Silica-based:

TSKgel Protein C_4-300	TSKgel ODS-140HTP	TSKgel ODS-100V
TSKgel ODS-100Z	TSKgel Super-ODS	TSKgel Super-Octyl
TSKgel Super-Phenyl	TSKgel CN-80Ts	TSKgel Octyl-80Ts
TSKgel ODS-80TM	TSKgel ODS-80Ts	TSKgel ODS-80Ts QA
TSKgel ODS-120A	TSKgel ODS-120T	TSKgel OligoDNA-RP
TSKgel TMS-250		

Polymer-based:

TSKgel Octadecyl-2PW	TSKgel Octadecyl-4PW	TSKgel Octadecyl-NPR
TSKgel Phenyl-5PW RP		

Reversed Phase Tips:

- TSKgel reversed phase columns are offered in stainless steel hardware. Stainless steel (SS) frits are embedded in the body of the column endfittings. The nominal frit size for SS columns is engraved in the endfittings.
- Halide salts corrode stainless steel tubing, fitting, and frits. Do not store SS columns in a mobile phase containing NaCl and, where possible, use another salt in the operating buffer.
- Good laboratory procedures demand that the analytical column be protected by a guard column. TSKgel guardgel kits, containing column hardware and gel packing, are available to pack your own guard column. In addition, guard cartridges, guardfilters, and packed guard columns are available for use with TSKgel reversed phase columns.
- Caution: The silica particles in TSKgel Super series columns have a relatively small pore volume, which results in shorter retention times than obtained on most other reversed phase columns. For instance, to achieve similar retention times as obtained on TSKgel ODS-100V, the percentage organic solvent in the mobile phase has to be reduced by about 5-10% on a TSKgel Super-ODS column.
- Optimizing results with the TSKgel Super series columns: TSKgel Super series columns can be used on a regular HPLC system if the dead volume is minimized, although optimal results are obtained with an HPLC system designed for 2 mm or smaller ID columns. The following recommendations are for 4.6 mm ID columns. Use proportionately lower values for 2 mm ID columns.

    1. A guard filter is highly recommended to reduce particulate contamination from the sample or system components.
    2. Keep sample volume less than 10 µL.
    3. To ensure minimal extra-column volume, keep tubing as short as possible (extra-column volume less than 5 µL between column and detector).
    4. Conventional 0.1 mm ID connecting tubing may be used (0.005”).
    5. The smallest detector time constant should be selected (if possible, less than 50 ms).
    6. The detector flow cell should be 2 µL or less for best results. A standard HPLC flow cell (10 µL) can be used as an alternative; however, it is recommended that the heating coil is removed.

- TSKgel reversed phase columns are supplied with an Inspection Data Sheet, which includes a QC chromatogram and test data, and an OCS Sheet summarizing the recommended operating conditions for optimum column performance.
- A separate TSKgel Column Instruction Manual that reviews general guidelines for column installation and care, as well as troubleshooting tips for commonly encountered problems, can be downloaded from the Tosoh Bioscience LLC website (www.tosohbioscience.com).

About

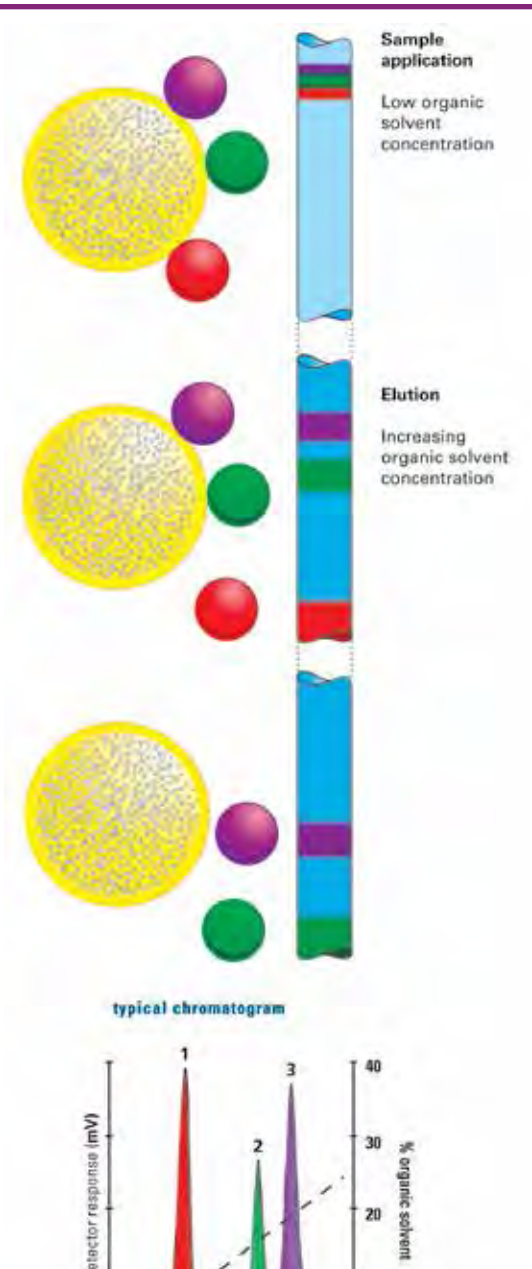
Reversed Phase Chromatography (RPLC or RPC) is the most efficient of all biomolecule separation techniques. It has been the technique of choice for the analysis of small molar mass compounds in both the pharmaceutical and chemical industries, as well as in biomedical research, since the late 1970s. More recently, RPC has become the accepted tool for the separation of peptides, proteins and other biopolymers, making it largely responsible for the widespread popularity of HPLC as a chromatographic technique.

The opposite of normal phase chromatography, RPC requires a non-polar stationary phase and a mobile phase that consists of a mixture of water and polar-solvent mobile phase. The so-called "hydrophobic effect" is the major driving force for retention in RPC. The hydrophobic effect is related to the non-polar surface area of the solute molecule, which varies as a function of mobile phase composition, while the strength of the hydrophobic bond is proportional to the decrease in molecular surface area when the solute associates with the carbon-based stationary phase. Mobile phase additives, such as trifluoroacetic acid, increase protein hydrophobicity by forming ion pairs that strongly adsorb to the stationary phase. Typically, the mobile phase consists of a mixture of water (buffer) and acetonitrile, methanol or, less common, THF, or 2-propanol. The biological molecules are eluted from the chromatographic support by a change in the polarity of the mobile phase.

Silica particles are most commonly used as the support, which then is derivatized with octadecylsilane (ODS). Polymer-based supports have been introduced as an alternative to silica-based reversed phase columns, particularly for analyzing basic compounds in their neutral state at high pH.

RPC columns can be applied to the analysis of a wide variety of compounds, ranging from neutral polar and non-polar solutes to acidic, basic, and amphoteric compounds. RPC is also an efficient technique for the analysis of derivatized amino acids, peptides and proteins, although protein structure is not always maintained due to the high concentration of organic solvent required for their elution.

Figure 1: Reversed Phase Chromatography



TSKgel Reversed Phase Chromatography Columns

Tosoh Bioscience offers 18 distinct Reversed Phase column types which are based on either silica or methacrylate particles, as discussed in Table 1.

Table 1: Overview of TSKgel RPC columns

Silica-based columns	Polymer-based columns
High purity Type B silica High efficiencies Excellent recoveries Low bleed for MS	Hydrophilic backbone to improve recovery and reduce secondary interactions. pH stable from 1 to 12. Compatibility with organic solvents eliminates swelling.
An excellent choice for analysis of small molecules and peptides. Grouped into 6 product families.	An excellent choice for large MW biomolecules (>1.0 × 10^4 Da) and for analyzing small MM compounds at high pH. Offered in 4 different chemistries.
• Protein C_4-300 • ODS-140HTP • ODS-100V and 100Z (10 nm) • Monomeric bonded silica (8 nm) • High efficiency (14 nm) • Specialty silica columns	• Octadecyl-2PW (12.5 nm) • Octadecyl-4PW (50 nm) • Phenyl-5PW RP (100 nm) • Octadecyl-NPR (nonporous)

The silica-based TSKgel reversed phase product line consists of ten stationary phases designed for the analysis of low molar mass compounds, including pharmaceutical drugs, forensic compounds, derivatized amino acids, carbohydrates, steroids, lipids, and fatty acids, as well as two stationary phases with larger pore size designed for protein analysis.

TSKgel silica packings consist of spherical particles with uniform pore sizes of 8, 10, 12, 14, 25, or 30 nm bonded with a monomeric or polymeric layer of octadecyl, octyl, cyano, trimethylsilyl, or phenyl groups. Several of the stationary phases are subsequently derivatized with trimethylsilyl groups by a proprietary method that deactivates residual but accessible silanol groups.

Polymethacrylate-based reversed phase columns are available in a range of pore and particle sizes. Although often not as efficient as and less robust than silica-based RPC columns, key advantages of polymer-based columns are the fact that they are chemically stable from pH 2 to 12, which allows many basic compounds to be analyzed in their uncharged form, thus reducing secondary adsorption and improving peak shape and improving recovery for peptides and proteins due to reduced secondary interactions.

Tables 2 and 3 feature the properties and applications of each of the TSKgel silica-based and polymer-based reversed phase columns.

Table 2: Properties of TSKgel silica-based RPC columns

Properties of Silica-Based TSKgel RPC Columns						
Column	**Functional group**	**End-capped**	**% Carbon**	**Particle size (µm)**	**Pore size (nm)**	**Application/Features**
Protein C_4-300	C4 alkyl, polymeric	Yes	3	3	30	For recovery and resolution of proteins
ODS-140HTP	C18 alkyl, polymeric	Yes	8	2.3	14	UHPLC applicable
ODS-100V	C18 alkyl, monomeric	Yes	15	3, 5	10	1st choice; High surface polarity, compatible with 100% aqueous eluents, strong retention of polar compounds
ODS-100Z	C18 alkyl, monomeric	Yes	20	3, 5	10	1st choice; More hydrophobic than ODS-100V; stronger retention and higher selectivity for non-polar compounds; higher steric selectivity
ODS-120T	C18 alkyl, polymeric	Yes	22	5, 10	12	Specialty column for analysis of peptides, small proteins, and small molar mass compounds in chemical and environmental samples
ODS-120A	C18 alkyl, polymeric	No	20	5, 10	12	Specialty column for analysis of polyaromatic hydrocarbons. Best choice for steric selectivity.
ODS-80Ts	C18 alkyl, monomeric	Yes	15	5, 10	8	Low molar mass pharmaceuticals, bases, nucleosides and nucleotides
ODS-80Ts QA	C18 alkyl, monomeric	Yes	15	5	8	Tighter specs than standard ODS-80Ts
ODS-80TM	C18 alkyl, monomeric	Yes	15	5, 10	8	Low molar mass pharmaceuticals, bases, nucleosides and nucleotides
Oligo-DNA RP	C18 alkyl, monomeric	No	10	5	25	For analysis and purification of oligonucleotides, RNA and DNA-fragments
Octyl-80Ts	C8 alkyl, monomeric	Yes	11	5	8	Reduced tailing when analyzing basic compounds

Table 2 Continued: Properties of TSKgel silica-based RPC columns

Properties of Silica-Based TSKgel RPC Columns						
Column	**Functional group**	**Endcapped**	**% Carbon**	**Particle size (µm)**	**Pore size (nm)**	**Application/Features**
Super-ODS	C18 alkyl, polymeric	Yes	8	2.3	14	High throughput analysis of hydrophilic or hydrophobic peptides, tryptic digests, peptide mapping, low molar mass pharmaceuticals, purines and pyrimidines, nucleosides, nucleotides
Super-Octyl	C8 alkyl, polymeric	Yes	5	2.3	14	
Super-Phenyl	Phenyl alkyl, polymeric	Yes	3	2.3	14	
CN-80Ts	CN, monomeric	Yes	8	5	8	Polar peptides, amino acids, and other pharmaceutical and food & beverage products
TMS-250	C1 alkyl, monomeric	Yes	5	10	25	Protein separations

Table 3: Properties of TSKgel polymer-based RPC columns

Properties of Polymer-Based TSKgel RPC Columns						
Column	**Functional group**	**Endcapped**	**% Carbon**	**Particle size (µm)**	**Pore size (nm)**	**Application/Features**
Octadecyl-2PW	C18 alkyl, monomeric	-	-	5	12.5	Peptides, low molar mass oligomers
Octadecyl-4PW	C18 alkyl, monomeric	-	-	7, 13	50	Large peptides, peptides unstable at low pH
Phenyl-5PW RP	Phenyl, monomeric	-	-	10, 13	100	Proteins, high pH applications
Octadecyl-NPR	C18 alkyl, monomeric	-	-	2.5	nonporous	Provides excellent stability in high pH buffer systems; medium and high molar mass peptides and proteins

About: TSKgel Protein C_4-300 Reversed Phase Chromatography Columns

TSKgel Protein C_4-300 columns are designed for the optimal recovery and resolution of proteins such as recombinant proteins, antibody fragments or PEGylated proteins.

The 30 nm pore size of the TSKgel Protein C_4-300 columns are ideal for the separation of proteins. A particle size of 3 µm and optimized ligand density and alkyl length result in better protein and peptide resolution compared to other leading RP-C4 HPLC phases.

The C4 short alkyl chain ligand and its controlled bonding density provide moderate hydrophobicity to the stationary phase, which results in protein separations with high recovery and less peak tailing. The large pore size, allowing macromolecules to enter the interior of the pore, provides higher peak capacities than reversed phase columns with 10 nm pore size.

Attributes and Applications

The silica-based, wide pore TSKgel Protein C_4-300 HPLC columns are suitable for highly efficient, reversed phase separations of large biomolecules such as proteins. Table 4 lists the attributes of TSKgel Protein C_4-300.

Table 4. Product attributes

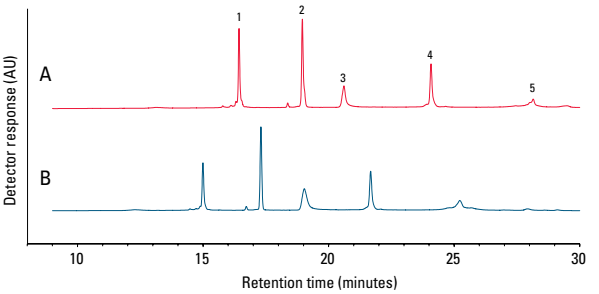
Attribute	Value
Pore size	30 nm
Endcapped	Yes (Trimethylsilyl)
Particle size	3 µm
pH stability	1.5-7.5
Ligand	C4 (Butyl)
Surface area (m^2/g):	100
% Carbon	3%

Standard Proteins

Figure 2 shows the separation of a mixture of standard proteins on the TSKgel Protein C_4-300 column compared to a competitor column with 3.5 µm particle size. The resolution between cytochrome c and lysozymes reaches 24.8 on the TSKgel Protein C_4-300 column compared to 18.6 on the competitor C_4 column.

Furthermore, the TSKgel column shows higher theoretical plates and less peak tailing, especially for BSA (Peak 3), and also a better resolution of minor peaks.

Figure 2. Comparison of standard protein separation



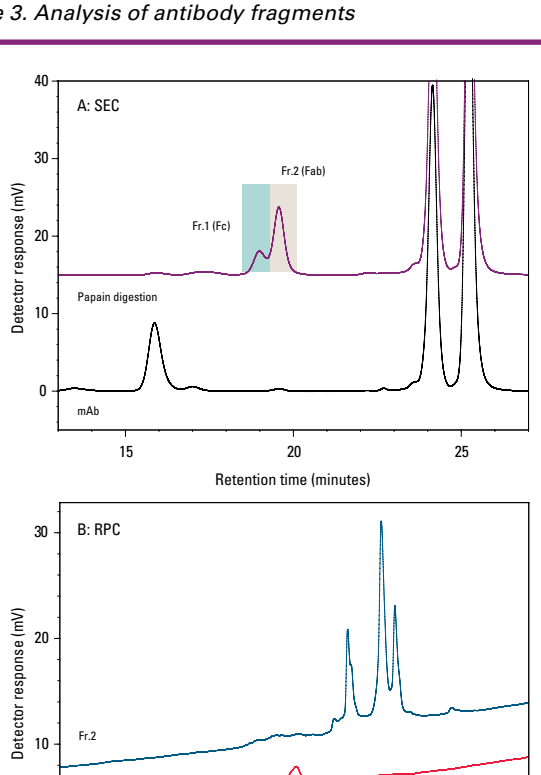
Columns:	**A. TSKgel Protein C_4-300, 3 µm, 4.6 mm ID × 15 cm**
	B. Competitor A, 3.5 µm, 4.6 mm ID × 15 cm
Mobile phase:	A: H_2O/CH_3CN/TFA = 90/10/0.05 (v/v/v)
	B: H_2O/CH_3CN/TFA = 20/80/0.05 (v/v/v)
Gradient:	0 min (0%B) 45 min (100%B)
Flow rate:	1.0 mL/min
Detection:	UV @ 210 nm
Temperature:	40 °C
Injection vol.:	10 µL
Samples:	1. cytochrome C 2. lysozyme
	3. BSA 4. α-chymotrypsinogen A
	5. ovalbumin (each 2 µg/10 µL)

Antibody Fragments

Figures 3A & 3B show the analysis of antibody fragments. The monoclonal antibody human IgG_1 was first papain digested and separated using a TSKgel G3000SWXL SEC column (Figure 3A). The intact form of the antibody, partially digested fragments, and completely digested fragments were separated on the basis of molecular size.

Two fractions were obtained from the SEC analysis and each fraction was analyzed with the TSKgel Protein C_4-300 reversed phase column, as shown in Figure 3B. Several peaks were observed in each chromatogram of the analysis of Fc (fragment 1) and Fab (fragment 2), indicating that the antibody used in this study was heterogeneous in hydrophobicity.

Figure 3. Analysis of antibody fragments



Conditions for SEC

Column:	**TSKgel G3000SWXL, 3 µm, 7.8 mm ID × 30 cm × 2**
Mobile phase:	20 mmol/L phosphate buffer, pH 7.0 + 0.3 mol/L NaCl
Flow rate:	1.0 mL/min
Temperature:	25 °C
Sample:	monoclonal antibody (human IgG_1)

Conditions for RPC

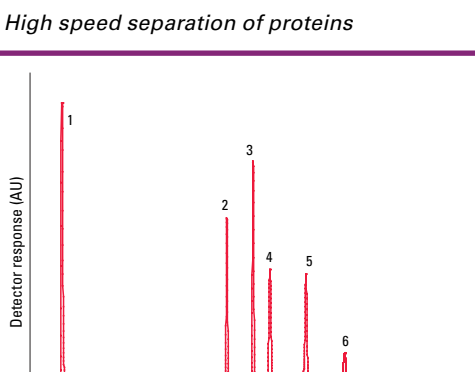
Column:	**TSKgel Protein C_4-300, 3 µm, 4.6 mm ID × 30 cm**
Mobile phase:	A: 0.05% TFA in H_2O B: 0.05% TFA in ACN
Gradient:	0 min (5%B) 20 min (50%B)
Flow rate:	1.0 mL/min
Temperature:	70 °C
Sample:	monoclonal antibody (human IgG_1)

Reduced Analysis Time in Protein Separation

For high speed separations, the analysis time can be reduced by more than eighty percent when using the short 5 cm TSKgel Protein C_4-300 column and increasing the flow rate to 3 mL/min (see Figure 4). The backpressure remains below 15 MPa, allowing the use of standard HPLC systems. The long term stability of the new C4 phase in acidic solution was tested by flushing the column with 30% acetonitrile, 0.2% TFA (4 times the standard TFA concentration) at 40 °C.

There was no change in theoretical plates even after 1,000 hours of run time under this chromatographic condition. Also retention times of standard proteins didn't have significant loss when compared to the initial values.

Figure 4. High speed separation of proteins



Column:	**TSKgel Protein C_4-300, 3 µm, 4.6 mm ID × 5 cm**
Mobile phase A:	$H_2O/CH_3CN/TFA$ = 90/10/0.05 (v/v/v)
Mobile phase B:	$H_2O/CH_3CN/TFA$ = 20/80/0.05 (v/v/v)
Gradient:	0 min (0%B) 5 min (100%B)
Flow rate:	3.0 mL/min
Detection:	UV @ 210 nm
Temperature:	40 °C
Injection vol.:	10 µL
Samples:	1. phenylalanine 2. cytochrome C 3. lysozyme 4. BSA 5. α-chymotrypsinogen A 6. ovalbumin (each 0.2 g/µL)

About: TSKgel ODS-140HTP Reversed Phase Chromatography Columns

TSKgel ODS-140HTP columns provide high resolution and short analyses times at moderate pressures, enabling high throughput separations. The polylayer bonding chemistry of the 2.3 µm particle size of these columns results in highly efficient and durable columns. The lower pressure drop reduces the burden on the hardware, allowing the TSKgel ODS-140HTP columns to be used with either UPLC® (up to 62 MPa) or conventional HPLC systems.

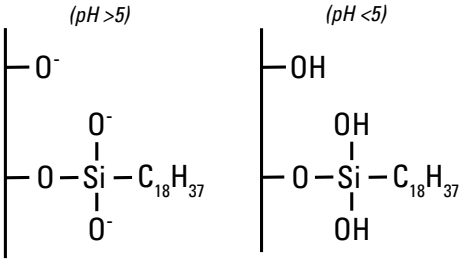
Attributes and Applications

Table 5 lists the attributes of TSKgel ODS-140HTP columns, while Figure 5 displays the structure. For use in high throughput applications, including drug discovery, pharmacokinetics and peptide digest separations, TSKgel ODS-140HTP columns offer excellent peak shape for basic compounds.

Table 5: Product attributes

Attribute	Value
Pore size (mean)	14 nm
Endcapped	Yes
Particle size	2.3 µm
pH stability	2.0-7.5
Functional group	C18 (polymeric bonding chemistry)
% Carbon	8

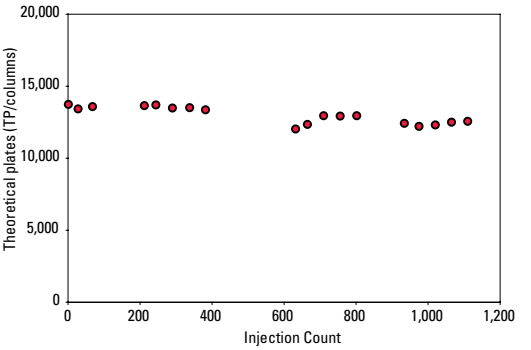
Figure 5: TSKgel ODS-140HTP structure



Column Stability

Figures 6 and 7 demonstrate that TSKgel ODS-140HTP columns are stable at high flow rates under demanding step gradient conditions. Figure 6 shows that consistent theoretical plate values were obtained on the TSKgel ODS-140HTP column during 1,110 gradient cycles consisting of five minute step gradients from 10% to 50% and from 50% to 100% methanol at 0.6 mL/min. During each cycle, pressure fluctuated between 30 and 60 MPa.

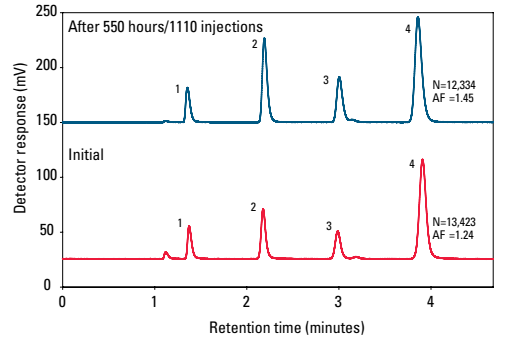
Figure 6: Stability of TSKgel ODS-140HTP columns



Column:	**TSKgel ODS-140HTP, 2.3 µm, 2.1 mm ID × 10 cm**
Mobile phase:	A: H_2O/MeOH = 90/10 B: H_2O/MeOH = 50/50 C: MeOH
Gradient:	A→B→C (5 min., Step gradient)
Flow rate:	0.6 mL/min
Temperature:	25 °C
Pressure:	A: 45 MPa B: 59 MPa C: 32 MPa
Sample:	napthalene

Figure 7 shows injections of test solutes after the first step gradient cycle and after 1,110 cycles. The results clearly demonstrate the durability of the TSKgel ODS-140HTP columns when operated at high flow rate and high pressure.

Figure 7: Durability of TSKgel ODS-140HTP columns

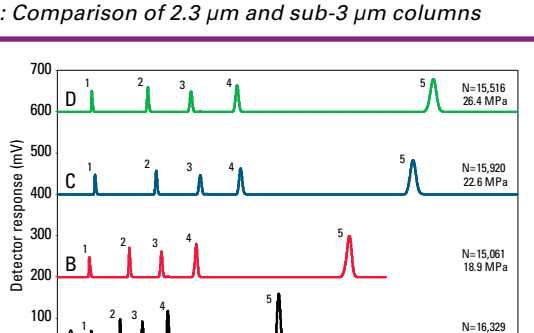


Column:	**TSKgel ODS-140HTP, 2.3 µm, 2.1 mm ID × 10 cm**
Mobile phase:	H_2O/MeOH = 30/70
Flow Rate:	0.2 mL/min
Detection:	UV @ 254 nm
Temperature:	25 °C
Injection vol.:	2 µL
Samples:	1. phenol 2. benzene 3. toluene 4. napthalene

Performance Data

Column efficiency of a TSKgel ODS-140HTP column compares favorably with other sub-3 µm ODS columns (see Figure 8). Higher efficiency and a shorter retention time make the TSKgel ODS-140HTP column more suitable for high throughput separations.

Figure 8: Comparison of 2.3 µm and sub-3 µm columns

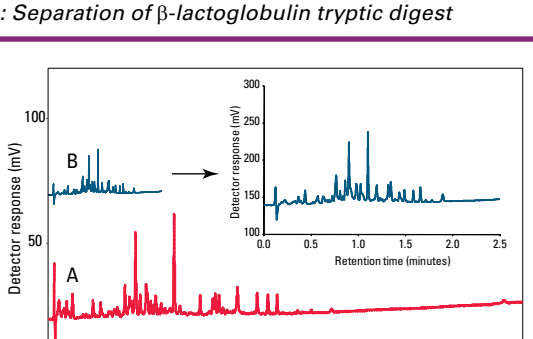


Columns:	**A. TSKgel ODS-140HTP, 2.3 µm, 2.1 mm ID × 10 cm**
	B. Ascentis® Express C18, 2.7 µm, 2.1 mm ID × 10 cm
	C. Luna C18(2)-HST, 2.5 µm, 2 mm ID × 10 cm
	D. YMC UltraHT® Pro C18, 2 µm, 2 mm ID × 10 cm
Mobile phase:	H_2O/MeOH = 30/70
Flow Rate:	0.2 mL/min
Detection:	UV @ 254 nm
Temperature:	25 °C
Injection vol.:	2 µL
Samples:	1. uracil
	2. benzene
	3. toluene
	4. napthalene
	5. fluorene

Tryptic Digest

Excellent resolution at high speed was achieved on a TSKgel ODS-140HTP column for the separation of a β-lactoglobulin tryptic digest (see Figure 9). As expected, peak capacity improved when using a longer gradient time.

Figure 9: Separation of β-lactoglobulin tryptic digest

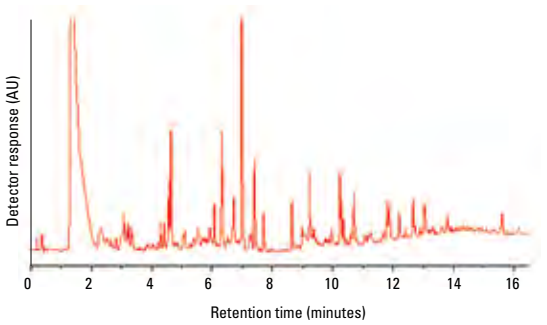


Column:	**TSKgel ODS-140HTP, 2.3 µm, 2.1 mm ID × 5 cm**
Mobile phase:	A: H_2O/ACN (95/5) + 0.1% TFA
	B: H_2O/ACN (50/50) + 0.1% TFA
Gradient:	0-100%B (linear gradient)
Gradient time:	A: 10 min
	B: 2.5 min
Flow rate:	1.0 mL/min
Detection:	UV @ 220 nm
Temperature:	40 °C
Injection vol.:	10 µL
Sample:	β-lactoglobulin tryptic digest

Herbal Extract

In Chinese traditional medicine, an extract of *Crinum latifolium L.* is used to invigorate blood circulation. It is thought to possess antiviral and immunostimulative properties and shows immunomodulatory properties in human peripheral blood mononuclear cells. The analysis of products derived from plant extracts is a challenging chromatographic task. Due to the high number of components, the column needs to provide high peak capacity. As shown in Figure 10, a TSKgel ODS-140HTP column is an excellent choice for plant extract separations.

Figure 10: Separation of Crinum latifolium L.

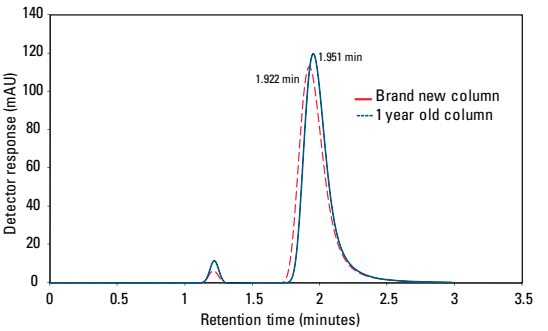


Column:	**TSKgel ODS-140HTP, 2.3 µm, 2.1 mm ID × 10 cm**
Mobile phase:	A: H_2O B: ACN
Gradient:	0 min (5%B) 0.08 min (5%B) 7.47 min (40%B) 13.66 min (100%B) 16.13 min (100%B) 16.14 min (5%B)
Flow rate:	0.523 mL/min
Detection:	UV @ 220 nm
Temperature:	35 °C
Injection vol.:	2 µL
Sampling rate:	80 Hz
Sample:	50 g/L extract of *Crinum latifolium L* by 95% ethanol
Instrument:	Acquity UPLC® System with TUV detector

Caffeine Analysis

HPLC methods are commonly used for the analysis of caffeine in beverages. A caffeine USP standard eluted from a TSKgel ODS-140HTP, 2.3 µm column within two minutes under isocratic chromatographic conditions using a conventional HPLC system. The durability of the column was tested under these isocratic conditions using a fresh TSKgel ODS-140HTP column and one run frequently for over a year (more than 1,000 injections). No significant change in elution profile was noted. Caffeine eluted at 1.922 minutes from the new column while the used column yielded a retention time of 1.951 minutes (Figure 11).

Figure 11: Isocratic elution of caffeine USP and test of column stability

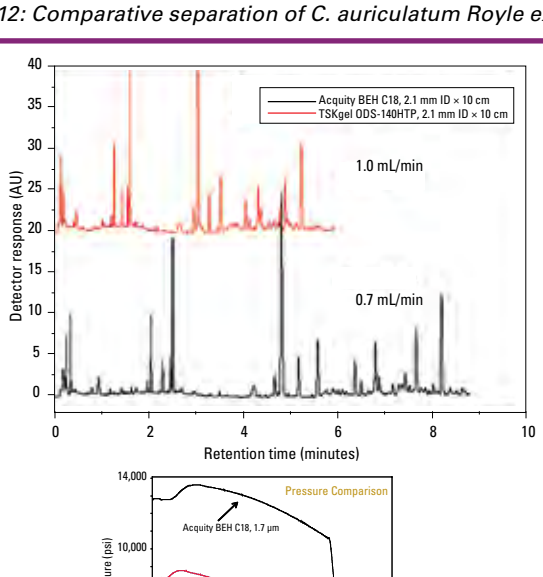


Column:	**TSKgel ODS-140HTP, 2.3 µm, 2.1 mm ID × 5 cm**
Mobile phase (Isocratic):	10% ACN in H_2O containing 0.15% TFA
Flow rate:	0.2 mL/min
Detection:	UV @ 275 nm
Temperature:	40 °C
Injection vol.:	10 µL
Sample:	caffeine USP (1.427 mg/mL)

Root Extract

Figure 12 details the separation using a TSKgel ODS-140HTP column of a root tuber extract of *cynanchum auriculatum* Royle ex Wight. This weed, also known as climbing milkweed, is used in traditional Chinese medicine for its anti-tumor and anti-gastric lesion activity. The TSKgel ODS-140HTP column delivers a faster analysis at a higher flow rate under a lower pressure compared to a competitive sub-2 µm column when run on an Acquity UPLC system.

Figure 12: Comparative separation of C. auriculatum Royle ex Wight



Columns:	**TSKgel ODS-140HTP, 2.3 µm, 2.1 mm ID × 10 cm** Acquity BEH C18, 1.7 µm, 2.1 mm ID × 10 cm
Mobile phase:	A: H_2O B: ACN
Flow rate:	1.0 mL/min (TSKgel ODS-140HTP) 0.7 mL/min (Acquity BEH C18)
Detection:	UV @ 220 nm
Temperature:	40 °C
Injection vol.:	1 µL
Sampling rate:	80 Hz
Sample:	10 g/L extract of *C. auriculatum* Royle ex Wight by 95% ethanol
Instrument:	Acquity UPLC System with TUV detector

Optimum gradient for Acquity BEH C18: 0 min (5%B) 0.68 min (5%B) 2.28 min (30%B) 8.57 min (68%B) 8.70 min (100%B) 20 min (100%B)

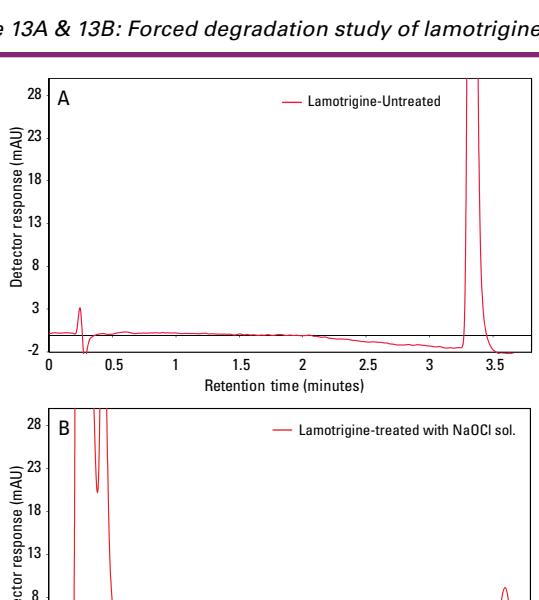
Optimum gradient for TSKgel ODS-140HTP: 0 min (5%B) 0.48 min (5%B) 1.6 min (30%B) 6.0 min (68%B) 6.1 min (100%B) 20 min (100%B)

Forced Degradation of Off-Patent Drug

In 2007, more than two thirds of all prescriptions in the United States were filled with generic drugs (http://www.nytimes.com/2009/01/06/us/06healthcare.html?r=1) Like the manufacturers of brand name drugs, generic manufacturers need to develop validated methods to meet regulatory compliance. Forced degradation studies are designed to determine the degradation products formed during accelerated pharmaceutical studies and long-term stability studies.

A TSKgel ODS-140HTP column was used to study the degradation of lamotrigine, an anti-epileptic drug that lost patent protection in 2009. Figure 13A shows the analysis of untreated lamotrigine. Lamotrigine is known to form two different N-chloro products when in contact with a 6% NaOCl solution. Upon treatment with NaOCl, the lamotrigine peak disappeared, leaving only evidence of degradation products (as demonstrated in Figure 13B).

Figure 13A & 13B: Forced degradation study of lamotrigine

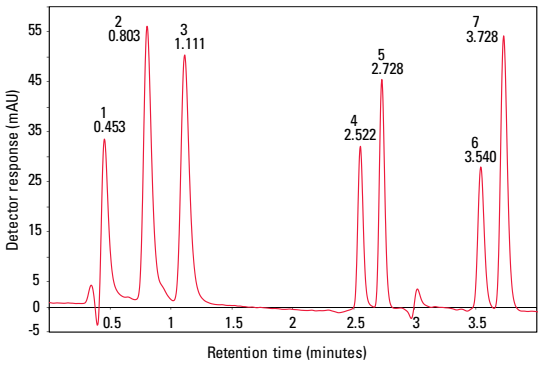


Column:	**TSKgel ODS-140HTP, 2.3 µm, 2.1 mm ID × 5 cm**
Mobile phase:	A: H_2O + 0.15% TFA B: 100% ACN with 0.15% TFA
Gradient:	0 min (4%A) 15 min (100%B)
Flow rate:	0.8 mL/min
Detection:	UV @ 215 nm
Temperature:	40 °C
Injection vol.:	10 µL
Sample:	lamotrigine (25 mg/L, 750 µL) in mobile phase A treated with 750 µL of 6% NaOCl solution for 1 minute. Final concentration of lamotrigine: 12.5 mg/L

OTC Cold, Sinus and Allergy Medications

Six cold and sinus drug standards (Figure 14) were separated as sharp peaks with good resolution within 3.8 minutes using a TSKgel ODS-140HTP column. The peak labeled (1) was identified as maleate originating from the drug standard chlorpheniramine maleate (5). Diphenhydramine is considerably shorter retained than that reported using an ACQUITY UPLC HSS T3, 1.8 µm, 2.1 mm ID × 10 cm column (Mazzeo JR, LCGC Asia Pacific, Volume 10, Issue 1, May1, 2007). The two drug substances diphenhydramine and dextromethorphan have very similar and strong hydrophobic properties with a tendency to co-elute or elute with considerable overlap. These substances were separated with a resolution of 1.9.

Figure 14: Analyses of six cold and sinus drug standards



Column:	**TSKgel ODS-140HTP, 2.3 µm, 2.1 mm ID × 5 cm**
Mobile phase:	A: H_2O with 0.15% TFA
	B: 100% ACN with 0.15% TFA
Gradient:	Time (min) Solvent B (%) Flow (mL/min)
	1.4 2.0 0.6
	1.5 24.0 1.4
	2.1 1.4
	2.2 0.8
	4.2 5.0 0.8
	4.1 1.0 0.6
Detection:	UV @ 215 nm
Temperature:	50 °C
Injection vol.:	10 µL
Samples:	1. maleate peak
	2. phenylephrine
	3. acetaminophen
	4. doxylamine succinate
	5. chlorpheniramine maleate
	6. dextromethorphan HBr
	7. diphenhydramine HCl

About: TSKgel ODS-100V Reversed Phase Chromatography Columns

TSKgel ODS-100V reversed phase columns are general purpose columns suitable for the most demanding separations in quality control as well as in research and development. Containing a unique surface property utilizing highly efficient bonding and endcapping procedures, secondary interactions of basic, acidic, and chelating compounds are limited.

TSKgel ODS-100V columns provide strong retention for polar compounds as these types of compounds are retained by hydrophobic association, plus by enhanced interaction of their polar groups with the more polar surface of the TSKgel ODS-100V column. In addition to the strong retention, these columns also provide higher selectivity for polar compounds. Monomeric bonded phase chemistry of the TSKgel ODS-100V packing material provides complete wetting and retention stability in 100% aqueous mobile phases (see Figure 16).

TSKgel ODS-100V columns are available in 3 µm particle size in addition to the traditional 5 µm size. The 3 µm columns are well suited for high throughput LC/MS applications, providing fast and efficient separations.

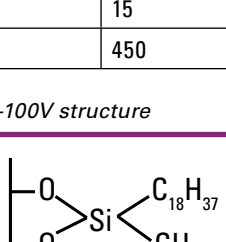
Attributes and Applications:

Product attributes of TSKgel ODS-100V columns are listed in Table 6. The structure is displayed in Figure 15. TSKgel ODS-100V columns are the best choice for challenging compounds, including acidic, basic, zwitterionic, and chelating compounds.

Table 6: Product attributes

Attribute	Value
Pore size (mean)	10 nm
Molar mass limit	1.0×10^4 Da
Endcapped	Yes
Particle size	3 µm and 5 µm
pH stability	2.0-7.5
Functional group	octadecylmethylsilane
% Carbon	15
Surface area (m^2/g)	450

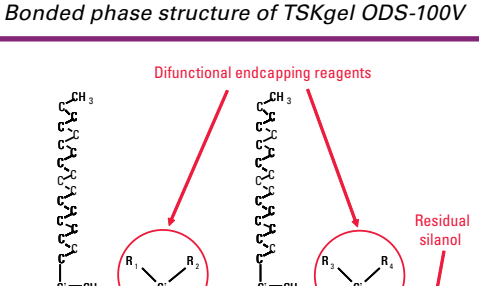
Figure 15: TSKgel ODS-100V structure



Novel Bonding Chemistry

The novel bonding chemistry employed in the preparation of TSKgel ODS-100V is depicted in Figure 16. The TSKgel ODS-100V bonded phase 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. This material is made under conditions that promote the formation of a monomeric bonded phase layer.

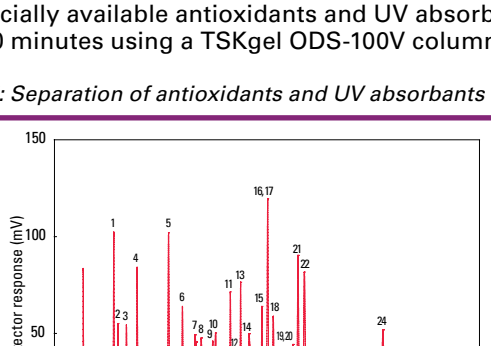
Figure 16: Bonded phase structure of TSKgel ODS-100V



Antioxidants and UV Absorbants

Small quantities of antioxidants and UV stabilizers are often added to commercial plastics to prevent or reduce degradation. It is of vital importance in the manufacturing process to accurately control these additives. The chromatogram in Figure 17 shows the separation of 26 commercially available antioxidants and UV absorbants in about 50 minutes using a TSKgel ODS-100V column.

Figure 17: Separation of antioxidants and UV absorbants

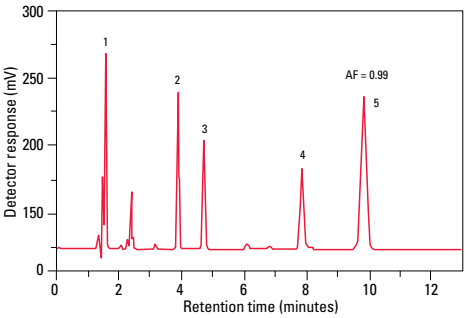


Column:	**TSKgel ODS-100V, 5 µm, 4.6 mm ID × 25 cm**
Mobile phase:	A: H_2O B: CH_3CN
Gradient:	0 min (60%B) 20 min (100%B)
Flow rate:	1.0 mL/min
Temperature:	50 °C
Detection:	UV @ 225 nm
Injection Vol.:	10 µL
Concentration:	10 mg/L each
Samples:	1. Cyasorb® UV-24 2. BHA 3. Ionox 100 4. Seesorb 101 5. 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

Bonded Phase Characterization

Standard Reference Material SRM 870 was developed by NIST (National Institute of Standards and Technology) as a means to classify the many commercially available reversed phase columns into closely-related groups. Amitriptyline, a tertiary amine, and quinizarin, a strong chelating compound, are included in the SRM 870 mixture, together with more traditional compounds. As shown in Figure 18, symmetrical peaks are obtained on a TSKgel ODS-100V column for all compounds in this test mixture, clearly demonstrating the superior performance of this column for the analysis of basic and chelating compounds as well as for less challenging compounds.

Figure 18: Separation of SRM 870

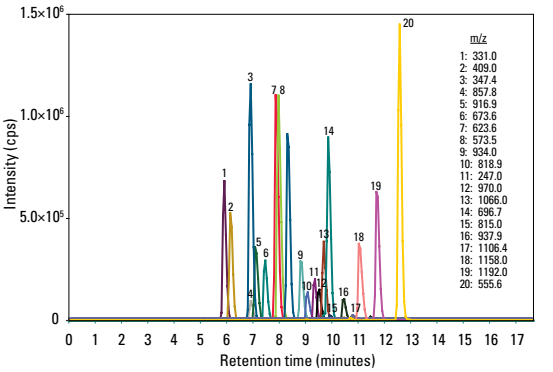


Column:	**TSKgel ODS-100V, 3 µm, 4.6 mm ID × 15 cm**
Mobile phase:	20 mmol/L phosphate buffer, pH 7.0/MeOH (20/80)
Flow rate:	1.0 mL/min
Detection:	UV @ 254 nm
Temperature:	40 °C
Injection vol.:	10 µL
Samples:	1. uracil 2. toluene 3. ethyl benzene 4. quinizarin 5. amitriptyline

Tryptic Digest

The rapid identification of 20 peptides using a TSKgel ODS-100V column is detailed in Figure 19. The high speed analysis and symmetrical peaks of basic compounds in low concentration ammonium formate buffer make this column an excellent choice for LC/MS work.

Figure 19: Rapid identification of 20 peptide fragments

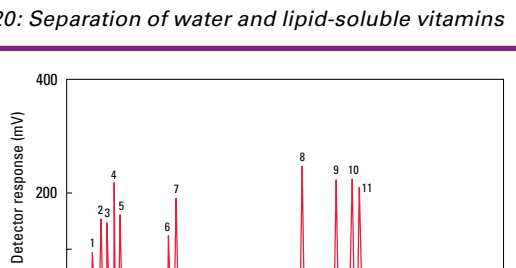


Column:	**TSKgel ODS-100V, 3 µm, 2.0 mm ID × 15 cm**
Mobile phase:	A: 0.1% TFA in H_2O B: 0.1% TFA in ACN
Gradient:	0 min (10%B) 15 min (70%B) 17 min (70%B)
Flow rate:	0.2 mL/min
Injection vol.:	2 µL
Sample:	β-lactoglobulin tryptic digest
Instrument:	Q TRAP, ESI+

Vitamins

Water and lipid-soluble vitamins were separated in a single run on a TSKgel ODS-100V column as demonstrated in Figure 20. The sample is a mixture of vitamins ranging from the very polar water-soluble vitamin ascorbic acid to the very hydrophobic tocopherol derivatives. 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 high resolution for the polar compounds in the mixture, while at the same time delivers a short analysis time for the late eluting non-polar compounds.

Figure 20: Separation of water and lipid-soluble vitamins

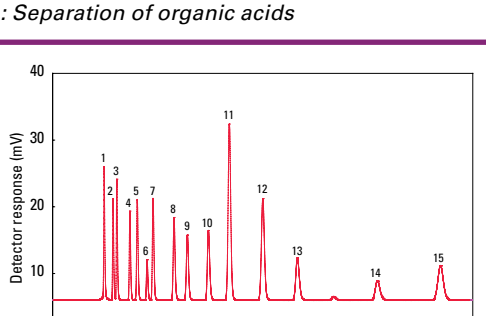


Column:	**TSKgel ODS-100V, 5 µm, 4.6 mm ID × 15 cm**
Mobile phase:	A: 0.1% TFA in H_2O
	B: 0.1% TFA in ACN
Gradient:	0 min (0%B) 20 min (40%B)
	22 min (100%B) 50 min (100%B)
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Temperature:	40 °C
Injection vol.:	5 µL
Samples:	1. L-ascorbic acid
	2. nicotinic acid
	3. thiamine
	4. pyridoxal
	5. pyridoxine
	6. caffeine
	7. riboflavine
	8. retinol
	9. δ-tocopherol
	10. α-tocopherol
	11. α-tocopherol acetate

Organic Acids

Organic acids play an important role in many metabolic processes, fermentation and food products. Figure 21 shows a baseline separation of 15 organic acids in less than 25 minutes using a simple 0.1% phosphoric acid mobile phase with a TSKgel ODS-100V column.

Figure 21: Separation of organic acids

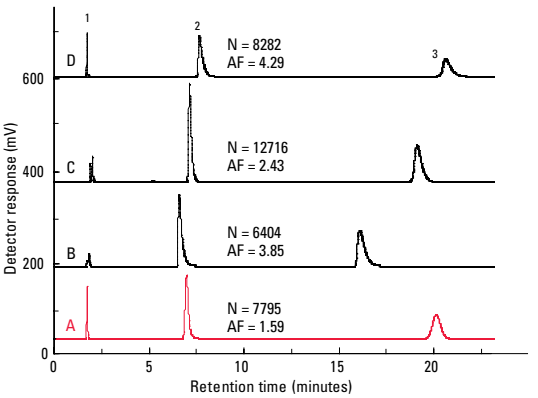


Column:	**TSKgel ODS-100V, 5 µm, 4.6 mm ID × 25 cm**
Mobile phase:	0.1% H_3PO_4
Flow rate:	1.0 mL/min
Temperature:	40 °C
Injection vol.:	10 µL
Samples:	1. oxalic acid (0.1 g/L)
	2. l-Tartaric acid (0.5 g/L)
	3. formic acid (1.0 g/L)
	4. l-Malic acid (1.0 g/L)
	5. l-Ascorbic acid (0.1 g/L)
	6. lactic acid (1.0 g/L)
	7. acetic acid (1.0 g/L)
	8. maleic acid (0.01 g/L)
	9. citric acid (1.0 g/L)
	10. succinic acid (1.0 g/L)
	11. fumaric acid (0.025 g/L)
	12. acrylic acid (0.1 g/L)
	13. propionic acid (2.0 g/L)
	14. glutaric acid (1.0 g/L)
	15. itaconic acid (0.025 g/L)

Performance Data

To demonstrate the absence of accessible silanol groups, Figure 22 compares retention and peak shape for two tricyclic antidepressant drugs on four water-wettable columns including TSKgel ODS-100V and three competitive C18 reversed phase columns. The ability to provide symmetrical peak shapes for basic compounds makes TSKgel ODS-100V the column of choice for method development and quantitative analysis of small molar mass compounds using from 100% aqueous to 100% organic mobile phase conditions.

Figure 22: Comparison of C18 columns



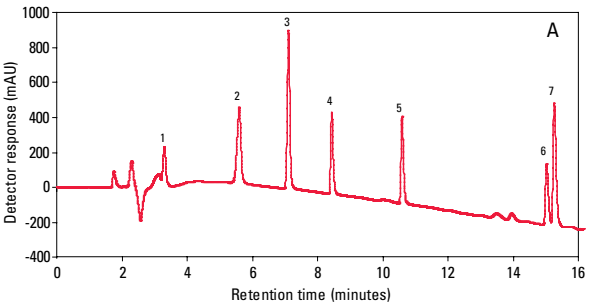
Columns:	**A. TSKgel ODS-100V, 5 µm, 4.6 mm ID × 15 cm**
	B. CAPCELL PAK C18AQ®, 5 µm, 4.6 mm ID × 15 cm
	C. Hydrosphere® C18, 5 µm, 4.6 mm ID × 15 cm
	D. Atlantis® dC18, 5 µm, 4.6 mm ID × 15 cm
Mobile phase:	50 mmol/L phosphate buffer, pH 7.0/MeOH (30/70)
Flow rate:	1.0 mL/min
Detection:	UV @ 254 nm
Temperature:	40 °C
Injection vol.:	10 µL
Samples:	1. uracil
	2. desipramine
	3. imipramine

Cold, Sinus and Analgesic Medications

Because of FDA-mandated changes to the regulation of drugs containing the popular decongestant pseudoephedrine, many pharmaceutical companies reformulated their products using phenylephrine as a substitute. To support the need to revalidate test methods, we used a TSKgel ODS-100V column to separate phenylephrine from some of the most common combinations of cold and sinus medications on the market today.

Figure 23A shows the separation of a cold mixture containing six common ingredients using a TSKgel ODS-100V, 3 µm column. The TSKgel ODS-100V column produced a single sharp peak for the analysis of phenylephrine and also a single peak for doxylamine. All compounds were resolved by this column in less than 17 minutes.

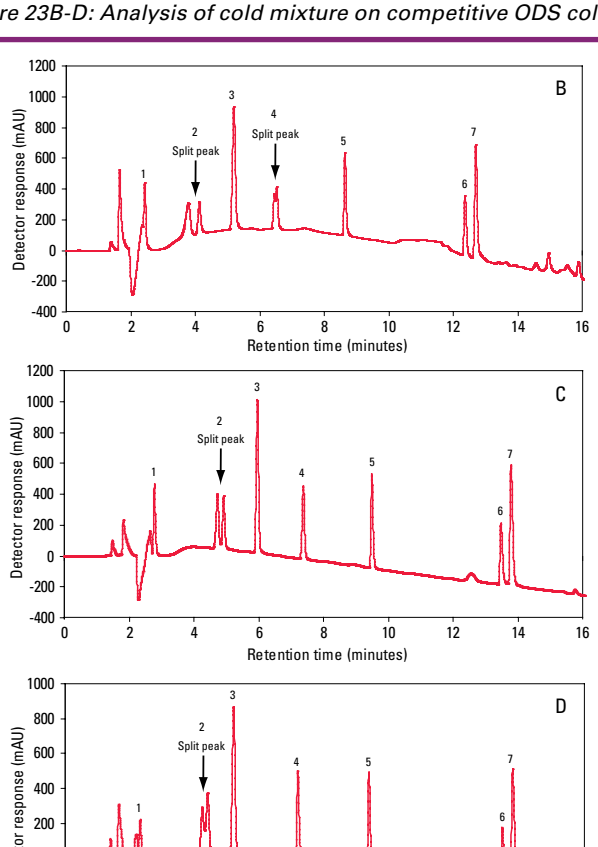
Figure 23A: Analysis of cold mixture on TSKgel ODS-100V column



Column:	**A. TSKgel ODS-100V, 3 µm, 4.6 mm ID × 15 cm**
Mobile phase	A: 0.15% TFA in H_2O
	B: 0.02% TFA in ACN/MeOH (75/25)
Gradient:	0 min (96%A, 4%B)
	15 min (40%A, 60%B)
	17 min (40%A, 60%B)
Flow rate:	1.0 mL/min
Detection:	UV @ 210 nm
Temperature:	40 °C
Injection vol.:	20 µL
Samples:	1. maleate
	2. phenylephrine HCl
	3. acetaminophen
	4. doxylamine succinate
	5. chlorpheniramine
	6. dextromethorphan HBr
	7. diphenhydramine HCl

Figures 23B-D shows the same cold mixture run on three competitive ODS columns under the same chromatographic conditions. On all three columns, phenylephrine eluted as two distinct peaks with each peak having approximately half the area as the single peak produced on the TSKgel ODS-100V column. Also, one of the competitive columns exhibited peak-splitting on the doxylamine peak.

Figure 23B-D: Analysis of cold mixture on competitive ODS columns



Columns:	B. Symmetry® C18, 3.5 µm, 4.6 mm ID × 15 cm
	C. Luna C18(2), 3 µm, 4.7 mm ID × 15 cm
	D. Zorbax® Eclipse Plus C18, 3.5 µm, 4.7 mm ID × 15 cm
Mobile phase:	A: 0.15% TFA in H_2O
	B: 0.02% TFA in ACN/MeOH (75/25)
Gradient:	0 min (96%A, 4%B)
	15 min (40%A, 60%B)
	17 min (40%A, 60%B)
Flow rate:	1.0 mL/min
Detection:	UV @ 210 nm
Temperature:	40 °C
Injection vol.:	2 0 µL
Samples:	1. maleate
	2. phenylephrine HCl
	3. acetaminophen
	4. doxylamine succinate
	5. chlorpheniramine
	6. dextromethorphan HBr
	7. diphenhydramine HCl

About: TSKgel ODS-100Z Reversed Phase Chromatography Columns

TSKgel ODS-100Z reversed phase columns are a great choice when a change of selectivity from the TSKgel ODS-100V columns is needed to resolve one or more overlapping pairs. The TSKgel ODS-100Z columns contain a high density monomeric C18 bonded phase (Figure 25) for maximum retention and selectivity of small molar mass compounds. Exhaustive endcapping prevents secondary interaction with residual silanol groups. Available in 3 and 5 µm particle size, TSKgel ODS-100Z columns stand out for lot-to-lot reproducibility (see Figure 26).

Containing a high carbon content of 20%, TSKgel ODS-100Z columns exhibit a high stability at both low and high pH. This stability at low pH is important when running peptides and proteins. At low pH conditions, silanol groups get removed first by acid hydrolysis before hydrolysis of the alkyl chains takes place. Because of their high bonded phase surface coverage, the TSKgel ODS-100Z columns can be expected to last longer before showing appreciable changes in retention due to increased silanol interaction.

TSKgel ODS-100Z columns provide longer retention for non-polar compounds and a slightly higher selectivity for non-polar compounds, for example when you need to separate homologues series, than the TSKgel ODS-100V columns. Steric selectivity is also higher for TSKgel ODS-100Z columns. This plays a role with complex 3-D molecules, such as aromatic hydrocarbons, steroids, etc.

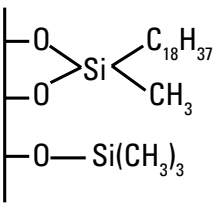
Attributes and Applications:

Table 7 lists the attributes of TSKgel ODS-100Z columns, while Figure 25 displays the structure. This general purpose column is the workhorse for analysis of small molar mass compounds in life science applications.

Table 7: Product attributes

Attribute	Value
Pore size (mean)	10 nm
Molar mass limit	1.0×10^4 Da
Endcapped	Exhaustive
Particle size	3 µm and 5 µm
pH stability	2.0-7.5
Functional group	octadecylmethylsilane
% Carbon	20
Surface area (m^2/g)	450

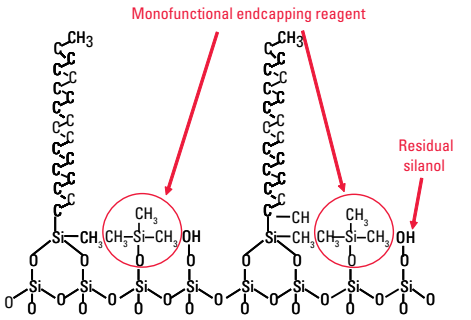
Figure 24: TSKgel ODS-100Z structure



Novel Bonding Chemistry

The novel bonding chemistry employed in the preparation of TSKgel ODS-100Z is depicted in Figure 25. TSKgel ODS-100V is prepared by reacting the surface with a difunctional octadecylsilane reagent, followed by repeated endcapping with monofunctional trimethylsilane reagent. The TSKgel ODS-100Z is prepared under conditions that promote the formation of a monomeric bonded phase layer.

Figure 25: Bonded phase structure of TSKgel ODS-100V

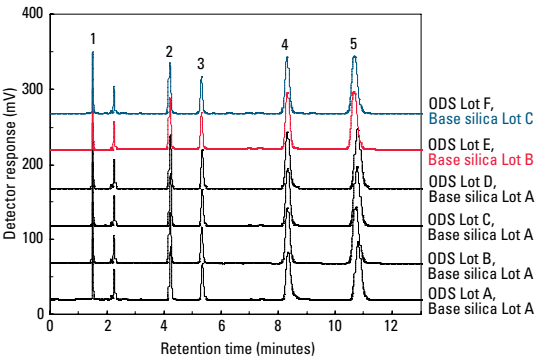


Lot-to-Lot Reproducibility

Figure 26 shows the chromatograms for SRM870 test mixture using 6 bonding lots of TSKgel ODS-100Z columns prepared from 3 different 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 the low activity towards chelating compounds and the very low activity towards organic bases, respectively, of TSKgel ODS-100Z columns.

Figure 26: TSKgel ODS-100Z lot-to-lot variability



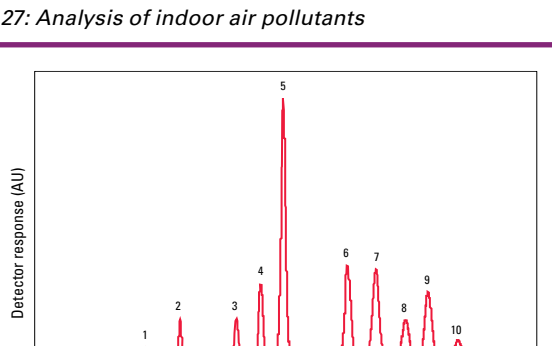
Column: **TSKgel ODS-100Z, 5 µm, 4.6 mm ID × 15 cm**
Mobile phase: 20 mmol/L phosphate buffer, pH 7.0/MeOH = 20/80
Flow rate: 1.0 mL/min
Detection: UV @ 254 nm
Temperature: 40 °C
Injection vol.: 10 µL
Samples: 1. uracil 2. toluene 3. ethyl benzene 4. quinizarin 5. amitriptyline

Indoor Air Pollutants

In the last several years, a growing body of scientific evidence has indicated that the air within homes and other buildings can be more seriously polluted than the outdoor air in even the largest and most industrialized cities. Other research indicates that people spend approximately 90 percent of their time indoors. Thus, for many people, the risks to health may be greater due to exposure to air pollution indoors than outdoors. This is the reason for the increased emphasis on the monitoring of indoor air pollutants.

Ten common indoor air pollutants were sharply resolved on a TSKgel ODS-100Z column (see Figure 27).

Figure 27: Analysis of indoor air pollutants

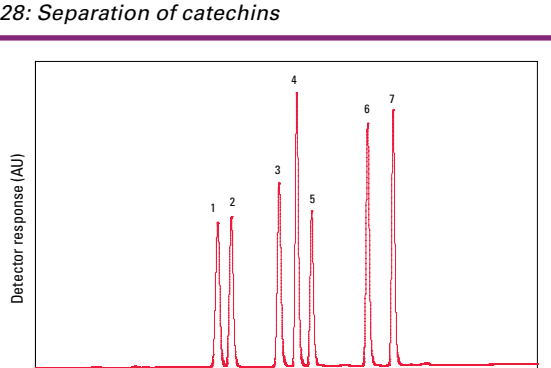


Column:	**TSKgel ODS-100Z, 5 µm, 4.6 mm ID × 15 cm**
Mobile phase:	H_2O/CH_3OH = 32/68
Flow rate:	1.0 mL/min
Detection:	UV @ 210 nm
Temperature:	40 °C
Injection vol.:	10 µL
Samples:	1. chloroform (1.0 g/L)
	2. benzene (0.1 g/L)
	3. trichloroethylene (0.05 g/L)
	4. toluene (0.05 g/L)
	5. styrene (0.05 g/L)
	6. o-dichlorobenzene (0.05 g/L)
	7. ethylbenzene (0.05 g/L)
	8. p-xylene (0.05 g/L)
	9. m-dichlorobenzene (0.05 g/L)
	10. tetrachloroethylene (0.05 g/L)

Polyphenols

Catechins, which are found in large quantities in tea, are polyphenols. Catechins have been extensively studied for their antioxidant properties. Figure 28 demonstrates the baseline separation of six catechins in the presence of caffeine on a 15 cm TSKgel ODS-100Z column.

Figure 28: Separation of catechins

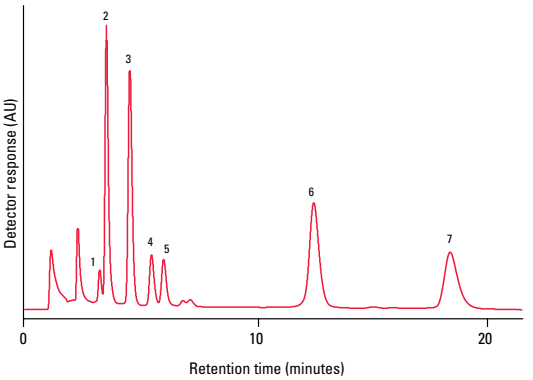


Column:	**TSKgel ODS-100Z, 5 µm, 4.6 mm ID × 15 cm**
Mobile phase:	A: 10 mmol/L KH_2PO_4, pH 2.5
	B: CH_3OH
Gradient:	0 min (18%B) 15 min (60%B)
Flow rate:	1.0 mL/min
Detection:	UV @ 270 nm
Temperature:	40 °C
Injection vol.:	5 µL
Samples:	1. (–)-epigallocatechin (175 mg/L)
	2. (–)-catechin (87 mg/L)
	3. (–)-epigallocatechin gallate (43 mg/L)
	4. caffeine (217 mg/L)
	5. (+)-epicatechin (87 mg/L)
	6. (–)-epicatechin gallate (43 mg/L)
	7. (–)-catechin gallate (43 mg/L)

Tetracycline Antibiotics

A 15 cm TSKgel ODS-100Z column was evaluated for its selectivity for a mixture of tetracycline-like chemical structures. Tetracycline is an impurity in oxytetracycline formulations. The two compounds have very similar structures and separation is difficult. As demonstrated in Figure 29, a TSKgel ODS-100Z column provides superior resolution for oxytetracycline (peak 2) and tetracycline (peak 3) within the mixture.

Figure 29: Separation of tetracycline antibiotics

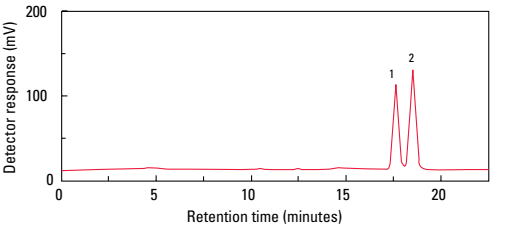


Column:	**TSKgel ODS-100Z, 5 µm, 4.6 mm ID × 15 cm**
Mobile phase:	10 mmol/L formic acid/ACN = 82.5/17.5
Flow rate:	1.0 mL/min
Detection:	UV @ 254 nm
Temperature:	10 °C
Injection vol.:	20 µL
Samples:	1. tetracycline derivative 2. oxytetracycline (20 mg/L) 3. tetracycline (20 mg/L) 4. doxycycline derivative 5. chlortetracycline derivative 6. chlortetracycline (30 mg/L) 7. doxycycline (30 mg/L)

Fat-Soluble Vitamins

Analysis of fat soluble vitamins D2 (ergocalciferol) and D3 (cholecalciferol) are critical because they differ only in one methyl group and one double bond. These compounds are very hydrophobic. As shown in Figure 30, separation was achieved using a TSKgel ODS-100Z column under isocratic conditions, demonstrating the ability of these columns to operate under non-aqueous reversed phase (NARP) conditions, in this case 100% acetonitrile.

Figure 30: Analysis of fat-soluble vitamins



Column:	**TSKgel ODS-100Z, 5 µm, 4.6 mm ID × 15 cm**
Mobile phase:	ACN
Flow rate:	1.0 mL/min
Detection:	UV @ 280 nm
Temperature:	25 °C
Injection vol.:	5 µL
Samples:	1. ergocalciferol 2. cholecalciferol

About: TSKgel Super-ODS Reversed Phase Chromatography Columns

TSKgel Super-ODS columns are packed with monodispersed 2 µm* spherical silica particles covalently bonded with octadecyl groups. The small particle size makes the Super series the highest efficiency reversed phase columns in the TSKgel product line. The monodispersed packing generates operational back pressures more typical of larger particles allowing the use of higher flow rates than other 2 µm packings

*nominal particle size; mean particle size is 2.3 µm.

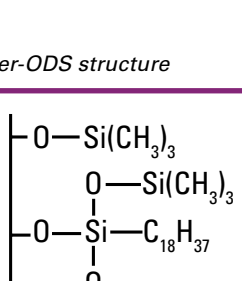
Attributes and Applications:

Table 8 lists the attributes of TSKgel Super-ODS columns, while Figure 31 displays the structure. TSKgel Super-ODS is an excellent choice for small peptides, amino acids, tryptic digests, nucleotides, pharmaceutical molecules, and food/ beverage samples.

Table 8: Product attributes

Attribute	Value
Pore size	14 nm
Exclusion limit	2.0×10^4 Da
Endcapped	Yes
Particle size	2.3 µm
pH stability	2.0-7.5
Functional group	C18 (polymeric bonding chemistry)
% Carbon	8

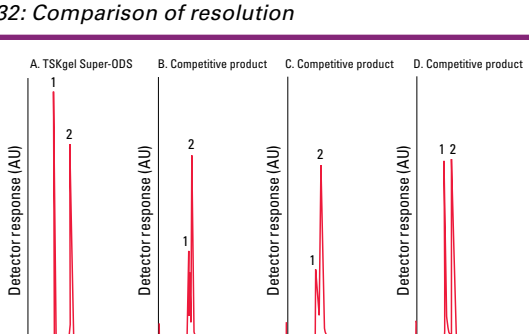
Figure 31: TSKgel Super-ODS structure



Superior Resolution

Figure 32 demonstrates the superior resolution of the TSKgel Super-ODS columns when compared with competitive 3 µm packings.

Figure 32: Comparison of resolution

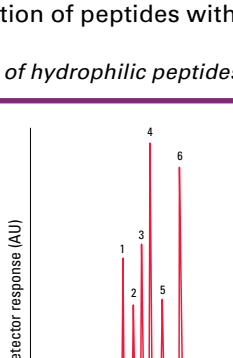


Columns: **A: TSKgel Super-ODS, 2.3 µm, 4.6 mm ID × 5 cm**
B, C & D: silica C18, 3 µm, 4.6 mm ID × 5 cm
Mobile phase: A: 30% CH_3CN B, C, D: 50% CH_3CN
Flow rate: 1.0 mL/min
Detection: UV @ 254 nm (2 mL cell)
Temperature: ambient
Samples: 1. pyradine 2. phenol

Peptide Separation

The chromatogram in Figure 33 shows the analysis of hydrophilic peptides using a TSKgel Super-ODS column. Since TSKgel Super-ODS has a large surface area, it shows favorable separation of peptides with high hydrophilicity.

Figure 33: Analysis of hydrophilic peptides



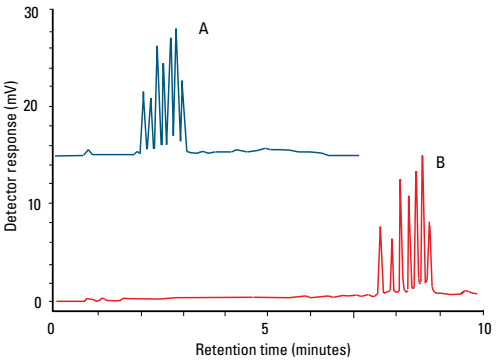
Column: **TSKgel Super-ODS, 2.3 µm, 4.6 mm ID × 5 cm**
Mobile phase: 13 mmol/L $HClO_4$/ACN
Linear gradient from 10% to 50% ACN over 10 minutes
Flow rate: 2 mL/min
Detection: UV @ 220 nm, micro-flow cell
Temperature: 25 °C
Samples: 1. oxytocin 2. a-endorphin
3. bombesin 4. Leu-enkephalin
5. gamma-endorphin 6. somatostatin
All peptides were injected at 0.1 to 0.2 µg each.

Oligonucleotides

Most synthesis protocols for oligonucleotides incorporate the use of a protective group on the 5′ terminal. Typically this protective group is dimethoxytrityl (DMT), which is a hydrophobic compound. One strategy for separating DMT on final products from DMT failures is the use of reversed phase chromatography.

The effect of gradient conditions on the separation of 12-18-mer polyadenylic oligonucleotides is shown in Figure 34. With the TSKgel Super-ODS column, this separation can be performed in less than five minutes under the conditions listed in Figure 34.

Figure 34: Separation of oligonucleotides

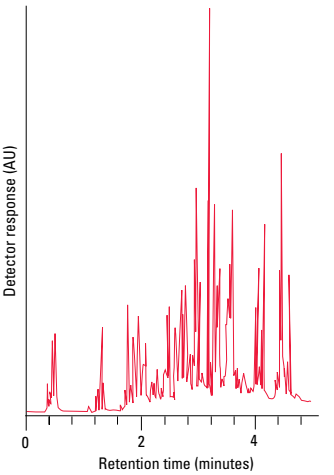


Column:	**TSKgel Super-ODS, 2.3 µm, 4.6 mm ID × 10 cm**
Mobile phase:	20 mmol/L phosphate buffer + 5 mmol/L t-butyl ammonium phospate, pH 6.0/CH_3CN
Gradient:	A: linear, 32-49% ACN in 5 minutes B: linear, 20-40% ACN in 10 minutes
Flow rate:	1.5 mL/min
Detection:	UV @ 260 nm
Temperature:	40 ˚C
Sample:	12-18-mer polyadenylic oligonucleotides

Trypsin Digest

A tryptic digest of α-chymotrypsinogen is separated on a TSKgel Super-ODS column as shown in Figure 35. The entire digest is separated in under five minutes.

Figure 35: Trypsin digest of α-chymotrypsinogen

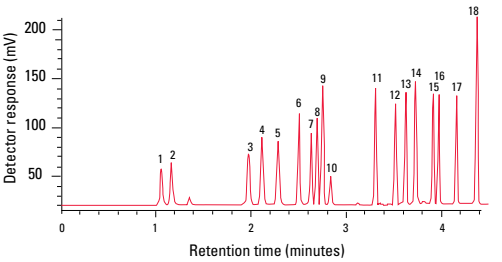


Column:	**TSKgel Super-ODS, 2.3 µm, 4.6 mm ID × 5 cm**
Mobile phase:	13 mmol/L $HClO_4$/CH_3CN; linear gradient of CH_3CN
Flow rate:	1.5 mL/min
Detection:	UV @ 220 nm
Temperature:	25 °C
Sample:	2 µL portion of trypsin digest of α-chymotrypsinogen

Amino Acids

The baseline separation of 18 PTC-derivatized amino acids in five minutes is demonstrated in Figure 36 using a TSKgel Super-ODS column.

Figure 36: PTC amino acids



Column:	**TSKgel Super-ODS, 2.3 µm, 4.6 mm ID × 10 cm**
Mobile phase:	A: ACN/50 mmol/L acetate buffer, pH 6.0 = 3/97 B: ACN/H_2O = 60/40
Flow rate:	1.5 mL/min
Detection:	UV @ 254 nm
Temperature:	25 °C
Injection vol.:	5 mL (250 pmol)
Samples:	1. Asp 2. Glu 3. Ser 4. Gly 5. His 6. Arg 7. Thr 8. Ala 9. Pro 10. PTC-NH_2 11. Try 12. Val 13. Met 14. Cys 15. Ile 16. Leu 17. Phe 18. Lys

About: TSKgel Super-Octyl Reversed Phase Chromatography Columns

TSKgel Super-Octyl columns are packed with monodispersed 2 µm* spherical silica particles covalently bonded with octyl groups. The small particle size makes the Super series the highest efficiency reversed phase columns in the TSKgel reversed phase column product line. The monodispersed packing generates operational back pressures more typical of larger particles allowing the use of higher flow rates than other 2 µm packings and offers less hydrophobicity than TSKgel Super-ODS.

* nominal particle size; mean particle size is 2.3 µm.

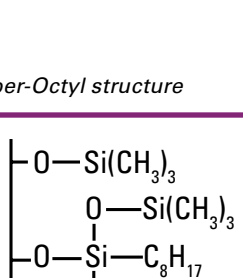
Attributes and Applications

Table 9 lists the attributes of TSKgel Super-Octyl columns, while Figure 37 displays the structure. TSKgel Super-Octyl columns are an excellent choice for peptides, proteins, amino acids, tryptic digests, nucleotides, pharmaceutical molecules, and food/beverage samples.

Table 9: Product attributes

Attribute	Value
Pore size	14 nm
Exclusion limit	2.0×10^4 Da
Endcapped	Yes
Particle size	2.3 µm
pH stability	2.0-7.5
Functional group	C8 (polymeric bonding chemistry)
% Carbon	5

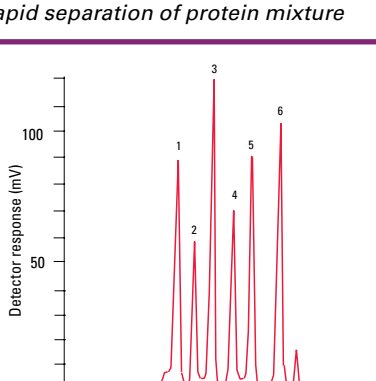
Figure 37: TSKgel Super-Octyl structure



Protein Mixture

The rapid analysis of a protein mixture using the TSKgel Super-Octyl column is shown in Figure 38. The separation was completed in one minute.

Figure 38: Rapid separation of protein mixture



Column:	**TSKgel Super-Octyl, 2.3 µm, 4.6 mm ID × 5 cm**
Mobile phase:	A: 13 mmol/L $HClO_4$, B: 13 mmol/L $HClO_4$/CH_3CN = 20/80 40%B to 100%B in a 1.5 min linear gradient
Flow rate:	2.0 mL/min
Detection:	UV @ 220 nm
Samples:	1. ribonuclease A 2. insulin 3. cytochrome C 4. lysozyme 5. α-lactalbumin 6. myoglobin

About: TSKgel Super-Phenyl Reversed Phase Chromatography Columns

TSKgel Super-Phenyl columns are packed with monodispersed 2 µm* spherical silica particles covalently bonded with phenyl groups. The small particle size makes the Super series the highest efficiency reversed phase columns in the TSKgel product line. The monodispersed packing generates operational back pressures more typical of larger particles allowing the use of higher flow rates than other 2 µm packings and offers less hydrophobicity than TSKgel Super-Octyl and TSKgel Super-ODS columns.

*nominal particle size; mean particle size is 2.3 µm.

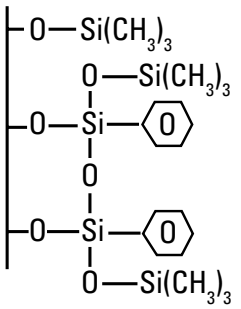
Attributes and Applications:

Table 10 lists the attributes of TSKgel Super-Phenyl columns; Figure 39 shows the structure. TSKgel Super-Phenyl is an excellent choice for peptides, proteins, amino acids, tryptic digests, nucleotides, pharmaceutical molecules, and food/beverage samples.

Table 10: Product attributes

Attribute	Value
Pore size	14 nm
Exclusion limit	2.0×10^4 Da
Endcapped	Yes
Particle size	2.3 µm
pH stability	2.0-7.5
Functional group	phenyl (polymeric bonding chemistry)
% Carbon	3

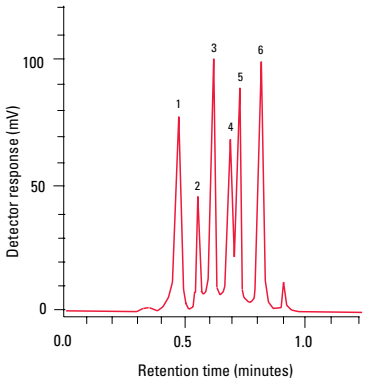
Figure 39: TSKgel Super-Phenyl structure



Protein Mixture

The chromatogram in Figure 40 shows retention and selectivity of TSKgel Super-Phenyl columns for proteins. The separation was achieved within one minute.

Figure 40: Rapid separation of protein mixture



Column:	**TSKgel Super-Phenyl, 2.3 µm, 4.6 mm ID × 5 cm**
Mobile phase:	A: 13 mmol/L $HClO_4$
	B: 13 mmol/L $HClO_4$/CH_3CN = 20/80
	40%B to 100%B in a 1.5 min linear gradient
Flow rate:	2.0 mL/min
Detection:	UV @ 220 nm
Samples:	1. ribonuclease A
	2. insulin
	3. cytochrome C
	4. lysozyme
	5. α-lactalbumin
	6. myoglobin

About: TSKgel CN-80Ts Reversed Phase Chromatography Columns

TSKgel CN-80Ts is an alternative to C18 (ODS) and C8 (Octyl) phases. The resin is based on a high-purity, metal-free 80Ts silica bonded to a C_3CN group. The cyano group is the least hydrophobic of the 10 nm phases available and in some cases is used under normal phase conditions.

The nomenclature for TSKgel reversed phase columns is based on the characteristics of the individual packing. In the case of TSKgel CN-80Ts, the "T" indicates endcapping with TMS groups while the subscript "S" denotes that endcapping is complete. Bonded phase pore size is indicated by the number in the product description, in this case TSKgel CN-80Ts has 8 nm nominal pore size. The pore size of the base silica is 10 nm.

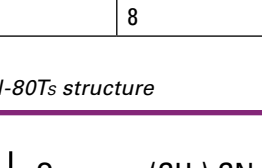
Attributes and Applications

Table 11 lists the attributes of TSKgel CN-80Ts columns, while Figure 41 displays the structure. TSKgel CN-80Ts is useful for the analysis of polar peptides, amino acids, and other pharmaceutical and food & beverage products. As with other 80Ts products, TSKgel CN-80Ts provides reproducible separations of molecules below 6,000 Da.

Table 11: Product attributes

Attribute	Value
Pore size	8 nm
Molar mass limit	6,000 Da
Endcapped	Yes - complete
Particle size	5 µm
pH stability	2.0-7.5
Functional group	cyano (monomeric bonding chemistry)
% Carbon	8

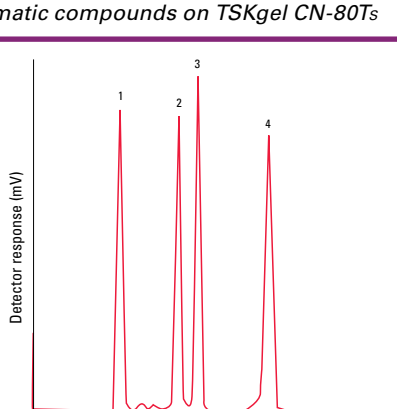
Figure 41: TSKgel CN-80Ts structure



Aromatic Compounds

The chromatogram in Figure 42 shows the symmetrical peaks obtained with the rapid separation of 3 aromatic compounds using a TSKgel CN-80Ts column.

Figure 42. Aromatic compounds on TSKgel CN-80Ts



Column:	**TSKgel CN-80Ts, 5 µm, 4.6 mm ID × 15 cm**
Mobile phase:	50% MeOH
Flow rate:	1.0 mL/min
Temperature:	25 °C
Samples:	1. uracil
	2. benzene
	3. toulene
	4. napthalene

About: TSKgel Octyl-80Ts Reversed Phase Chromatography Columns

The high-purity, metal-free silica particles in TSKgel Octyl-80Ts columns contain 8 nm pores and are bonded with octylmethyl silyl groups. Featuring a proprietary technique for complete endcapping of residual silanol groups, TSKgel Octyl-80Ts columns reduce tailing when analyzing basic compounds. TSKgel Octyl-80Ts columns have a lower carbon load and hydrophobicity than the corresponding ODS products. The C8 alkyl ligand provides a unique selectivity for the analysis of low molar mass pharmaceuticals, bases, nucleosides, and nucleotides.

The nomenclature for TSKgel reversed phase columns is based on the characteristics of the individual packing. In the case of TSKgel Octyl-80Ts, the "T" indicates endcapping with TMS groups while the subscript "S" denotes that endcapping is complete. The pore size of the bonded phase particles is indicated by the number in the product description; in this case TSKgel Octyl-80Ts has 8 nm nominal pore size. The pore size of the starting or base silica is 10 nm.

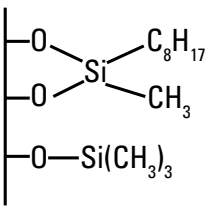
Attributes and Applications

Table 12 lists the attributes of TSKgel Octyl-80Ts columns. The stucture of the bonded phase is displayed in Figure 43. TSKgel Octyl-80Ts columns are recommended for molecules under 6,000 Da, such as amino acids, pharmaceuticals, nucleotides, and food and beverage components. Common applications include purity checks and peptide mapping.

Table 12: Product attributes

Attribute	Value
Pore size	8 nm
Molar mass limit	6,000 Da
Endcapped	Yes
Particle size	5 µm
pH stability	2.0-7.5
Functional group	C8 (monomeric bonding chemistry)
% Carbon	11

Figure 43: TSKgel Octyl-80Ts structure

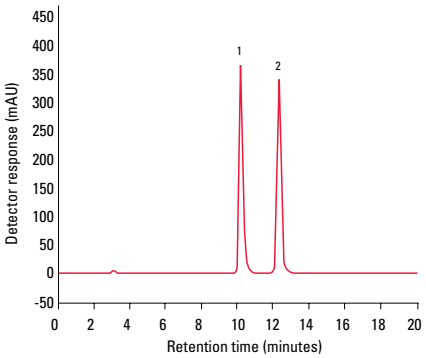


Asthma Medication

Pranlukast hydrate dry syrup is a medicine used in Japan that inhibits contraction of the airway and vascular permeability by binding with leukotriene receptors and blocking their action. It helps to prevent symptoms of bronchial asthma such as coughing, wheezing, and difficulty in breathing. Its action is similar to Merck & Co.'s Singulair (montelukast).

The Japanese Pharmaceutical Drug Standards recommends an octyl column for the analysis of pranlukast and the internal standard isoamyl p-oxybenzoate. Figure 44 shows the high resolution separation of puranlukast hydrate and isoamyl p-oxybenzoate using a TSKgel Octyl-80Ts column.

Figure 44: Analysis of puranlukast hydrate dry syrup



Column:	**TSKgel Octyl-80Ts, 5 µm, 4.6 mm ID × 15 cm**
Mobile phase:	20 mmol/L KH_2PO_4/ACN/ MeOH = 5/5/1(v/v/v)
Flow rate:	0.6 mL/min
Detection:	UV/VIS @ 260 nm
Temperature:	25 °C
Injection vol.:	4 µL
Samples:	1. pranlukast hydrate, 0.2 mg/L 2. isoamyl p-oxybenzoate (4-hydroxybenzoic acid isoamyl ester), 0.2 mg/L

Sample preparation:
Pranlukast solution: To 400 mg of pranlukast hydrate dry syrup, 10 mL of acetonitrile/dimethyl sulfoxide = 3/1(v/v) was added and shaken vigorously. Solution was centrifuged at 3000 rpm for 5 min. To 1 mL of supernatant, 9 mL of acetonitrile/dimethyl sulfoxide = 3/1(v/v) was added.

Isoamyl p-oxybenzoate solution (IS): To 4.03 mg of isoamyl p-oxybenzoate, 10 mL of acetonitrile/dimethyl sulfoxide = 3/1(v/v) was added and dissolved. 5 mL of both solutions were mixed and applied.
Sample: pranlukast hydrate dry syrup

About: TSKgel ODS-80TM Reversed Phase Chromatography Columns

TSKgel ODS-80TM is a packing with a C18 (ODS) group bonded to a 8 nm pore size, high-purity, metal-free silica. High endcapping of the TSKgel ODS-80TM bonded phase shields the silica surface from participating in solute retention through ionic interaction.

The nomenclature for TSKgel reversed phase columns is based on the characteristics of the individual packing. In the case of TSKgel ODS-80TM, the "T" indicates endcapping with TMS groups while the subscript "M" denotes a monolayer coverage of C18 groups. Bonded phase pore size is indicated by the number in the product description, in this case TSKgel ODS-80TM has 8 nm nominal pore size. The pore size of the base silica is 10 nm.

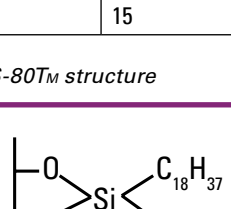
Attributes and Applications

The product attributes of TSKgel ODS-80TM columns are listed in Table 13; the structure is displayed in Figure 45. The TSKgel ODS-80TM column is a general purpose column for the analysis of low molar mass pharmaceuticals, basic compounds, nucleosides, nucleotides, purines, and pyrimidines. Common applications include purity checks and peptide mapping.

Table 13: Product attributes

Attribute	Value
Pore size	8 nm
Molar mass limit	6,000 Da
Endcapped	Yes
Particle size	5 µm and 10 µm
pH stability	2.0-7.5
Functional group	C18 (monomeric bonding chemistry)
% Carbon	15

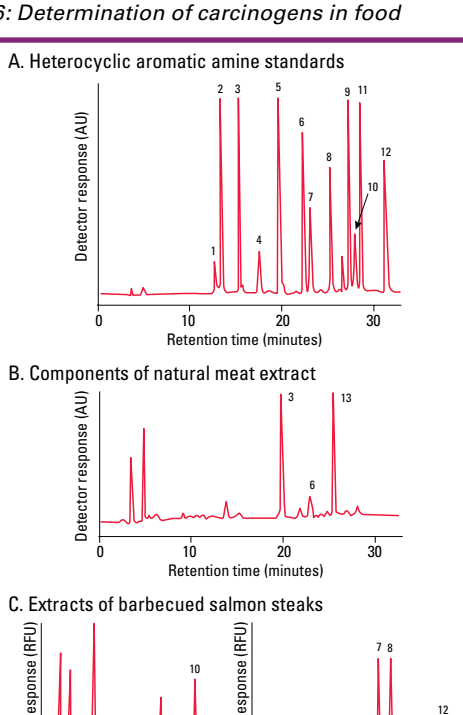
Figure 45: TSKgel ODS-80TM structure



Food Products

TSKgel ODS-80TM provides high efficiency and symmetrical peaks for basic, heterocyclic aromatic amines in food products, as shown in Figure 46. In this study, TSKgel ODS-80TM columns provided the best resolution of nanogram levels of the amines in barbecued food, known to be potential carcinogens.

Figure 46: Determination of carcinogens in food



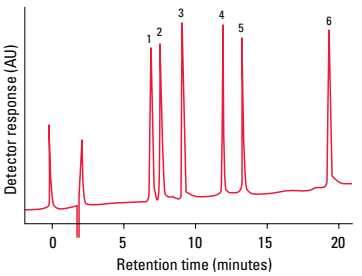
Column: **TSKgel ODS-80TM, 5 µm, 4.6 mm ID × 15 cm**
Mobile phase: 15 min linear gradient from 5% to 15% CH_3CN in 0.01 mol/L triethyl ammonium phosphate(TAP), pH 3.2; then switch to TAP buffer at pH 3.6 and conduct a 4 min linear gradient to 25% CH_3CN, followed by a 15 min linear gradient to 55% CH_3CN
Flow rate: 1.0 mL/min
Detection: A: UV @ 263 nm
B: UV @ 360 nm
C: fluoresence: Ex: 360 nm, Em: 450 nm
Samples: 1. Glu-P-2 (18 ng) 2. IQ (12 ng) 3. MeIQ (14 ng)
4. Glu-P-1 (18 ng) 5. MeIQx (12 ng)
6. 4,8-DiMeIQx (15 ng) 7. norharman (10 ng)
8. harman (15 ng) 9. Trp-P-2 (12 ng) 10. PhIP (15 ng)
11. Trp-P-1 (8 ng) 12. A-alpha-C (17 ng)
13. 4,7,8-TriMeIQx
Legend: see footnote below for explanation of abbreviations

Amino-imidazo-quinolines (IQ and MeIQ)
Amino-imidazo-quinoxalines (MeIQx and DIMEIQx)
Amino-pyrido-indoles (Trp-P-1 and Trp-P-2)
Amino-pyrido-imidazoles (Glu-P-1 and Glu-P-2)
Amino-alpha-carbolines (A-alpha-C and MeA-alpha-C)

Peptides

Figure 47 demonstrates the applicability of the TSKgel ODS-80TM column for the analysis of peptides. Very high resolution was achieved for each compound.

Figure 47: Peptide analysis

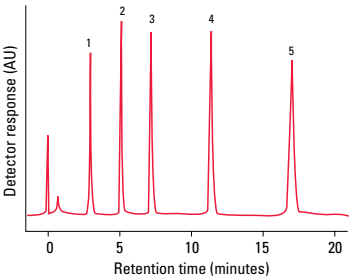


Column:	**TSKgel ODS-80TM, 5 µm, 4.6 mm ID × 15 cm**
Mobile phase:	90 min linear gradient from 23.5% to 100% CH_3CN in 0.1% TFA
Flow rate:	1.0 mL/min
Detection:	UV @ 220 nm
Samples:	1. bradykinin (2 µg) 2. α-endorphin (2 µg) 3. angiotensin II (1.5 µg) 4. angiotensin I (1.5 µg) 5. substance P (2 µg) 6. β-endorphin (3 µg)

Pharmaceuticals

The TSKgel ODS-80TM column was used successfully for the baseline separation of 5 common pharmaceuticals, as shown in Figure 48.

Figure 48: Common pharmaceuticals



Column:	**TSKgel ODS-80TM, 5 µm, 4.6 mm ID × 15 cm**
Mobile phase:	35% CH_3OH in 0.05 mol/L phosphoric acid, pH 2.5
Flow rate:	1.0 mL/min
Detection:	UV @ 254 nm
Samples:	1. p-aminoacetophenon (0.05 µg) 2. caffeine (0.25 µg) 3. salicylamide (0.6 µg) 4. aspirin I (1.56 µg) 5. phenacetin (0.16 µg)

About: TSKgel ODS-80Ts Reversed Phase Chromatography Columns

TSKgel ODS-80Ts columns contain packing that has C18 groups bonded to 8 nm pore size, high-purity, metal-free silica. The silica used in the OSD-80Ts is highly endcapped, which reduces cationic interactions. In addition, the silica does not contain metal ions or ammonium moieties that can broaden peaks of acidic compounds and chelating reagents.

The nomenclature for TSKgel reversed phase columns is based on the characteristics of the individual packing. In the case of TSKgel ODS-80Ts, the "T" indicates endcapping with TMS groups while the subscript "S" denotes that endcapping is complete. Bonded phase pore size is indicated by the number in the product description, in this case TSKgel ODS-80Ts has 8 nm nominal pore size. The pore size of the base silica is 10 nm.

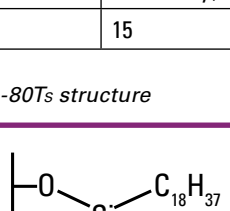
Attributes and Applications

Table 14 lists the attributes of TSKgel ODS-80Ts columns, while Figure 49 displays the structure. The TSKgel ODS-80Ts columns are useful for molecules in the 100-6,000 Da range, so small peptides and pharmaceuticals can be successfully separated on this column.

Table 14: Product attributes

Attribute	Value
Pore size	8 nm
Molar mass limit	6,000 Da
Endcapped	Yes
Particle size	5 µm and 10 µm
pH stability	2.0-7.5
Functional group	C18 (monomeric bonding chemistry)
% Carbon	15

Figure 49: TSKgel ODS-80Ts structure

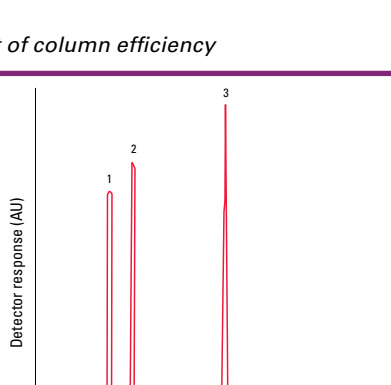


Food Products

Because of the stability of silica-based packings at acidic and neutral pH, most reversed phase separations are conducted in the pH range from 2.0 to 7.0. Under these pH conditions, however, organic bases have a charge and careful control of the eluent pH with buffers, and/or ion-pair liquid chromatography is employed to isolate them. An ion-pair reagent added to the buffer forms a complex with the stationary phase. For basic compounds, alkylsulfonic acids are most often used, while allyl amines are typical ion-pair reagents for strongly acidic analytes.

Since the ODS binding and trimethylsilyl endcapping techniques leave few residual silanol groups to cause tailing, the endcapped silica reduces cationic interactions, metal ion interactions, or ammonium moiety interactions that can broaden peaks of basic compounds, acidic compounds, and chelating reagents as shown in Figure 50 using a TSKgel ODS-80Ts column.

Figure 50: Test of column efficiency

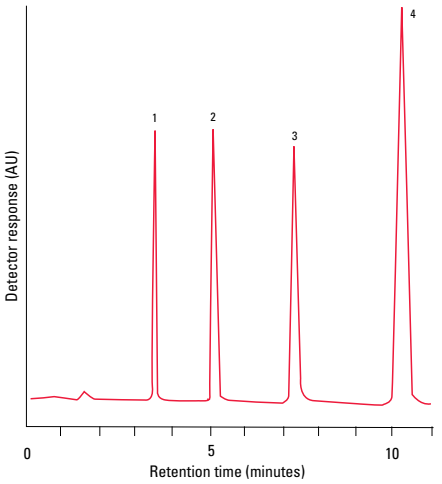


Column:	**TSKgel ODS-80Ts, 5 µm, 4.6 mm ID × 15 cm**
Mobile phase:	50% MeOH
Flow rate:	1.0 mL/min
Detection:	UV @ 254 nm
Temperature:	25 °C
Samples:	1. pyridine 2. phenol 3. methyl benzoate

Pharmaceuticals

Figure 51 shows simple pharmaceuticals analyzed using a 2 mm ID TSKgel ODS-80Ts column.

Figure 51: Analysis of pharmaceuticals

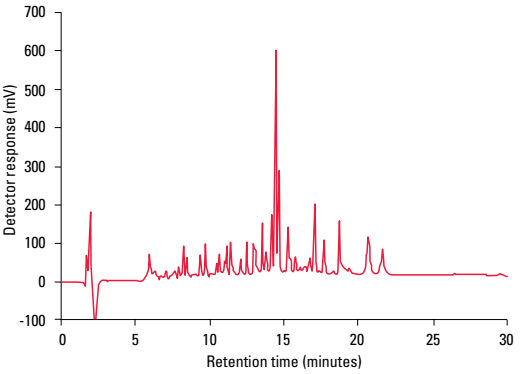


Column:	**TSKgel ODS-80Ts, 5 µm, 2 mm ID × 15 cm**
Mobile phase:	50 mmol/L phosphate buffer, pH 2.5/ MeOH= 60/40
Flow rate:	0.2 mL/min
Detection:	UV @ 254 nm
Temperature:	25 ˚C
Samples:	1. caffeine (12 ng) 2. salicylamide 3. aspirin (120 ng) 4. phenacetin (18 ng)

Trypsin Digest

Figure 52 shows the analysis of a trypsin digest of β-lactoglobulin on a TSKgel ODS-80Ts semi-micro column.

Figure 52: Trypsin digest



Column:	**TSKgel ODS-80Ts, 5 µm, 2.0 mm ID × 15 cm**
Mobile phase:	A: 0.1% TFA solution B: ACN + 0.1% TFA A (100%A) → A (30%) linear gradient (30 min)
Flow rate:	0.20 mL/min
Detection:	UV @ 215 nm, micro-cell
Temperature:	25 °C
Sample:	trypsin digest of β-lactoglobulin (10 µL)

About: TSKgel ODS-80Ts QA Reversed Phase Chromatography Columns

TSKgel ODS-80Ts QA columns were developed specifically for use by QA/QC departments that require highly reproducible separations. These columns are prepared from the same endcapped C18 packing material as TSKgel ODS-80Ts columns, but with narrower manufacturing specifications to meet the demand for high reproducibility.

The variation between different lots of TSKgel ODS-80Ts QA packing material is minimized by selecting batches of TSKgel ODS-80Ts that fall within a very narrow range of specifications, as demonstrated in Table 15 below. In addition, each column must pass demanding specifications for efficiency (N) and peak asymmetry, as are spelled out in the Operating Conditions and Specifications sheet. The end result is TSKgel ODS-80Ts QA columns that exhibit an unparalleled level of reproducibility for retention, selectivity (k´), efficiency (N), and peak symmetry.

Table 15: Product specifications

Attribute	Specification Range	Lot-to-Lot Reproducibility (CV%)
Particle Size: -Distribution (dp_{90}/dp_{10})	4.95-5.35 1.55 - 1.70	0.6 1.8
Surface area (m^2/g)	410 - 440	0.5
Pore size (nm) silica	9 - 10	0.5
Pore volume (mL/g silica)	0.96 - 1.04	0.7
Carbon content (wt%)	14.0 - 15.0	N/A
C18 coverage (µmol/m^2)	1.71 - 1.99	1.3
Metal ion content (ppm) -Na -Al -Fe -Ti	 <10 <10 <10 <10	 N/A N/A N/A N/A

Column classification:

TSKgel ODS-80Ts QA columns were submitted to several characterization tests to determine the level of hydrophobic retention, steric selectivity, and retention of basic compounds. The results of the characterization tests were used to establish specifications listed in Table 16.

Table 16: Characterization test results

Parameter	Specification	CV (%)	Test Conditions
k' naphthalene (hydrophobicity)	1.53 - 1.63	1.3	1
α triphenylene/*o*-terphenyl (steric selectivity)	1.21 - 1.25	0.4	1
k' procainamide (basic compounds)	1.35 - 1.55	2.6	2
k' phenol	9.25 - 9.85	0.9	2
k' oxine copper (inertness to chelating compounds)	1.13 - 1.35	3.5	3

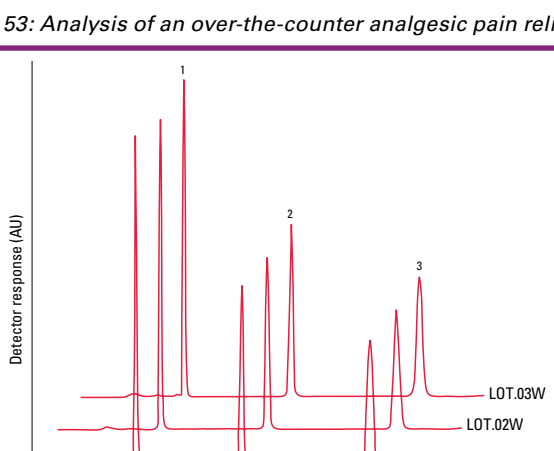
1. 80% CH_3OH/20% H_2O, T = 40 °C
2. 10% ACN/90% 20 mmol/L Na_2HPO_4 (pH 6.6, adjusted with 20 mmol/L NaH_2PO_4, T = 40 °C)
3. 7% ACN/93% 20 mmol/L H_3PO_4, T = 40 °C

Over-the-Counter Analgesic Pain Reliever

To demonstrate the lot-to-lot reproducibility that can be expected when using TSKgel ODS-80Ts QA columns, the contents of an over-the-counter analgesic pain reliever was analyzed on columns from three different lots of packing material. The results are shown in Figure 53 and Table 17 below. Excellent reproducibility of retention, peak height, peak area, efficiency, and peak shape is evident for all three ingredients.

Highly reproducible results can be achieved using the TSKgel ODS-80Ts QA columns as shown in this lot-to-lot reproducibility test.

Figure 53: Analysis of an over-the-counter analgesic pain reliever



Column:	**TSKgel ODS-80Ts QA, 5 µm, 4.6 mm ID × 15 cm**
Mobile phase:	50 mmol/L phosphate buffer, pH 2.5/ACN = 80/20
Flow rate:	1.0 mL/min
Detection:	UV @ 254 nm
Temperature:	40 °C
Injection vol.:	5 µL
Samples:	1. caffeine 2. salicylamide 3. acetylsalicylic acid

Table 17: Results demonstrating lot-to-lot reproducibility

Compound	Lot#	RT (min)	Peak Area (mv x sec)	Peak Height	N	AF
caffeine	01W 02W 03W	3.047 3.080 3.080	4.51575×10^2 4.50235×10^2 4.44175×10^2	99.26 94.04 95.63	10,396 9,597 10,143	1.10 1.13 1.13
salicylamide	01W 02W 03W	6.190 6.253 6.250	3.96172×10^2 3.94822×10^2 3.90617×10^2	54.06 52.23 52.40	16,441 15,797 16,333	1.03 1.07 1.06
acetylsalicylic acid	01W 02W 03W	9.983 10.080 10.063	4.34473×10^2 4.33835×10^2 4.27633×10^2	37.35 36.48 36.28	16,742 16,297 16,649	1.03 1.05 1.05

About: TSKgel ODS-120A Reversed Phase Chromatography Columns

TSKgel ODS-120A columns use a 15 nm pore size silica base support. The bonding method results in a polymeric coverage of C18 groups on the silica surface.

The "A" signifies that the material is not endcapped. For charged samples, the endcapped TSKgel ODS-120T column is a more suitable alternative.

Bonded phase pore size is indicated by the number in the product description, in this case TSKgel ODS-120A has 12 nm nominal pore size.

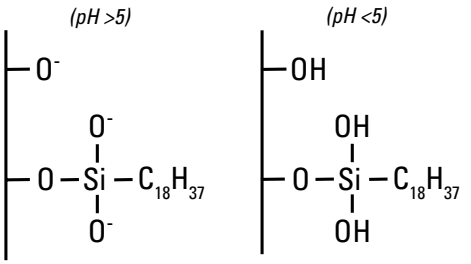
Attributes and Applications

Table 18 lists the attributes of TSKgel ODS-120A columns, while Figure 54 displays the structure. The silica base support's exclusion limit of 1.0×10^4 Da makes them a good choice for the reversed phase chromatography of peptides, small proteins, and environmental samples such as poly-aromatic hydrocarbons.

Table 18: Product attributes

Attribute	Value
Pore size (mean)	12 nm
Exclusion limit	1.0×10^4 Da
Endcapped	No
Particle size (mean)	5 µm and 10 µm
pH stability	2.0-7.5
Functional group	C18 (polymeric bonding chemistry)
% Carbon	20

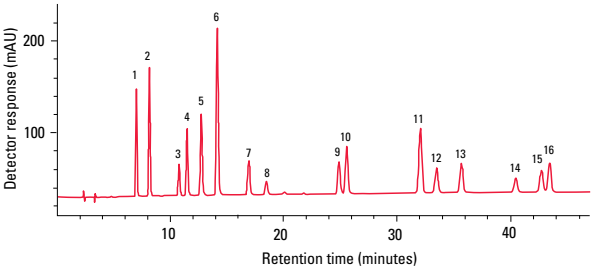
Figure 54: TSKgel ODS-120A structure



Polynuclear Aromatic Hydrocarbons

The polymeric stationary phase of the TSKgel ODS-120A column exhibits improved shape selectivity for the separation of complex geometric isomers, such as polynuclear aromatic hydrocarbons (PAH) as shown in Figure 55.

Figure 55: Separation of 16 poly-aromatic hydrocarbons



Column:	**TSKgel ODS-120A, 5 µm, 4.6 mm ID × 25 cm**
Gradient:	40 min linear from 75% MeOH/25% H_2O to 95% MeOH/5% H_2O, 5 min hold
Flow rate:	1.2 mL/min
Detection:	UV @ 254 nm
Temperature:	40 °C
Samples:	5 mL mixture of:

1. naphthalene
2. acenaphthylene
3. acenaphthene
4. fluorene
5. phenanthrene
6. anthracene
7. fluoranthene
8. pyrene
9. benzo(a)anthracene
10. chrysene
11. benzo(b)fluoranthene
12. benzo(k)fluoranthen
13. benzo(a)pyrene
14. dibenzo(a,h)anthracene
15. benzo(g,h,i)perylene
16. indeno(1,2,3-cd)pyrene

About: TSKgel ODS-120T Reversed Phase Chromatography Columns

TSKgel ODS-120T columns use a 15 nm pore size silica base support. The columns are endcapped with trimethyl silane groups to improve the peak shape of negatively charged analytes.

Bonded phase pore size is indicated by the number in the product description, in this case TSKgel ODS-120T has 12 nm nominal pore size.

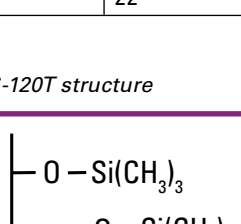
Attributes and Applications

Table 19 lists the attributes of TSKgel ODS-120T columns, while Figure 56 displays the structure. With an exclusion limit of 1.0×10^4 Da, the TSKgel ODS-120T are a good choice for the reversed phase chromatography of peptides, small proteins, and small molar mass compounds in organic and environmental samples.

Table 19: Product attributes

Attribute	Value
Pore size (mean)	12 nm
Exclusion limit	1.0×10^4 Da
Endcapped	Yes
Particle size	5 µm and 10 µm
pH stability	2.0-7.5
Functional group	C18 (polymeric bonding chemistry)
% Carbon	22

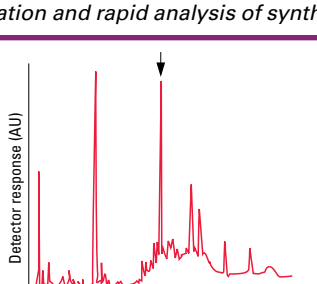
Figure 56: TSKgel ODS-120T structure



Peptides

Endcapped TSKgel ODS-120T is an alternative to TSKgel ODS-80TM for peptide and protein separation. Figure 57 demonstrates the applicability of the TSKgel ODS-120T column for the analysis of synthetic peptides.

Figure 57: Purification and rapid analysis of synthetic peptides

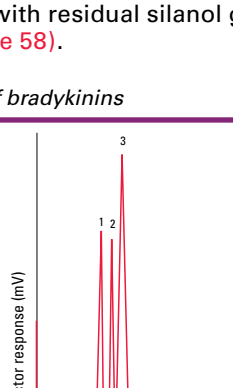


Column:	**TSKgel ODS-120T, 5 µm, 4.6 mm ID × 15 cm**
Mobile phase:	48 min linear gradient from 14% to 50% CH_3CN in 0.1% TFA
Flow rate:	1.0 mL/min
Detection:	UV @ 215 nm
Sample:	triacontadipeptide (EAEDLQVGQVELGGGPGAGSLQPLALEGSLQC) indicated by arrow; 50 µg in 50 µL

Bradykinins

The good peak shape of closely related bradykinins on TSKgel ODS-120T in the non-buffered eluent is due to reduced interaction with residual silanol groups as a result of endcapping (Figure 58).

Figure 58: Separation of bradykinins

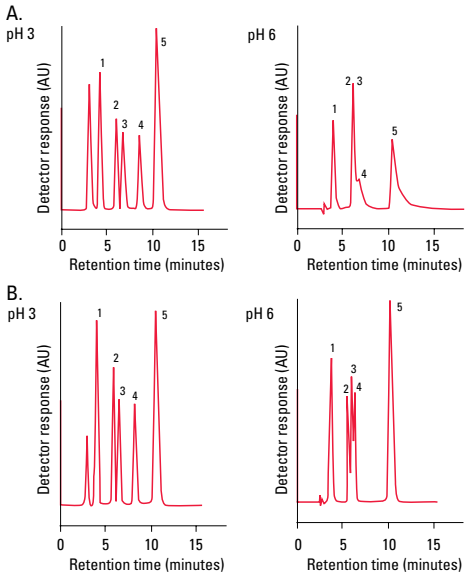


Column:	**TSKgel ODS-120T, 5 µm, 4.6 mm ID × 25 cm**
Mobile phase:	20% CH_3CN in 0.05% TFA
Flow rate:	1.0 mL/min
Detection:	UV @ 220 nm
Temperature:	25 °C
Samples:	1. Lys-bradykinin 2. Met-Lys-bradykinin 3. bradykinin

Catecholamines

In Figure 59, the effect of pH on endcapped and non-endcapped packings is shown for the same columns in the separation of catecholamines. When the pH of the eluent is above the pKa of the non-endcapped silanol groups, the TSKgel ODS-120A packing is negatively charged, and the catecholamine peaks tail. However, notice the similar resolution on TSKgel ODS-120A and TSKgel ODS-120T columns when the eluent is buffered at an acidic pH, where the silanol groups will be protonated.

Figure 59: Separation of catecholamines



Columns:	**A. TSKgel ODS-120A, 5 µm, 4.6 mm ID × 25 cm** **B. TSKgel ODS-120T, 5 µm, 4.6 mm ID × 25 cm (endcapped)**
Mobile phase:	0.1 mol/L phosphate buffer, pH 3.0 or 6.0
Flow rate:	1.0 mL/min
Detection:	UV @ 254 nm
Samples:	1. norepinephrine 2. epinephrine 3. 3,4-dihydroxybenzylamine 4. D,L-DOPA 5. dopamine-HCl

About: TSKgel OligoDNA-RP Reversed Phase Chromatography Columns

Specifically designed for the purification of oligonucleotides, and RNA and DNA fragments (up to 500-mer), TSKgel OligoDNA-RP columns can provide excellent separations of samples with very similar sequences. The packing is prepared by monomeric binding of octadecyl silyl groups to 5 µm spherical silica gel with 25 nm pores. This packing is not endcapped and it has a relatively low carbon content of 10%.

The 25 nm pore size of the TSKgel OligoDNA-RP column provides excellent kinetics for molecules with helix shape structures processing large radii of gyration. The 5 µm particle size provides a minimum of 7,000 plates per 15 centimeter column.

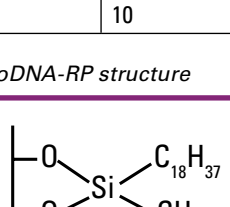
Attributes and Applications

Table 20 lists the attributes of TSKgel OligoDNA-RP columns, while Figure 60 displays the structure.

Table 20: Product attributes

Attribute	Value
Pore size (mean)	25 nm
Exclusion limit	500-mer
Endcapped	No
Particle size (mean)	5 µm
pH stability	2.0-7.5
Functional group	C18 (monomeric bonding chemistry)
% Carbon	10

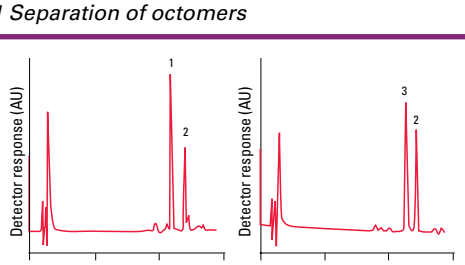
Figure 60: TSKgel OligoDNA-RP structure



Octamers

TSKgel OligoDNA-RP columns possess high-resolving power for octamers of similar sequence, as demonstrated in Figure 61.

Figure 61 Separation of octomers

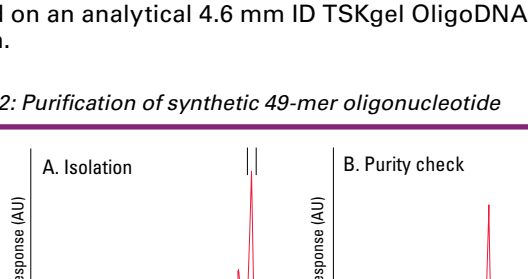


Column:	**TSKgel OligoDNA-RP, 5 µm, 4.6 mm ID × 5 cm**
Mobile phase:	120 min linear gradient from 5% to 25% CH_3CN in 0.1 mol/L ammonium acetate, pH 7.0
Flow rate:	1.0 mL/min
Detection:	UV @ 260 nm
Sample:	1. linker EcoR I, d(CGAATTCG) 2. *Hpa* I, d(CGTTAACG) 3. linker EcoR V, d(CGATATCG)

Oligonucleotides

The semi-preparative isolation of a 49-mer oligonucleotide from the crude synthetic reaction mixture using a 7.8 mm ID TSKgel OligoDNA-RP column is shown in Figure 62. The purity of the isolated oligonucleotide was subsequently verified on an analytical 4.6 mm ID TSKgel OligoDNA-RP column.

Figure 62: Purification of synthetic 49-mer oligonucleotide



Columns:	**A. TSKgel OligoDNA-RP, 5 µm, 7.8 mm ID × 15 cm** **B. TSKgel OligoDNA-RP, 5 µm, 4.6 mm ID × 15 cm**
Mobile phase:	A. 120 min linear gradient from 6.25% to 25% CH_3CN (7.8 mm ID) column B. 90 min linear gradient from 7.5% to 25% CH_3CN (4.6 mm ID) column both in 0.1 mol/L ammonium acetate, pH 7.0
Flow rate:	A. 2.8 mL/min (7.8 mm ID) B. 1.0 mL/min (4.6 mm ID)
Detection:	UV @ 260 nm
Sample:	synthetic 49-mer oligonucleotide, d(AGCTTGGGCTGCAGGTCGTCTCTAGAGGATCCCC GGGCGAGCTCGAATT)

About: TSKgel TMS-250 Reversed Phase Chromatography Columns

TSKgel TMS-250 columns contain a unique C1 bonded phase. The packing is prepared by monomeric binding of trimethyl silyl groups to a 25 nm pore size spherical silica.

Due to the low hydrophobicity of the ligand, excellent recoveries are common even when used with large proteins. Proteins such as adolase (158 kDa) exhibit sharp peaks relative to wide pore C18 columns.

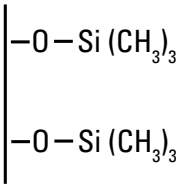
Attributes and Applications

Table 21 lists the attributes of TSKgel TMS-250 columns; Figure 63 displays the structure. TSKgel TMS-250 columns are an excellent choice for analysis of larger proteins by reversed phase HPLC.

Table 21: Product attributes

Attribute	Value
Pore size (mean)	25 nm
Exclusion limit	2.0 × 10^5 Da
Endcapped	Yes
Particle size	10 µm
pH stability	2.0-7.5
Functional group	C1 (monomeric bonding chemistry)
% Carbon	5

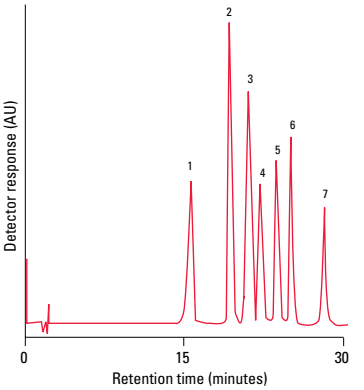
Figure 63: TSKgel TMS-250 structure



Protein Analysis

The resolution of proteins on TSKgel TMS-250 columns is shown in Figure 64. The wide pore packing of these columns can accommodate such large proteins as adolase.

Figure 64: High resolution protein separation

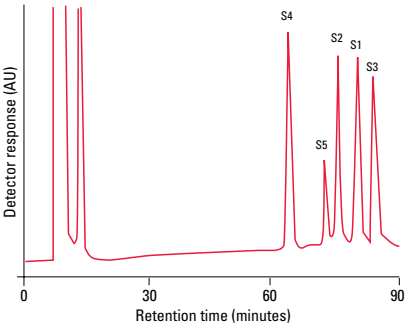


Column: **TSKgel TMS-250, 10 µm, 4.6 mm ID × 7.5 cm**
Mobile phase: 60 min linear gradient from 20% to 95% CH_3CN in 0.05% TFA, pH 2.2
Flow rate: 0.61 mL/min
Detection: UV @ 220 nm
Samples: 5 µg each of: 1 ribonuclease A 2. cytochrome C 3. lysozyme 4. bovine serum albumin 5. aldolase 6. carbonic anhydrase 7. ovalbumin

Protein Subunits

Figure 65 illustrates the high resolution and efficiency of TSKgel TMS-250 for the isolation of B. pertussis toxin (PT) subunit proteins. Five distinct PT subunits of molar mass ranging from 1.0 × 10^4 to 2.6 × 10^4 Da were resolved without significant cross-contamination using a specially packed 7.5 mm ID × 30 cm TSKgel TMS-250 column.

Figure 65: Separation of protein subunits



Column: **TSKgel TMS-250, 10 µm, 7.5 mm ID × 30 cm**
Mobile phase: Load and 12 min wash with 34% CH_3CN in 0.1% TFA, followed by a 100 min linear gradient from 34% to 47% CH_3CN in 0.1% TFA
Flow rate: 1.5 mL/min
Detection: UV @ 210 nm and 280 nm (not shown)
Injection vol.: 100 µg in 500 mL
Sample: purified *Bordetella pertussis* toxin in 0.1 mol/L phosphate buffer, pH 7.2, with 0.5 mol/L NaCl and 30% glycerol

About: TSKgel Octadecyl-2PW Reversed Phase Chromatography Columns

The highly cross-linked polymethacrylate base material of TSKgel Octadecyl-2PW columns provides excellent stability in high pH buffer systems and can withstand rigorous cleaning with either acid or base. The 12.5 nm pore size of TSKgel Octadecyl-2PW columns makes them ideally suited for peptides and small proteins. Large pores allow unhindered access to proteins and other large molar mass biopolymers. The TSKgel Octadecyl-2PW columns demonstrate faster analysis than other competitive reversed phase polymeric columns.

Attributes and Applications

Table 22 lists the attributes of TSKgel Octadecyl-2PW columns, while Figure 66 displays the structure. The 12.5 nm pores allow for analysis of peptides up to 8,000 Da.

Table 22: Product attributes

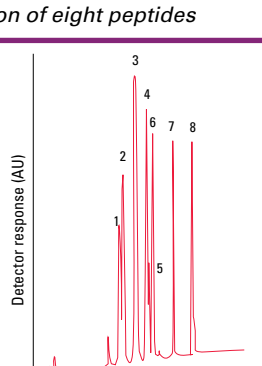
Attribute	Value
Pore size (mean)	12.5 nm
Exclusion limit	8,000 Da
Particle size (mean)	5 µm
pH stability	2.0-12.0
Functional group	C18 (monomeric bonding chemistry)

Figure 66: TSKgel Octadecyl-2PW structure

Neuropeptides

The rapid separation of a mixture of eight peptides using a TSKgel Octadecyl-2PW column is shown in Figure 67. The complexity of these peptides, found in neural tissue, requires an efficient column that is robust under low pH mobile phase conditions. A TSKgel Octadecyl-2PW column delivers symmetrical peaks and a sharp elution profile.

Figure 67: Separation of eight peptides

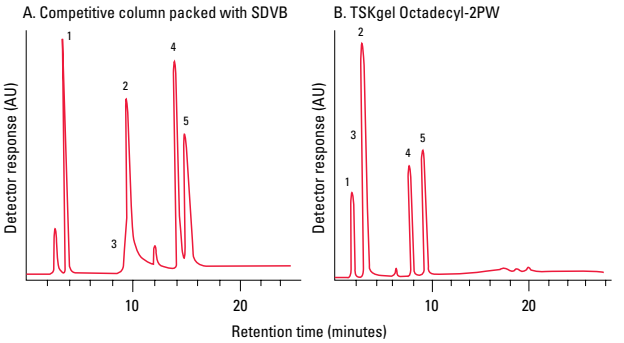


Column:	**TSKgel Octadecyl-2PW, 5 µm, 4.6 mm ID × 15 cm**
Mobile phase:	30 min linear gradient from 0.1% TFA/CH_3CN from 90/10 to 30/70
Flow rate:	1.0 mL/min
Detection:	UV @ 215 nm
Temperature:	ambient
Samples:	1. met-enkephalin 2. bradykinin 3. leu-enkephalin 4. neurotensin 5. bombesin 6. angiotensin I 7. somatostatin 8. insulin (bovine)

Common Drugs

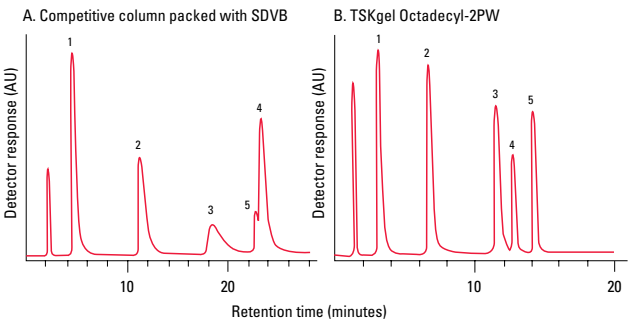
The polymeric backbone of TSKgel Octadecyl-2PW gives this column better pH stability than silica-based columns so the separations can be optimized over a wider pH range, as shown in Figure 68. A pH of 7.0 gives excellent resolution of a mixture of common drugs on the TSKgel Octadecyl-2PW column, while they tail or are unresolved on a competitive PSDVB column.

Figure 68: Comparison over a wide pH range



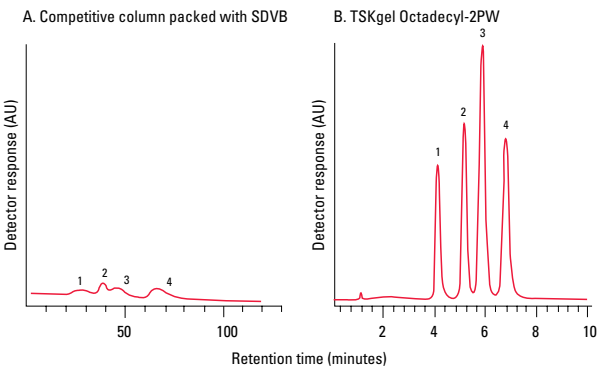
1. pH 2.5

Columns:	A. competitive column with styrene divinylbenzene (SDVB), 5 µm packing
	B. TSKgel Octadecyl-2PW, 5 µm, 4.6 mm ID × 15 cm
Mobile phase:	20 mmol/L phosphate buffer, pH 2.5/ACN, 80/20 to 0/100, 30 min linear gradient
Flow rate:	A. 0.5 mL/min B. 1.0 mL/min
Detection:	UV @ 254 nm
Temperature:	25 °C
Samples:	1. sulfide 2. disopyramide 3. chlorphenirmin 4. ciltrazem 5. hydroxyzine



2. pH 7.0

Columns:	A. competitive column with styrene divinylbenzene (SDVB), 5 µm packing
	B. TSKgel Octadecyl-2PW, 5 µm, 4.6 mm ID × 15 cm
Mobile phase:	20 mmol/L phosphate buffer, pH 7.0/ACN, 80/20 to 0/100, 30 min linear gradient
Flow rate:	A. 0.5 mL/min B. 1.0 mL/min
Detection:	UV @ 254 nm
Temperature:	25 °C
Samples:	1. sulfide 2. disopyramide 3. chlorphenirmin 4. ciltrazem 5. hydroxyzine



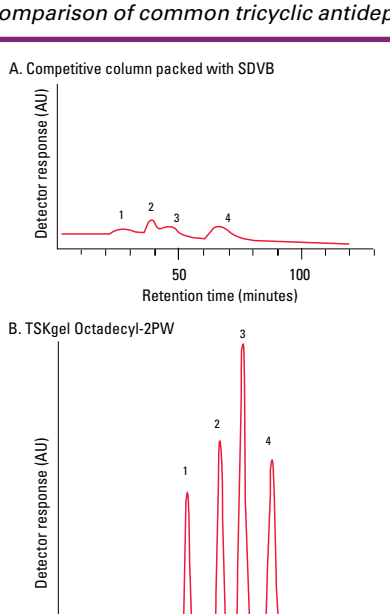
3. pH 11.0

Columns:	A. competitive column with styrene divinylbenzene (SDVB), 5 µm packing
	B. TSKgel Octadecyl-2PW, 5 µm, 4.6 mm ID × 15 cm
Mobile phase:	20 mmol/L phosphate buffer, pH 11.0/ACN, 40/60, 30 min linear gradient
Flow rate:	A. 0.5 mL/min B. 1.0 mL/min
Detection:	UV @ 254 nm
Temperature:	25 °C
Samples:	1. desipramine 2. imiptamine 3. amitriptyline 4. trimipramine

Tricylic Antidepessant Drugs

Figure 69 shows a comparison of four tricyclic antidepressant drugs on a column packed with styrene-divinylbenzene and a TSKgel Octadecyl-2PW column, both operated at pH 11. Recovery of sample analytes is high with the TSKgel Octadecyl-2PW column due to the modest hydrophobic nature of the polymethacrylate base matrix in comparison to a competitive polystyrene-based column.

Figure 69: Comparison of common tricyclic antidepressant drugs



Columns:	A. competitive column with styrene divinylbenzene (SDVB), 5 µm packing
	B. TSKgel Octadecyl-2PW, 5 µm, 4.6 mm ID × 15 cm
Mobile phase:	20 mmol/L phosphate buffer, pH 11.0/ ACN, 40/60
Flow rate:	A. 0.5 mL/min B. 1.0 mL/min
Detection:	UV @ 254 nm
Temperature:	25 °C
Samples:	1. desipramine
	2. imipramine
	3. amitriptyline
	4. trimipramine

About: TSKgel Octadecyl-4PW Reversed Phase Chromatography Columns

The highly cross-linked polymethacrylate base material of TSKgel Octadecyl-4PW provides excellent stability in high pH buffer systems and can withstand rigorous cleaning with either acid or base. The large pore size of TSKgel Octadecyl-4PW columns, 50 nm, allows unhindered access to proteins and other large molar mass biopolymers. The particle size offerings allow for analytical and semi-preparative scale separations.

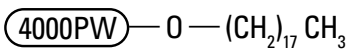
Attributes and Applications

Table 23 lists the attributes of TSKgel Octadecyl-4PW columns, while Figure 70 displays the structure. TSKgel Octadecyl-4PW columns are for the analysis of proteins up to 200 kDa.

Table 23: Product attributes

Attribute	Value
Pore size (mean)	50 nm
Exclusion limit	1,000 - 2.0 × 10^5 Da
Estimated ligand density	1 eq/L
Particle size (mean)	7 µm and 13 µm
pH stability	2.0-12.0
Functional group	C18 (monomeric bonding chemistry)

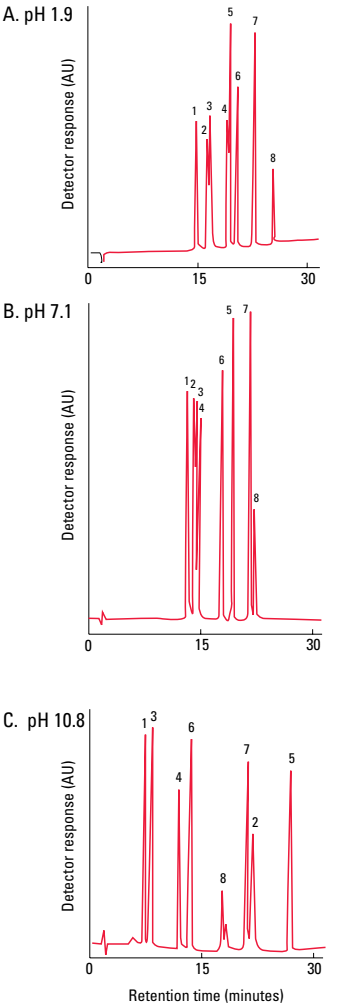
Figure 70: TSKgel Octadecyl-4PW structure



Peptides in Neural Tissue

The retention of eight peptides on a TSKgel Octadecyl-4PW column was compared under acidic, neutral, and basic pH conditions, as shown in Figure 71. This peptide mixture is well resolved only under high pH elution conditions that cannot be used with silica-based ODS columns. These high pH conditions also allow different selectivities of the eight peptides.

Figure 71: Comparison of pH conditions



Column:	**TSKgel Octadecyl-4PW, 5 µm, 4.6 mm ID × 15 cm**
Mobile phase:	A. 0.2% TFA, pH 1.9
	B. 0.05 mol/L phosphate buffer, pH 7.1
	C. 0.2 mol/L NH_3, pH 10.8
Gradient:	50 min. linear gradient from 0% to 80% CH_3CN
Flow rate:	1.0 mL/min
Detection:	UV @ 220 nm
Samples:	1. met-enkephalin
	2. bradykinin
	3. leu-enkephalin
	4. neurotensin
	5. bombesin
	6. angiotensin I
	7. somatostatin
	8. insulin

About: TSKgel Octadecyl-NPR Reversed Phase Chromatography Columns

The highly cross-linked polymethacrylate base material of TSKgel Octadecyl-NPR provides excellent stability in high pH buffer systems and can withstand rigorous cleaning with either acid or base.

NPR, nonporous resin, columns are prepared from nonporous methacrylate particles of uniform 2.5 µm size, which provides high efficiency separations and fast analyses of peptides and proteins. The nonporous particle structure limits product isolation to sub-microgram loads.

Attributes and Applications

Table 24 lists the attributes of TSKgel Octadecyl-NPR columns, while Figure 72 displays the structure. TSKgel Octadecyl-NPR columns are for the high efficiency purification of proteins and peptides at sub-microgram loads.

Table 24: Product attributes

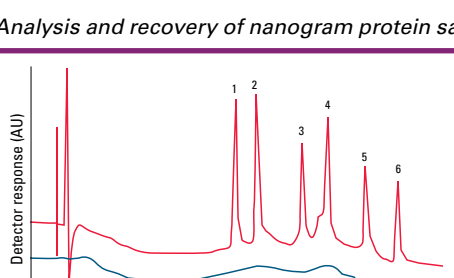
Attribute	Value
Pore size (mean)	nonporous
Exclusion limit	>1.0 × 10^6 Da
Estimated ligand density	1 eq/L
Particle size (mean)	2.5 µm
pH stability	2.0-12.0
Functional group	C18 (monomeric bonding chemistry)

Figure 72: TSKgel Octadecyl-NPR structure

Nanogram Protein Samples

Protein mass and activity recovery is a principal objective in protein purifications. Non-specific protein binding is minimized on the hydrophilic backbone of both porous and nonporous TSKgel polymeric packings, thus making high mass recovery for proteins and peptides possible. Sub-microgram protein loads eluted quickly with high resolution and high sample recovery rates from a TSKgel Octadecyl-NPR column, shown in Figure 73. This example also shows the excellent baseline stability of perchloric acid at low wavelengths. When sensitive detection is needed, perchloric acid is preferred over trifluoroacetic acid.

Figure 73: Analysis and recovery of nanogram protein samples

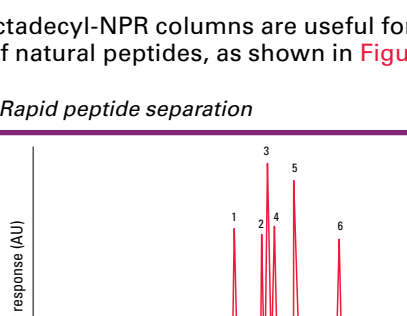


Column:	**TSKgel Octadecyl-NPR, 2.5 µm, 4.6 mm ID × 3.5 cm**
Mobile phase:	10 min linear gradient from 15% to 80% CH_3CN in 5 mmol/L $HClO_4$
Flow rate:	1.5 mL/min
Detection:	UV @ 220 nm
Samples:	50 ng each of 1. ribonuclease A 2. insulin 3. cytochrome C 4. lysozyme 5. transferrin 6. myoglobin
Note:	Blank gradient trace also shown

Natural Peptides

TSKgel Octadecyl-NPR columns are useful for the rapid analysis of natural peptides, as shown in Figure 74.

Figure 74: Rapid peptide separation

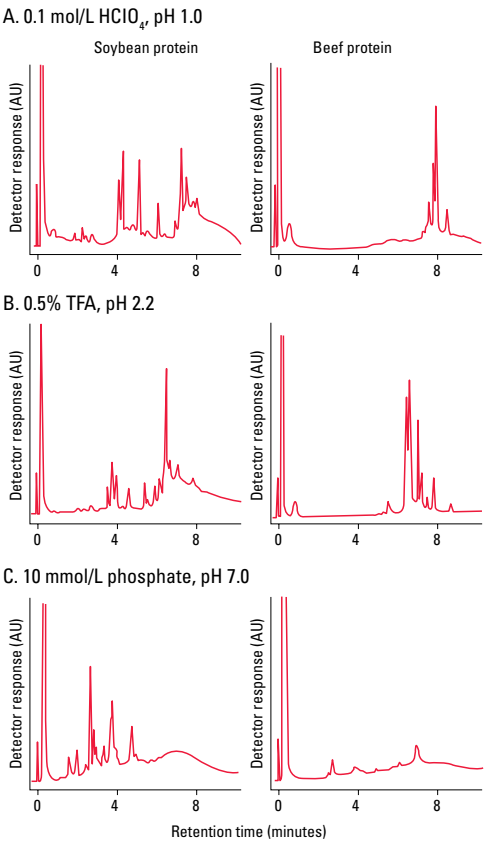


Column:	**TSKgel Octadecyl-NPR, 2.5 µm, 4.6 mm ID × 3.5 cm**
Mobile phase:	10 min linear gradient from 0% to 80% CH_3CN in 0.2% TFA
Flow rate:	1.5 mL/min
Detection:	UV @ 220 nm
Samples:	1. α-endorphin 2. bombasin 3. γ-endorphin 4. angiotensin 5. somatosatin 6. calcitonin

Method Development

Method development is expedient with TSKgel Octadecyl-NPR columns. In Figure 75, two protein extracts were analyzed under three different elution conditions in a relatively short time.

Figure 75: Rapid method development

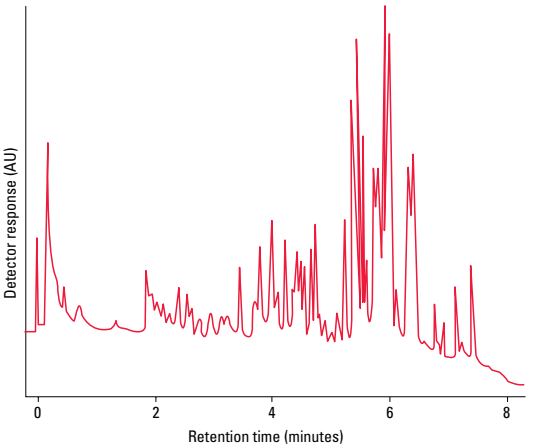


Column:	**TSKgel Octadecyl-NPR, 2.5 µm, 4.6 mm ID × 3.5 cm**
Mobile phase:	A. 10 min linear gradient from 0% to 80% CH_3CN in 0.1 mol/L $HClO_4$ B. 10 min linear gradient from 0% to 80% CH_3CN in 0.05% TFA, pH 2.2 C. 10 min linear gradient from 0% to CH_3CN in 10 mmol/L phosphate buffer to 80% CH_3CN in 0.5 mmol/L phosphate buffer, pH 7.0
Flow rate:	1.5 mL/min
Detection:	UV @ 220 nm
Samples:	left column: water extract of soybean flour right column: water extract of beef

Tryptic Digests

The 2.5 µm particle size of TSKgel Octadecyl-NPR columns also provides high resolution of tryptic digests, see Figure 76. The addition of a small quantity of surfactant to the mobile phase was necessary in this application to enhance retention of hydrophilic peptide fragments.

Figure 76: Fast, high resolution analysis



Column:	**TSKgel Octadecyl-NPR, 2.5 µm, 4.6 mm ID × 3.5 cm**
Mobile phase:	10 min linear gradient from 0% to 60% CH_3CN in 0.05 mol/L phosphate buffer, pH 2.8, containing 1 mmol/L sodium dodecyl sulfate
Flow rate:	1.5 mL/min
Detection:	UV @ 210 nm
Sample:	tryptic digest of reduced and S-carboxymethylated bovine serum albumin, 10 µg

About: TSKgel Phenyl-5PW RP Reversed Phase Chromatography Columns

TSKgel Phenyl-5PW RP columns are prepared by chemically bonding a high density of phenyl groups with an ether linkage to the base matrix of TSKgel G5000PW, a 10 µm high performance gel filtration packing. The TSKgel Phenyl-5PW RP column is structurally similar to the TSKgel Phenyl-5PW column used in hydrophobic interaction chromatography (HIC), but the RP column packing is prepared by bonding a higher density of phenyl groups. The greater level of hydrophobicity makes the packing more suitable for reversed phase chromatography.

The highly cross-linked polymethacrylate base material provides an advantage over silica when high pH buffer systems are needed. Additionally, TSKgel Phenyl-5PW RP can withstand rigorous cleaning protocols using either acid or base.

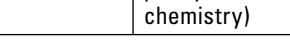
Attributes and Applications

Table 25 lists the attributes of TSKgel Phenyl-5PW RP columns, while Figure 77 displays the structure. The 100 nm pore size of the TSKgel Phenyl-5PW RP columns accommodates globular protein samples up to 1.0×10^6 Da.

Table 25: Product attributes

Attribute	Value
Pore size (mean)	100 nm
Exclusion limit	1.0×10^6 Da
Estimated ligand density	1 eq/L
Particle size (mean)	10 µm and 13 µm
pH stability	2.0-12.0
Functional group	phenyl (monomeric bonding chemistry)

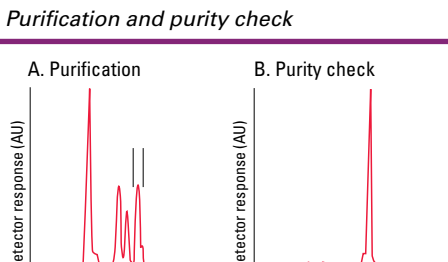
Figure 77: TSKgel Phenyl-5PW RP structure



Protein Analysis

Based on 100 nm pore size methacrylate resin, TSKgel Phenyl-5PW RP columns allow proteins unrestricted access to the available pore structure. Large proteins and biomolecules up to 1,000 kDa can be retained without being excluded from the pore structure, resulting in excellent peak symmetry and sharpness. For example, crude lactate dehydrogenase (approximately 120 kDa) eluted as a sharp peak during the purification and purity check performed on a TSKgel Phenyl-5PW RP column, as shown in Figure 78.

Figure 78: Purification and purity check

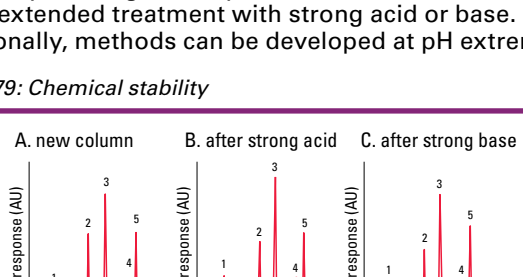


Column:	**TSKgel Phenyl-5PW RP, 10 µm, 4.6 mm ID × 7.5 cm**
Mobile phase:	2 min linear gradient from 5% to 20% CH_3CN in 0.05% TFA, followed by (A - 48 min/B - 32 min) linear gradient to (80%A/60%B) CH_3CN in 0.05% TFA
Flow rate:	1.0 mL/min
Detection:	UV @ 220 nm
Sample:	lactate dehydrogenase A. 40 µg in 100 µL B. purity check of fraction collected in part A

Chemical Stability

The chromatograms in Figure 79 show the retention and selectivity of TSKgel Phenyl-5PW RP columns are stable under extended treatment with strong acid or base. Additionally, methods can be developed at pH extremes.

Figure 79: Chemical stability



Column:	**TSKgel Phenyl-5PW RP, 10 µm, 4.6 mm ID × 7.5 cm**
Mobile phase:	60 min linear gradient from 5% to 80% CH_3CN in 0.05% TFA
Flow rate:	1.0 mL/min
Detection:	UV @ 220 nm
Samples:	10 µg each of 1. ribonuclease A 2. cytochrome C 3. lysozyme 4. bovine serum albumin 5. myoglobin