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If you have questions about applying methodology described in this article to a current application, please contact our technical service chemists.

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2 μ m Silica Packing Ensures Faster HPLC Analyses and Superior Resolution of Problem Analytes

Small (2 μ m) high purity, metal-free base silica and a unique endcapping procedure are used to prepare TSK-GEL Super-ODS columns. These properties ensure superior resolution and analysis speed, greater sensitivity, and reduced solvent consumption for analyses of small molecular weight compounds, such as amino acids, peptides, tryptic digests, nucleotides, pharmaceuticals, and food and beverage components. Basic compounds, chelating compounds, and other difficult analytes are eluted as sharp, well-separated peaks.

TSK-GEL® Super-ODS columns contain a C18 polymeric phase bonded to a 2-micron silica. The small particle size ensures superior resolution and analysis speed, as well as improved sensitivity and reduced solvent consumption. 110Å pores provide an exclusion limit of 100-20,000 Dalton. Thus, Super-ODS columns are ideal for analyses of small molecular weight compounds, such as amino acids, peptides, tryptic digests, nucleotides, pharmaceuticals, and components of food and beverage samples. A tryptic digest of α -chymotrypsinogen, for example, can be separated in less than 5 minutes on a 5cm x 4.6mm ID Super-ODS column (Figure A).

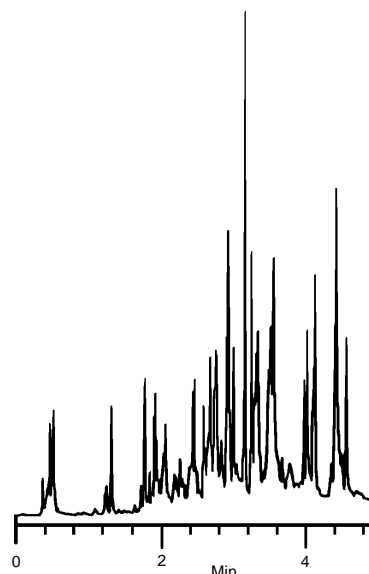
Table 1 lists the physical and operating properties of these columns. The high purity, metal-free base silica and unique endcapping procedure used to prepare TSK-GEL Super-ODS columns prevent unfavorable interactions between solutes and the column packing. Figure B shows the elution profiles for typical difficult-to-analyze compounds. The sharp peak for pyridine, a basic compound, indicates that few ionic sites exist on the Super-ODS packing material. In contrast, basic compounds are strongly adsorbed to incompletely endcapped C18 materials. The sharp peaks for chelating compounds also demonstrate the inertness of the Super-ODS material. Metallic impurities in a packing affect the

Table 1. Physical and Operating Properties of TSK-GEL Super-ODS Columns

Pore Volume:	0.25mL/g
Specific Surface Area:	96.8m ² /g
Mean Pore Diameter:	11.2nm
Particle Size (mean \pm SD):	2.29 \pm 0.27 μ m
Theoretical Plates/Column:	5cm: \geq 8000
	10cm: \geq 16,000
Standard Flow Rate:	1.5-2.5mL/min
Maximum Flow Rate:	4.0mL/min
Maximum Pressure:	300kg/cm ²
pH Range:	2-7.5
Temperature Range:	10-50°C

Figure A. Tryptic Digest of α -Chymotrypsinogen

Column: TSKgel Super-ODS,
5cm x 4.6mm ID, 2 μ m particles
Cat. No.: 818154
Mobile Phase: 13mM HClO₄, linear gradient of 0-80% acetonitrile (10 min)
Flow Rate: 1.5mL/min
Det.: UV, 220nm
Inj.: 2 μ L tryptic digest of α -chymotrypsinogen



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elution profiles of chelating and oxidizing compounds through the formation of chelation complexes or by redox reaction.

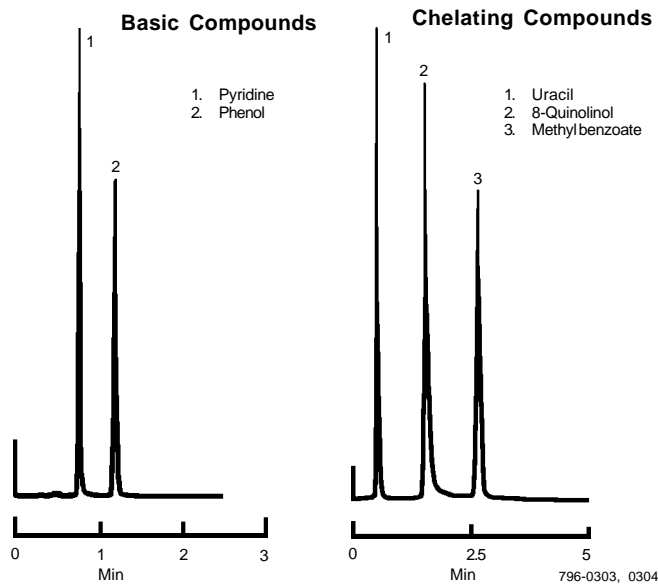
Figure C shows the relationship between HETP and the linear velocity of an acetonitrile:water mobile phase. At a flow rate of 29cm/min (5mL/min) the operating pressure drop is approximately 30MPa. With a 5cm column, the flow rate for methanol:water, 50:50 can be accelerated to approximately 2mL/min (or to 5mL/min for acetonitrile:water, 50:50) without exceeding the recommended maximum pressure drop. Figure D shows the effect of flow rate on the separation of a peptide mixture on a 5cm Super-ODS column. With flow rates of up to 5mL/min, analysis time is progressively reduced without reducing the quality of the analysis or creating an excessive pressure drop.*

We recommend using TSK-GEL Super-ODS columns for rapid analyses and superior elution profiles of small, difficult-to-analyze compounds. A 5cm column is suitable for most analyses, but a 10cm column is available for the most demanding applications.

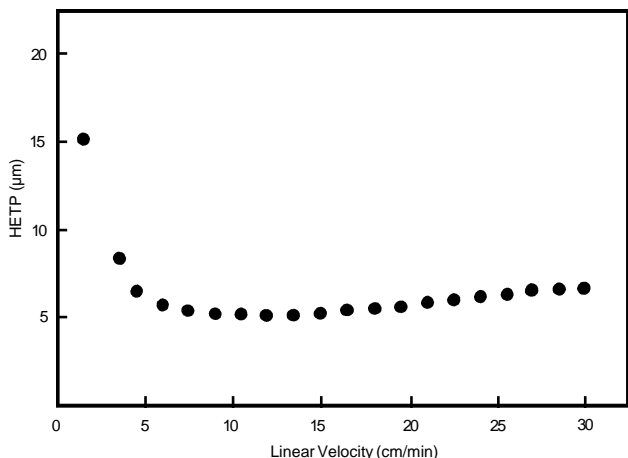
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Figure B. Difficult Analytes Elute as Sharp Peaks

Column: **TSKgel Super-ODS,**
5cm x 4.6mm ID, 2 μ m particles
Cat. No.: **818154**
Mobile Phase: acetonitrile:water, 30:70 (basic compounds) or
acetonitrile:20mM Na₂HPO₄ (pH to 6.8 with H₃PO₄), 30:70
(chelating compounds)
Flow Rate: 1mL/min
Det.: UV, 254nm

**Figure C. Effect of Mobile Phase Linear Velocity on Column Efficiency**

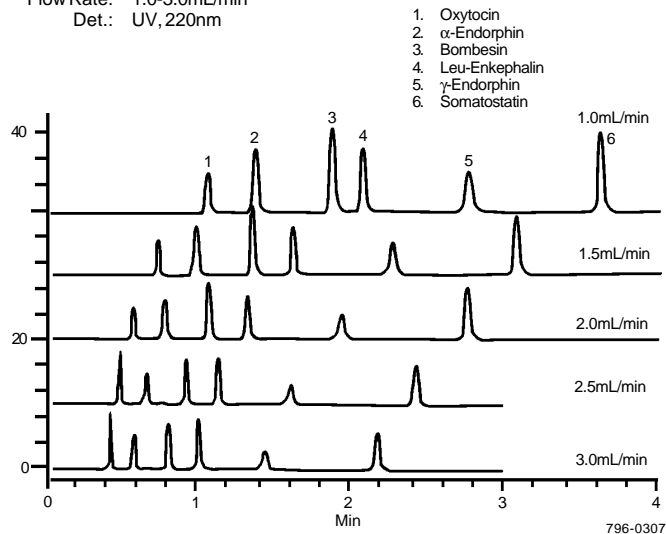
Column: **TSKgel Super-ODS,**
5cm x 4.6mm ID, 2 μ m particles
Cat. No.: **818154**
Mobile Phase: acetonitrile:water, 50:50
Flow Rate: 0.34-5.0mL/min
Det.: UV, 254nm
Inj.: fluorene



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Figure D. Higher Flow Rates Reduce Analysis Time Without Affecting the Quality of the Analysis

Column: **TSKgel Super-ODS,**
5cm x 4.6mm ID, 2 μ m particles
Cat. No.: **818154**
Mobile Phase: 13mM HClO₄, linear gradient of 23-56% acetonitrile
Flow Rate: 1.0-3.0mL/min
Det.: UV, 220nm



To maximize the efficiency of a Super-ODS column, you must control three major variables: the void volume, the detector time constant, and the sample volume. Excess void volume in connecting tubing and the detector cell can reduce column efficiency. The total void volume should be less than 3 μ L. As with all high efficiency columns, a faster time constant for the detector is needed to obtain highest efficiency. The time constant should be less than 50msec. Sample volume should be no more than 10 μ L. Additionally, the integrator sampling rate can affect column efficiency. Use a sampling rate greater than 10 times/second.

Ordering Information:

Description	Cat. No.
TSK-GEL Super ODS Columns	
5cm x 4.6mm ID	818154
10cm x 4.6mm ID	818197
TSK-GEL Super ODS Guard Filter	
pk. of 3	818207
Holder for Filter	818206

*When using a high-viscosity buffer, you might have to reduce the flow rate to avoid exceeding the maximum pressure drop. When changing solvents, use a flow rate equal to 25% of the maximum flow rate for the initial solvent.

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