TOSOH

SEPARATION REPORT NO. 89

TSKgel SUPER-ODS PACKED COLUMNS FOR ULTRA-FAST REVERSED PHASE LIQUID CHROMATOGRAPHY

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1. Introduction

It is widely accepted that reversed phase columns are the most popular type of HPLC columns due to their broad applicability, high physical stability, high efficiency, and ease of use. An additional reason for the popularity of reversed phase columns is that the separation conditions can simply be adjusted to match the sample properties. For instance, selectivity can be changed by adjusting the organic solvent composition to any value between 0 and 100% or by varying the density of the C18 bonded phase ligand.

Although the advantages of using narrow bore columns were long understood in terms of reducing solvent consumption and disposal cost, it was not until the acceptance of the mass spectrometer as an HPLC detection method that micro- or narrow bore columns became widely accepted. The optimum flow rate for most LC/MS applications is lower than for standard 4.6mm ID columns; the necessary lower flow rate is possible with narrow bore columns.

The particle size of reversed phase columns has declined over the years from about 10μm to somewhere between 3μm and 5μm. Although chromatographic theory has long predicted that higher efficiencies and shorter analysis times can be obtained by further reducing the particle size of HPLC packing materials, this obvious leap forward was not taken until 1995.

In 1995 Tosoh Corporation crossed the conventional 3μm particle size barrier by introducing an ultra-fast reversed phase packing material, TSKgel Super-ODS, containing spherical silica particles of 2μm nominal particle size. This Separation Report describes the features of TSKgel Super-ODS packed columns.

2. Specifications

Table 1 shows the efficiency and product specifications of the TSKgel Super-ODS columns. The available column diameters are 4.6mm, 2mm and 1mm ID. All diameters are offered in 5cm and 10cm lengths. A guard filter to protect the 4.6mm ID columns and a guard cartridge for the 2mm ID columns are also available.

3. Properties of the Packing Material

Table 2 compares the physical properties of TSKgel Super-ODS to TSKgel ODS-80T$_s$. The pore volume and specific surface area of TSKgel Super-ODS, which is a conventional reversed phase packing material, are about 1/3rd that of TSKgel ODS-80T$_s$. On the other hand, pore size is larger for TSKgel Super-ODS. The reason for the smaller pore volume and specific surface area is to ensure sufficient physical stability of the particles under high pressure, while the larger pore size ensures easy sample access after introducing a polymeric C18 bonded phase.

The average particle size of the TSKgel Super series columns is about 2.3μm, or 20% smaller than 3 micron packing materials. By employing such fine particles, high theoretical plates can be achieved. Moreover, the fact that the standard deviation of particle size distribution is smaller than that for conventional 3 or 5 micron products is also an essential factor for achieving high theoretical plates under relatively low pressure.

Table 1: Efficiency Specifications for TSKgel Super-ODS columns

Product name	Part No.	Column size	Minimum Theoretical Plates	Asymmetry Factor (10%)
TSKgel Super-ODS	18154	4.6mm ID × 5cm	8,000	0.8-1.6
TSKgel Super-ODS	18197	4.6mm ID × 10cm	16,000	0.8-1.6
TSKgel Super-ODS	19541	2.0mm ID x 5cm	6,000	0.8-1.5
TSKgel Super-ODS	19542	2.0mm ID x 10cm	12,000	0.8-1.5
TSKgel Super-ODS	20015	1.0mm ID x 5cm	1,500	0.8-2.0
TSKgel Super-ODS	20016	1.0mm ID x 10cm	N/D	0.8-2.0
Guardfilter for 4.mm ID columns, 3pk	18207	4mm ID x 0.4cm	----	—
Guardfilter Holder	18206	----	----	----
Gurard Cartridge for 2mm ID columns, 3pk	19672	2mm ID x 1cm	----	----
Guard Cartridge Holder	19308	2mm ID × 1cm	----	—

Table 2: Physical Properties of Reversed-phase Packing Materials (After introducing octadecyl groups)

TSK-GEL Packing material	Pore volume (mL/g)	Specific surface area (m^2/g)	Average pore size* (nm)	Average particle size x, SD(um)	Carbon content (C%)
Super-ODS	0.25[1]	96.8[1]	11.2[1]	2.29 +/- 0.27[2]	Approx. 8
ODS-80T$_s$	0.63	312.8	8.2	5.06 +/- 0.87	Approx. 15

* Pore sizes were determined for the bonded phase. The pore sizes before bonding are 120Å for Super-ODS and 100Å for ODS-80T$_s$.

1) Mercury porosimetry, 2) Scanning Electron Microscope (SEM)

4. Chromatographic Characteristics

4-1 Column Efficiency

Table 3 compares theoretical plate values of a TSKgel Super-ODS column with commercial 3μm packed columns. It is evident that commercial 3μm columns have theoretical plates (N or TP) in the range of 6,000 to 8,000 per 4.6mm ID x 5cm column, while the TSKgel Super-ODS column exhibited more than 10,000 plates. Surprisingly, the TSKgel Super-ODS column had a lower or similar pressure drop than the 3μm columns, although it had higher column efficiency. We contribute the modest pressure drop to the narrow particle size distribution of TSKgel Super-ODS.

Table 4 shows similar comparison data for 4.6mm ID x 10cm columns. It is clear that the TSKgel Super-ODS column has a lower pressure drop and higher theoretical plates than the two competitive columns. This table also shows that TSKgel Super-ODS has a shorter retention time than other commercial ODS packing materials. This is due to the difference in specific surface area, as was reported in Table 2.

Figure 1 shows a comparison of retention among TSKgel Super-ODS 5cm and 10cm columns and a 15cm TSKgel ODS-80T$_s$ column, which contains spherical 5μm particles.

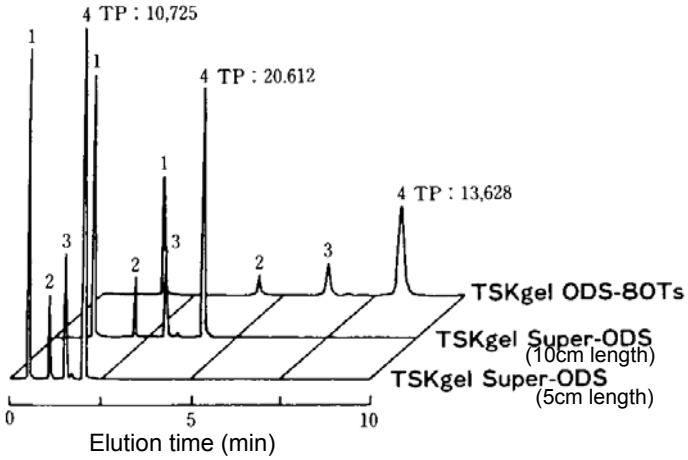

Figure 1: Retention Comparison with Conventional Columns (Isocratic Elution)

Column: TSKgel Super-ODS (4.6mm ID ×5cm)
TSKgel Super-ODS (4.6mm ID ×10cm)
TSKgel ODS-80T$_s$ (4.6mm ID ×15cm)
Eluent: 70% MeOH
Flow rate: 1.0mL/min
Temp.: 25°C
Detection: UV@254nm, micro-flow cell
Samples: 1. uracil 2. benzene 3. toluene 4. naphthalene

Column	Rs (1/2)	Rs (2/3)	Rs (3/4)
Super-ODS (5cm)	16.44	8.09	7.56
Super-ODS (10cm)	24.42	11.43	10.70
ODS-80T$_s$	21.88	9.53	7.39

4-2 Steric Selectivity

Table 5 shows the separation factors for the solute pair o-terphenyl (OT)/triphenylene(TR) on TSKgel Super-ODS and TSKgel ODS-80T$_s$. Compared to TSKgel ODS-80T$_s$, larger values were obtained on TSKgel Super-ODS for the separation factor and resolution, although retention, and thus capacity factor (k'), was smaller. The higher value for the separation factor is due to the polymeric nature of the bonded phase on TSKgel Super-ODS. In contrast, TSKgel ODS-80T$_s$ contains a monomeric bonded phase structure. It is known that steric selectivity is favored on polymer bonded phases.

Table 3: Comparison of Column Efficiency for 4.6mm ID x 5cm ODS Columns

Column	Particle size (μm)	Fluorene		Separation Factor α (NAP/FLU)[1]	Pressure[2] (kg/cm^2)
		tR (min)	N/column		
TSKgel Super-ODS	2	3.71	10728	2.30	97
ODS by company A	3	6.10	7453	2.38	98
ODS by company B	3	4.70	8701	2.27	124
ODS by company C	3	6.58	5893	2.39	116
ODS by company D	3	6.61	7652	2.38	94

(1)NAP: naphthalene, FLU: fluorene, (2)70% methanol, 1mL/min,

Table 4: Comparison of Column Efficiency for 4.6mm ID x 10cm ODS Columns

Column	Particle size (μm)	Naphthalene		Pressure* (kg/cm^2)
		tR (min)	N/column	
TSKgel Super-ODS	2	4.06	20612	191
ODS by company A	3	4.46	10651	262
ODS by company B	3	3.47	11685	191

* 70% methanol, 1mL/min

Table 5: Comparison of Steric Selectivity

TSKgel Column	o-terphenyl		triphenylene		Separation factor α (OT/TR)	Resolution Rs (OT/TR)
	k'	N	k'	N		
Super-ODS	2.19	9596	3.84	6059	1.98	13.53
ODS-80T$_s$	6.65	14163	8.00	14571	1.27	5.53

Eluent: 80% methanol (TSKgel Super-ODS)
85% methanol (TSKgel ODS-80T$_s$)
Flow rate: 1mL/min
Detection: UV@254nm

4-3 Residual Silanol Groups

One disadvantage of using a silica gel support is that only about half of all silanol groups can be reacted with the C18 bonding reagent. As a result, even after performing a secondary endcapping reaction, many silanol groups remain unreacted. Not all of these residual silanol groups are accessible, but those that are accessible can now participate in interacting with ionic, particularly basic, substances.

Except at low pH, residual silanol groups are negatively charged and can interact with basic substances. In general, if there are residual silanol groups on the packing material surface, charged acidic solutes can be repulsed from the C18 surface. This causes earlier than expected elution, while basic substances can be adsorbed, often leading to tailing peaks in the chromatogram. Figure 2 shows a comparison of elution of pyridine, which is a basic substance, between a TSKgel Super-ODS column and a TSKgel ODS-80T$_s$ column. In either packing material it is evident that pyridine elutes from the columns with reasonable peak shape. Therefore, it can be concluded that sufficient end-capping has been achieved in both column types.

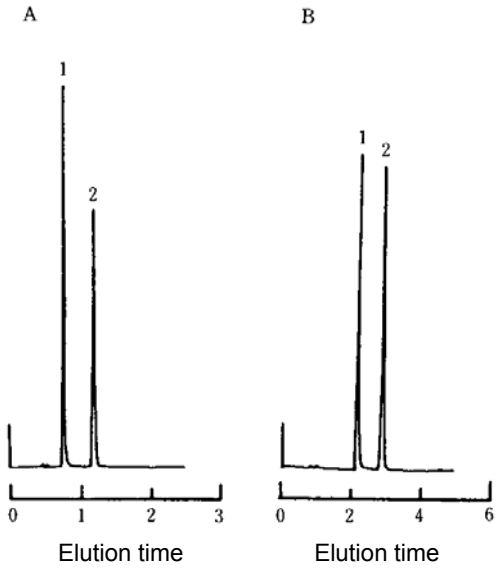

Figure 2: Comparison of Elution in TSKgel Super-ODS and TSKgel ODS-80T$_s$

Column: A. TSKgel Super-ODS (4.6mm ID ×5cm)
B. TSKgel ODS-80T$_s$ (4.6mm ID ×15cm)
Eluent: A. 30% acetonitrile
B. 50% acetonitrile
Flow rate: 1.0mL/min
Temp.: 25°C
Detection: UV@254nm, micro-flow cell
Samples: 1. pyridine 2. phenol

4-4 Elution of Metal Chelating Compounds

The presence of metal ions on the silica surface (iron, copper, etc.) may cause deterioration in sample recovery or severe distortion of peak shape through the formation of metal complexes with sample molecules. In addition, substances that can easily be oxidized or reduced may degrade and cause a change in peak shape or sample loss, both leading to low run-to-run reproducibility. TSKgel Super-ODS and TSKgel ODS-80T$_s$ are prepared from high-purity silica gels that are prevented from contact with metal species during the bonding reactions and subsequent processing. Therefore, TSKgel Super-ODS and TSKgel ODS-80T$_s$ show minimal interaction, even when analyzing known chelating compounds or oxidizable substances. Figure 3 shows the separation of 8-quinolinol, which is a metal chelating compound, using a TSKgel Super-ODS column. The chromatogram shows minimal peak distortion.

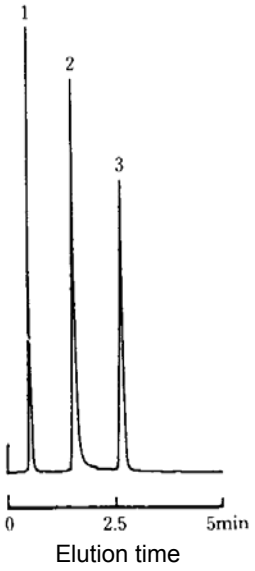

Figure 3: Chromatogram of Chelating Agent

Column: TSKgel Super-ODS (4.6mm ID ×5cm)
Eluent: 20mmol/L phosphate buffer, pH 6.8/ acetonitrile = 70/30
Flow rate: 1.0mL/min
Temperature: 40°C
Detection: UV@245nm, micro-flow cell
Samples: 1. uracil 2. 8-quinolinol 3. methylbenzoic acid

4-5 Relationship between Flow Rate and Column Efficiency

Column efficiency is expressed as the sum of the contributions of all sample dispersion mechanisms within the column. This is shown in the formula below.

Height of a theoretical plate (HETP):

$$H = H_{eddy} + H_{diff} + H_{stationary} + H_{mobile}$$

Here, H_{eddy} indicates dispersion by eddy diffusion, H_{diff} indicates dispersion by diffusion in longitudinal direction within the mobile phase, $H_{stationary}$ indicates dispersion by delay from mass transfer within the stationary phase, and H_{mobile} indicates dispersion by delay from mass transfer within the mobile phase. H_{diff} and H_{mobile} are the terms related to particle size. The effect of reducing particle size is most significant with H_{mobile}, which is proportional to the square of the particle size. Furthermore, although the terms related to flow rate are H_{diff}, $H_{stationary}$ and H_{mobile}, H_{diff} becomes smaller and $H_{stationary}$ and H_{mobile} become larger when the flow rate is increased. In general, column efficiency deteriorates at higher flow rates. However, H_{mobile} declines when particle size is reduced, suppressing the loss of efficiency at high flow rates.

Figure 4 shows the relationship between flow rate and column efficiency for 4.6mm ID TSKgel Super-ODS and TSKgel ODS-80T$_s$ columns under different eluent compositions. In the eluent containing methanol, the optimal flow rate is found near a linear velocity of 4cm/min (approximately 0.6mL/min) for TSKgel ODS-80T$_s$, while it is found near 6cm/min (approx. 1mL/min) for TSKgel Super-ODS. Although column efficiency starts to deteriorate rapidly at 4cm/min or greater with TSKgel ODS-80T$_s$, column efficiency for TSKgel Super-ODS deteriorates only gradually.

Meanwhile, in the eluent containing acetonitrile, optimal flow rate range lies near 6cm/min (1mL/min) for TSKgel ODS-80T$_s$ and near 12cm/min (2mL/min) for TSKgel Super-ODS. The optimal flow rate range shifts toward higher flow rates compared to the methanol eluent for both columns. This is due to the difference in the speed of mass transfer, which is inversely proportional to solvent viscosity. The deterioration of column efficiency at higher flow rates is small when using a low viscosity solvent system, enabling analysis at a high flow rate. The relationship between the flow rate in various solvent compositions and pressure drops is shown in Figure 5.

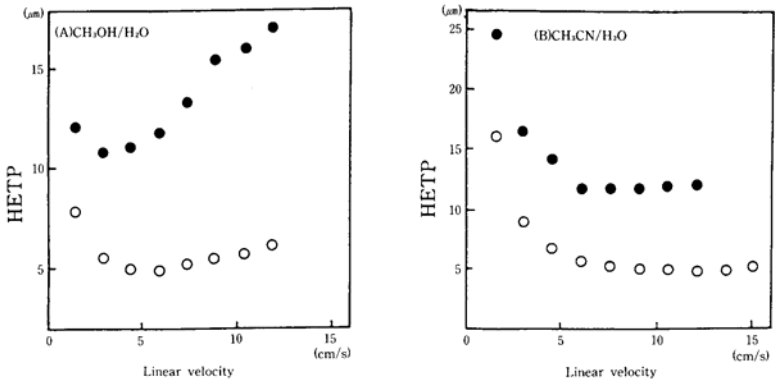

Figure 4: H/v Curve in Various Eluent Compositions

Column: (❍) TSKgel Super-ODS (4.6mm ID × 5cm)
(●) TSKgel ODS-80T$_s$ (4.6mm ID × 15cm)
Eluent: (A) 70% methanol
(B) 50% acetonitrile
Flow rate: 0.25 to 2.5mL/min
Detection: UV@254nm
Temperature: 25 °C
Sample: fluore ne

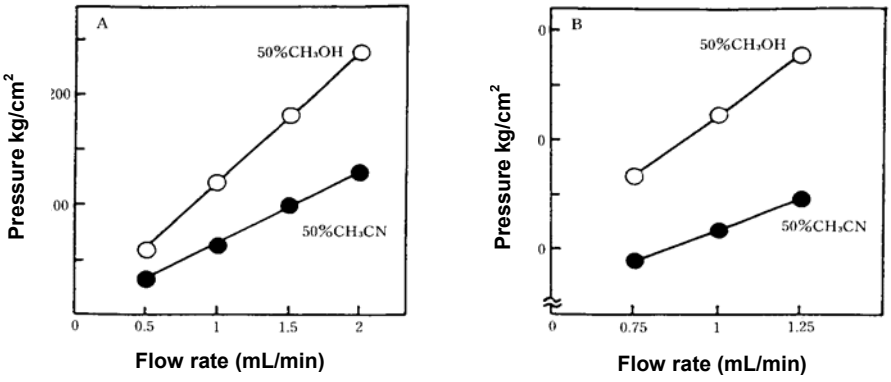

Figure 5: Relationship between Eluent Composition and Pressure

Column: A. TSKgel Super-ODS (4.6mm ID × 5cm)
B. TSKgel Super-ODS (4.6mm ID × 10cm)
Eluent: 50% MeOH, 50% CH3CH
Flow rate: 0.5 to 0.2mL/min
Temperature: Ambient

5. Factors Affecting the Separation

Although the factors affecting retention and selectivity are similar to those for conventional columns, various factors begin affecting column efficiency when column efficiency increases for the same column dimension. In this section, these factors are examined.

The factors affecting the column efficiency are largely divided into the following:

- o Extra-column band broadening
- o Detector response
- o Sample injection volume

5.1 Extra-Column Band Broadening

The column efficiency that is measured ideally would be only the efficiency of the packed column. In reality, the design of the column plays a role in the column efficiency that is measured. Assuming that the fittings and the tubing show the column at "its best", in other words they do not detract from the efficiency of the packed bed itself, then the efficiency outside the column must be maintained as is. Thus, the effect of all processes that would broaden the sample bands after they leave the column have to be minimized. In other words, dispersion of the sample outside of the column needs to be reduced. Some of the factors to consider are tubing and detector cell volume outside the column.

Table 6 shows the effect on observed column efficiency of the volume of tubing between injector/column and between column/detector. As is clear from the data in the table, column efficiency deteriorates by approximately 10% when the volume of tubing exceeds 2μL. It is also evident that the effect of tubing volume between injector and column on column efficiency is larger than that of tubing volume between column/detector.

Table 6: Effect of Tubing Volume on Efficiency

Injector/column*			Column/detector**		
Length of the tubing (cm)	Volume of the tubing (μl)	HETP (μm)	Length of the tubing (cm)	Volume of the tubing (μl)	HETP (μm)
10	0.79	4.66	10	0.79	4.66
15	1.19	4.70	15	1.19	4.70
30	2.36	5.23	30	2.36	4.74
50	3.93	5.51	50	3.93	5.35
70	5.50	5.89	70	5.50	5.54

Tubing with 0.1mm or 0.004" ID were used.
* Distance between column/detector 0.1mm ID ×10cm
**Distance between injector/column 0.1mm ID ×10cm
Column: TSKgel Super-ODS (4.6mm ID ×5cm)
Eluent: 70% methanol
Flow rate: 1mL/min
Detection: UV@254nm, micro-flow cell
Sample: fluorene

The detector cell volume may also affect the width of the peak when it travels through the cell. Table 7 shows the effect of detector cell volume on column efficiency. For detector cells, a 2μL micro-cell, a 10μL standard cell or a low dead volume-type cell was used.

Although column efficiency decreased by 6% in the low dead volume-type cell compared to the micro-cell, it decreased by as much as 70% with the standard cell. This is because the cell contained a heat sink to stabilize temperature fluctuations. The volume of the heat sink was approximately 30μL. It is evident from Table 7 that the cell volume needs to be minimized when using short TSKgel Super-ODS columns.

Table 7: Effect of Detector Cell Volume on Column Efficiency

Cell volume (μL)	Column theoretical plates (rate of deterioration in theoretical plates) TP/5cm column
2 (micro-flow cell)	10769 (0%)
10 (low dead volume-type)	10150 (6%)
10 (standard flow cell)	3104 (71%)

Eluent: 70% methanol, Sample: fluorene

5.2 Detector Response

Detector response may also affect column efficiency in high speed separations. Table 8 shows the relationship between detector response and column efficiency. It is apparent that resolution deteriorates and theoretical plates decrease drastically when the time constant is 1 second or larger. Therefore, it is necessary that a time constant is selected so that its effect on column efficiency is as small as possible. In Figure 6, chromatograms are shown for three time constant values. It is clear that the peak width becomes enlarged at 3 seconds, causing extreme deterioration in resolution. However even at 1 second, peak heights are smaller than what can be obtained at 50 milliseconds. The smallest time constant will have the least effect on column efficiency, although noise increases with decreasing time constants.

Table 8: Relationship between Detector Response and Column Efficiency

Time constant	Naphthalene theoretical plates TP/column (rate of deterioration)	Resolution α (TOP/NAP)
50msec	10529 (0%)	13.37
1sec	6996 (34%)	10.37
3sec	3420 (68%)	6.87

Eluent: 70% methanol
Sample: toluene (TOL), naphthalene (NAP)

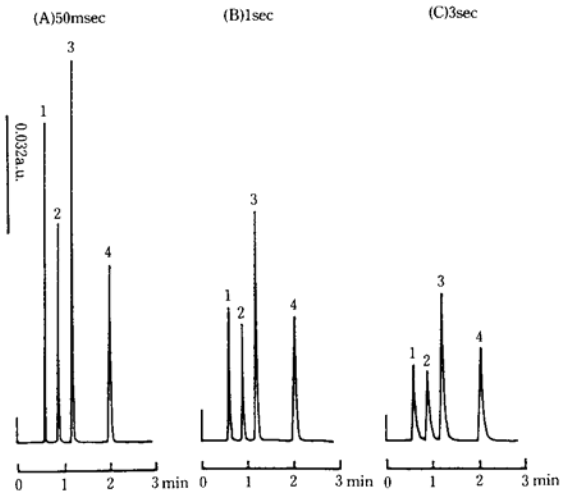

Figure 6: Effect of Detector Time Constant on Theoretical Plates

Column: TSKgel Super-ODS (4.6mm ID ×5cm)
Eluent: 70% methanol

Flow rate: 1.0mL/min
Sample: Fluorene
Temp.: 2 5°C
Detector: UV@254nm, micro-flow cell
Time constant: (A) 50msec (B) 1sec (C) 3sec

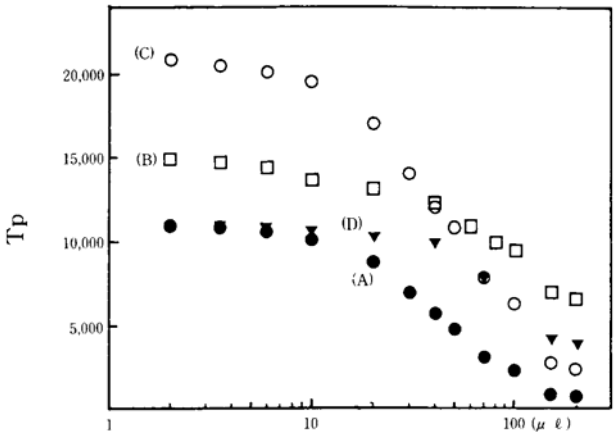

Figure 7: Sample Injection Volume and Column Efficiency (Theoretical Plates)

Column: A, D- TSKgel Super-ODS, 4.6mm ID × 5cm
B- TSKgel ODS-80T$_s$ (4.6mm ID ×15cm)
C- TSKgel Super-ODS (4.6mm ID ×10cm)
Eluent: 70% methanol
Flow rate: 1.0mL/min
Temperature: 25°C
Detection: UV (254nm)
Samples: naphthalene (0.1g/L), (A) (B) (C) dissolved in 70% methanol, (D) dissolved in 40% methanol, (0.1 g/L)

5.3 Sample Injection Volume

For every packed column dimension there is a maximum injection volume below which the volume of the eluting peak is not significantly impacted by the volume of the injected sample. When injecting more than the maximum injection volume, column efficiency decreases due to volume overload. This discussion assumes that the injection solvent is not stronger than the solvent strength of the mobile phase.

Assuming equally well-packed columns, when the length of a column is reduced by a factor of 2 (e.g., from 10cm to 5cm), the resulting peak volume decreases by the square root of 2. If the same packing material is used in both columns, the number of plates is reduced two-fold. However, as we have seen in data presented in this report, a 5cm TSKgel Super-ODS column can be as efficient as a 10cm column packed with 3micron packing material. It is clear then why we need to pay special attention to injection volume when working with the highly efficient TSKgel Super-ODS columns.. A large amount of efficiency is packed into this short column, which as a result is easier overloaded than the less efficient, longer conventional columns.

Figure 7 shows the relationship between sample injection volume and column efficiency. When the sample is dissolved in the mobile phase, the column efficiency begins deteriorating at a small injection volume on TSKgel Super-ODS (5cm length) because the column volume is 1/3rd of the 15cm TSKgel ODS-80T$_s$ column. For TSKgel Super-ODS columns, it is recommended to inject 10μL or smaller volumes. However, it is known that the injection volume can be increased significantly (~5X) without impacting column efficiency by reducing the percent organic solvent in the sample solution below the content of organic modifier in the eluent.

6. Applications

Figure 8 shows an example of the use of TSKgel Super-ODS for high speed separations. In this example, flow rate is changed from 1mL/min to 4mL/min. As a result, analysis time is reduced from 3 minutes to less than 1 minute. It is shown that resolution gradually decreases with increasing flow rate, while, of course, pressure drop increases proportionally with increasing flow rate.

Figure 9 shows the analysis of vitamins D_2 and D_3. When using a TSKgel ODS-80T$_s$ column, which contains a monomeric ODS bonded phase structure, it is not possible to obtain a baseline separation. A baseline separation in half the time was obtained on a TSKgel Super-ODS column, which contains a polymeric ODS bonded phase structure.

An example of a peptide separation is shown in Figure 10. Although TSKgel Octadecyl-NPR, a polymer-based, non-porous reversed phase packing material (2.5μm), is available for high speed separation of peptides and proteins, it has weak retention for hydrophilic peptides due to its low surface area. On the other hand, since TSKgel Super-ODS has a much larger surface area, it shows favorable separation of peptides with high hydrophilicity.

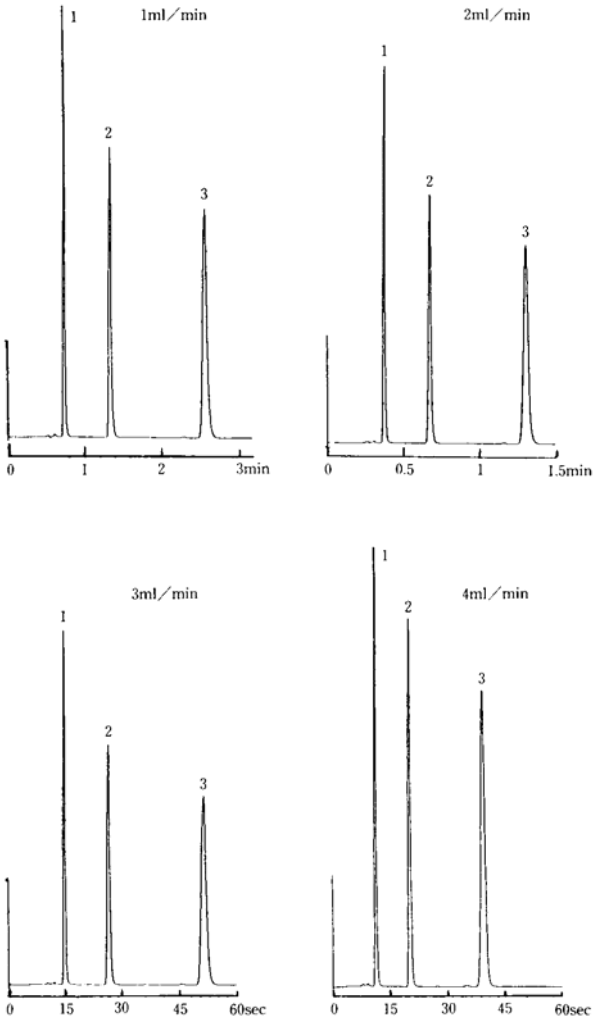

Figure 8: Relationship between Flow Rate and Separation on TSKgel Super-ODS

Column: TSKgel Super-ODS (4.6mm ID × 5cm)
Eluent: 20mmol/L phosphate buffer, pH 2.5/ ACN = 80/20
Flow rate: 1 to 4mL/min
Temp.: 2 5°C
Samples: 1. caffeine, 2. salicylamide, 3. phenacetin
Detection: UV@254nm, micro-flow cell

Flow rate	Elution point (min)			Resolution	
	Sample 1	Sample 2	Sample 3	Rs (1/2)	Rs (2/3)
1mL/min	0.73	1.33	2.58	14.45	16.17
2mL/min	0.37	0.67	1.30	13.04	15.08
3mL/min	0.25	4.44	0.86	12.01	14.20
4mL/min	0.19	0.33	0.66	10.34	12.95

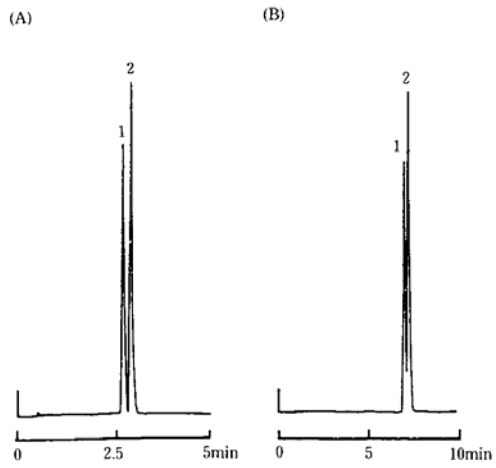

Figure 9: Vitamin D_2 and D_3 Separation

Column: (A) TSKgel Super-ODS (4.6mm ID × 5cm)
(B) TSKgel ODS-80T_s (4.6mm ID × 15cm)
Eluent: Methanol
Flow rate: 1mL/min
Temperature: 25 °C
Detection: UV@254nm, micro-flow cell
Samples: 1. vitamin D_2 2. vitamin D_3

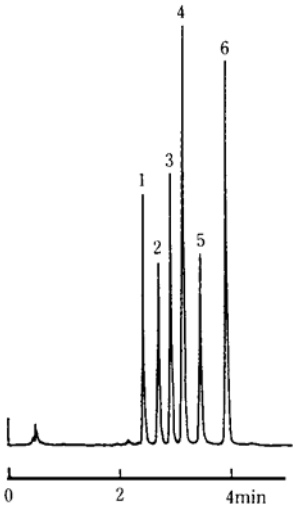

Figure 10: Analysis of Hydrophilic Peptides

Column: TSKgel Super-ODS (4.6mm ID × 5cm)
Eluent: 13mmol/L $HClO_4$/acetonitrile
Linear gradient from 10% to 50% acetonitrile over 10 minutes
Flow rate: 2mL/min
Temperature: 25°C
Detection: UV@220nm, micro-flow cell
Samples: 1. oxytocin
2. α-endorphin
3. bombesin
4. Leu-enkephalin
5. gamma-endorphin
6. somatostatin
All peptides are injected at 0.1 to 0.2μg each.

7. Operating Instructions in HPLC System, etc.

As described above, it is important to reduce the impact of extra-column band broadening effects on column efficiency. Follow the guidelines in Table 9 in order to optimize your system and thus obtain maximum column performance.

8. Conclusion

TSKgel Super-ODS is a reversed phase packing material which is capable of achieving high speed, high resolution separations by employing 2.3um, spherical silica particles with low pore volume for enhanced physical stability. The pores are functionalized with a polymerized C18 bonded phase structure that limits the influence of residual silanol groups and low level metal ion impurities on the separation of basic drugs and chelating compounds.

In order to take full advantage of the high performance of TSKgel Super-ODS columns, it is recommended to optimize the HPLC system to limit possible contributors to extra-column band broadening.

Table 9: Operating Instructions When Using TSKgel Super-ODS

In general:

- Suppress peak broadening in connecting tubing, detector, etc.
- Prevent the sample from overloading.
- Use caution in setting up detection and data processing, since analysis time is short (5 minutes or less).

Tubing:

- Use 0.004" ID (0.1mm) tubing, when available. A length of 30cm or less is desired. When using slightly larger ID tubing (for example 0.005" ID), make sure to reduce the length of the tubing beyond the recommendations given in the text.
- The following sections require 0.004" ID (0.1mm) tubing:
    - Between injection valve and guard filter inlet (see below), and between guard filter outlet and column
    - Between column outlet and detector inlet

Autosampler (sample injection):

- Sample injection volume should not exceed 10 μL, unless the injection solvent is weaker than the mobile phase. Sample concentration should be approximately 50μg/L.

Column protection:

- Always use a guard filter/guard cartridge to protect the column.
    - The guard holder is supplied with two pieces of tubing to connect to the column and injector.

Column oven:

- Use a constant temperature of 25 °C or higher. Pressure decreases and theoretical plates increase at 40°C compared to room temperature. Using elevated temperature also benefits run-to-run reproducibility.

Detector:

- UV detector requires micro-flow cells or low dead volume-type cells. Set the response to 50msec or 150msec.

Data processing:

- Use a sampling rate of 50msec.