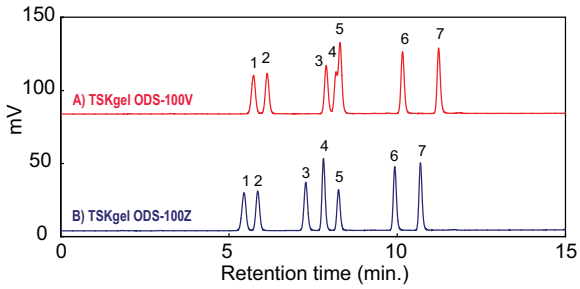
The analysis of a mixture of organic acids is shown in *Figure 24.* Organic acids play important roles in many metabolic processes, in fermentation and in food products. *Figure 24* shows an excellent separation of 25 organic acids within 25 minutes in a simple 0.1% phosphoric acid mobile phase.

Figure 22. Vitamins



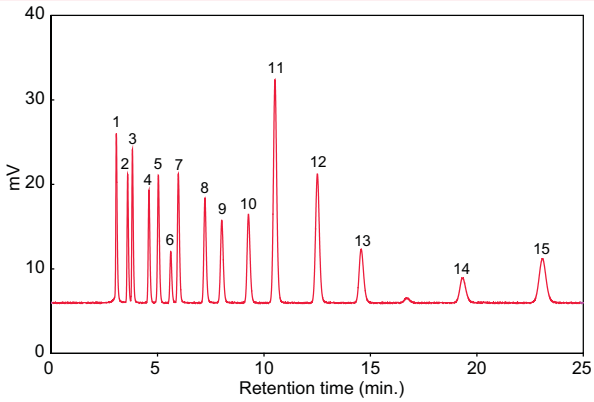
Columns:	A) TSKgel ODS-100V (4.6mmID x 15cm)
	B) TSKgel ODS-100Z (4.6mmID x 15cm)
Eluent:	A) 0.1% TFA in H_2O
	B) 0.1% TFA in CH_3CN
Gradient:	0 min (B: 0%) -- 20 min (B: 40%) --
	22min (B: 100%) -- 50min (B: 100%)
Flow rate:	1.0mL/min.
Temp.:	40˚C
Detection:	UV @ 280nm
Inj. volume:	5µL
Samples:	1. l-Ascorbic acid, 2. Nicotinic acid,
	3. Thiamine, 4. Pyridoxal, 5. Pyridoxine,
	6. Caffeine, 7. Riboflavin, 8. Retinol,
	9. Δ-tocopherol, 10. α-tocopherol,
	11. α-tocopherol acetate

Figure 23. Catechins



Columns:	A) TSKgel ODS-100V (4.6mmID x 15cm)
	B) TSKgel ODS-100Z (4.6mmID x 15cm)
Mobile phases:	A) 10mmol/L KH_2PO_4
	B) CH_3CN
Gradient:	0 min (B: 18%) -- 15 min (B: 60%) --
	20 min (B: 60%) -- 21 min (B: 18%)
Flow rate:	1.0mL/min
Temp:	40°C
Inj. Volume:	5µL
Samples:	1. (-)-Epigallocatechin
	2. (-)-Catechin,
	3. (-)-Epigallocatechin gallate
	4. Caffeine
	5. (+)-Epicatechin
	6. (-)-Epicatechin gallate
	7. (-)-Catechin gallate

Figure 24. Organic acids

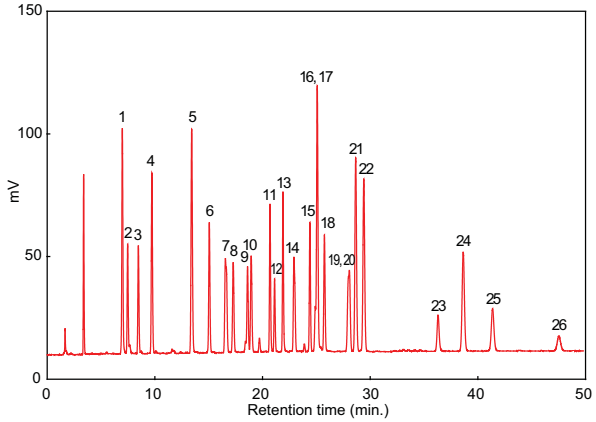


Column:	TSKgel ODS-100V (4.6mmID × 25cm)
Mobile phase:	0.1% H_3PO_4
Flow rate:	1.0mL/min
Temp:	40°C
Inj. Volume:	10µL
Samples:	1. Oxalic acid (0.1 g/L)
	2. l-Tartaric acid (0.5)
	3. Formic acid (1.0)
	4. l-Malic acid (1.0)
	5. l-Ascorbic acid (0.1)
	6. lactic acid (1.0)
	7. Acetic acid (1.0)
	8. Maleic acid (0.01)
	9. Citric acid (1.0)
	10. Succinic acid (1.0)
	11. Fumaric acid (0.025)
	12. Acrylic acid (0.1)
	13. Propionic acid (2.0)
	14. Glutaric acid (1.0)
	15. Itaconic acid (0.025)

A baseline separation of 26 well known polymer additives is shown in *Figure 25*. Note that while a simple linear acetonitrile gradient is used, the column temperature was increased to 50°C to achieve the required baseline separation on a TSKgel ODS-100V column.

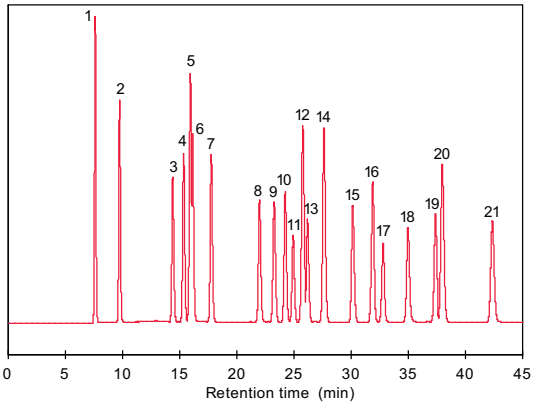
The analysis of mono-, di-, and tri-phosphorylated nucleotides on a TSKgel ODS-100V column is shown in *Figure 26*. The separation is accomplished by adding a short chain ion pairing agent, t-butylamine, and adjusting the mobile phase pH to 6.8, under which conditions the phosphate groups are fully charged. Acetonitrile is used as the organic modifier. As expected, nucleotides containing a single phosphate group elute first, followed by di- and tri-phosphorylated nucleotides. A gradient is required to elute the later eluting solutes from the column. The nucleotides are detected at 260nm UV.

Figure 25. Polymer additives



Column: TSKgel ODS-100V (4.6mmID × 15cm)
Mobile phases: A) H_2O
B) CH_3CN
Gradient: 0 min (B: 60%) -- 20 min (B: 100%)
Flow rate: 1.0mL/min
Temp: 50°C
Detection: UV (225nm)
Inj. Volume: 10µL
Concentration: 10mg/L each
Samples: 1. Cyasorb UV-24, 2. BHA, 3. Ionox 100,
4. Seesorb 101, 5. Tinuvin P, 6. Yoshinox SR,
7. Seesorb 202, 8. BHT, 9. Noclizer M-17,
10. Yoshinox 2246R, 11. Topanol CA,
12. Yoshinox 425, 13. Cyanox 1790,
14. Cyasorb UV-531, 15. Ionox 220,
16. Nonflex CBP, 17. Tinuvin 326, 18. Tinuvin 120,
19. Irganox 3114, 20. Uvtex OB, 21. Tinuvin 327,
22. Tinuvin 328, 23. Irganox 1010, 24. Irganox 1330,
25. Irganox 1076, 26. Irgafos 168

Figure 26. Nucleotides



Column: TSKgel ODS-100V (4.6mmID × 25cm)
Mobile phases: A) 20 mmol/L t-butylamine + H_3PO_4 (pH 6.8)
B) A/MeOH (90/10)
Gradient: 0 min (B: 0%) -- 35 min (B: 100%)
Flow rate: 1.0mL/min
Temp: 25°C
Detection: UV (260nm)
Inj. Volume: 2µL
Concentration: 0.3g/L each
Samples: 1. CMP, 2. UMP, 3. CDP, 4. dUMP, 5. GMP,
6. IMP, 7. UDP, 8. CTP, 9. TMP, 10. GDP,
11. IDP, 12. AMP, 13. UTP, 14. dGMP,
15. TDP, 16. GTP, 17. ITP, 18. ADP,
19. TTP, 20. dAMP, 21. ATP

6. Conclusions

As mentioned above, since TSKgel ODS-100V and TSKgel ODS-100Z columns are prepared from the same base silica, they have similar column efficiency (theoretical plate number) and back pressure properties. Also, because of highly efficient endcapping, residual ion-exchange activity is low, and symmetric peaks with minimal tailing can be obtained for basic, acidic and metal-chelating compounds.

Since the surface modification methods for TSKgel ODS-100V and TSKgel ODS-100Z columns differ, however, the sample selectivity is different. In general, TSKgel ODS-100V columns are best suited for the analysis of hydrophilic compounds, while TSKgel ODS-100Z columns are more appropriate for the analysis of hydrophobic compounds. With these two ODS columns, a wide variety of samples can be optimally separated.