TOSOH BIOSCIENCE

Separations Business Unit

Reversed Phase Chromatography

Reversed Phase is
never the same!

Why we are different

- unique chemical bonding with exhausting endcapping
- broadest range of use
- large range of polymer based RP columns for high pH separations
- alternatives to RP chromatography in case of HILIC

Tosoh Bioscience offers a broad line of analytical and preparative TSK-GEL RPC columns for the most complex separation problems and additional new guard column system !

To select the appropriate TSK-GEL RPC column from the Tosoh Bioscience portfolio, make your choice according to the complexity and the variety of your samples!

TSK-GEL ODS columns

best for separating small pharmaceutical compounds and monodisperse biopolymers, like proteins and peptides.
They exhibit high resolving power and sensitivity.

- TSK-GEL ODS-100V and Z
- TSK-GEL ODS-120
- TSK-GEL ODS-80

TSK-GEL - polymer RP-columns

compatible with a wide range of mobile phase pH conditions, for robust reproducible methods at high pH!

- TSK-GEL OligoDNA RP
- TSK-GEL Octadecyl-NPR
- TSK-GEL Octadecyl-2PW
- TSK-GEL Octadecyl-4PW

TSK-GEL - SuperSeries

designed for fast analysis or high throughput screening with high selectivity and high peak capacity. Exclusion limit of 20.000 daltons, and up to 200.000 theoretical plates/meter!

- TSK-GEL Super-ODS
- TSK-GEL Super-Octyl
- TSK-GEL Super-Phenyl

For polar compounds that will not be retarded on standard RP columns, the HILIC column Amide-80 is a must-have!

TSK-GEL HILIC columns

- TSK-GEL Amide-80

If help is needed, contact our technical support specialists to offer you assistance at +49 (0)11 13257-0.

Reversed Phase Chromatography

"REVERSED PHASE IS NEVER THE SAME"

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Difficult Separations

Polar and non-polar compounds in one sample?

Necessity of at least two different separation runs?

Tosoh Bioscience introduces the latest development in RP bonding chemistry. Adding polarity to the bonded alkyl phase will overcome “bonded phase collapse”.

Our solution for complex separations:

1 . TSKgel ODS-100

HIGHLIGHTS

- Differences in carbon content level and surface optimization to serve a wide range of samples.
- Ultra-pure based silica with 5 µm and 3 µm particle size, 100 Å pores, functionalized with C18.
- Compatible to pure aqueous and pure organic eluents.
- Enable simultaneous separation of water and fat soluble vitamins.
- Especially capable for analysis for pharmaceutical and food industries.

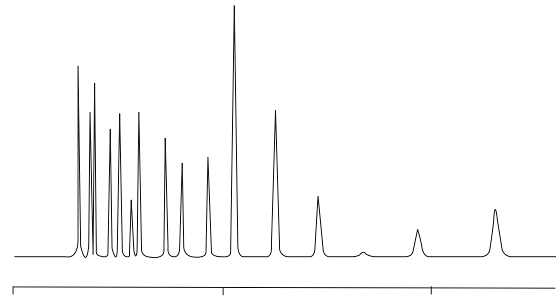
Newly developed RP material for the separation of small compounds, like organic acids (see Figure 1) and to simplify difficult separations (see Figure 2).

Figure 3 displays, that with their high surface polarity, the TSKgel ODS-100 columns are an excellent choice for methods development.

	TSKgel ODS-100V	TSKgel ODS-100Z
Matrix	ultra-pure silica	ultra-pure silica
Particle size	3 µm, 5 µm	3 µm, 5 µm
Pore size	100 Å	100 Å
Specific surface area	450 m^2/g	450 m^2/g
Functional group	C18	C18
Carbon content	15%	20%
Bonding phase	monomeric	monomeric
End-capping	yes	yes
Sample type	polar	hydrophobic

Table 1 Physical properties of TSKgel ODS-100V and TSKgel ODS-100Z

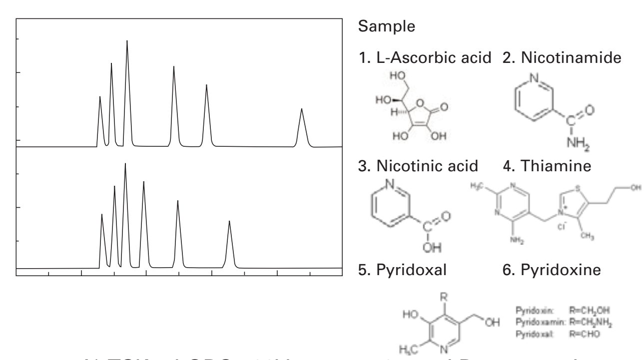
Isocratic separation of organic acids with TSKgel ODS-100V



Column: TSKgel ODS-100V, 5 µm, 4.6 mm ID x 25.0 cm L
Eluent: 0.1 % H_3PO_4
Flow rate: 1.0 ml/min
Inj. vol.: 10 µl
Temp.: 40°C
Detection: UV @ 210 nm

Figure 1

Isocratic separation of water soluble vitamins with TSKgel ODS-100V and TSKgel ODS-100Z



Column: A) TSKgel ODS-100V, 5 µm, 4.6 mm I.D. x 15 cm L
B) TSKgel ODS-100Z, 5 µm, 4.6 mm I.D. x 15 cm L
Eluent: $H_2O/CH_3CN(99/1)$ + 0.1 % TFA
Flow rate: 1.0 mL/min
Inj. vol.: 5 µL
Temp.: 40 °C
Detection: UV @ 280 nm

Figure 2

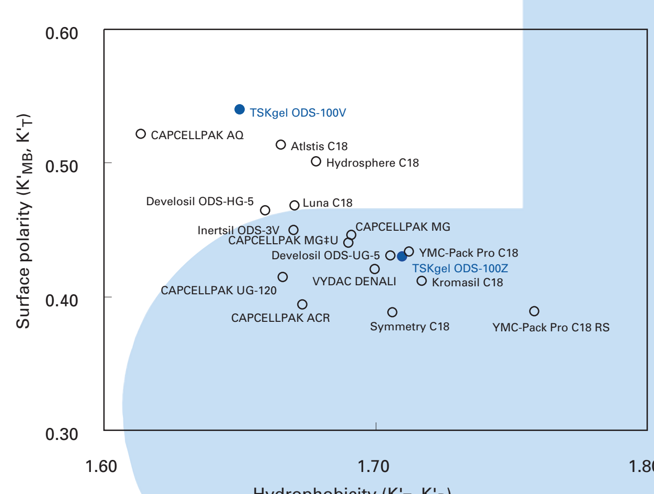
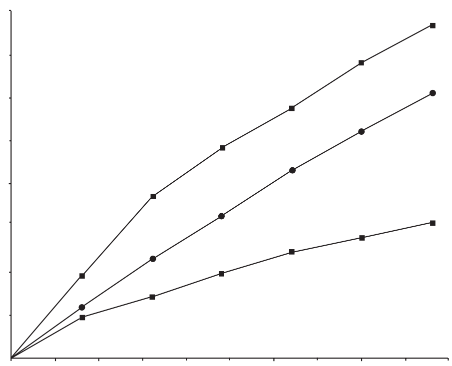


Figure 3

Difficult Separations

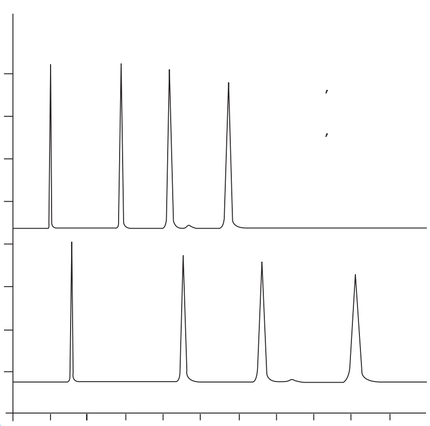
Pressure flow rate curves with different acetonitrile concentrations within the eluent



Column: TSKgel Super-ODS, 2 µm, 4.6 mm ID x 5.0 cm L

Figure 4

Chromatographic comparison between 3 µm and 5 µm TSKgel ODS-100V

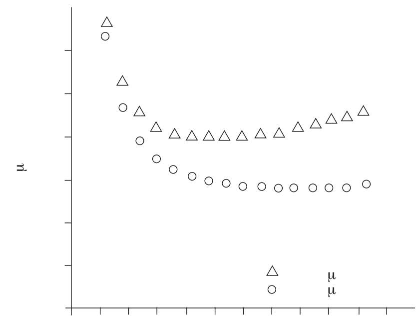


Column:	A) TSKgel ODS-100V, 3 µm, 2.0 mm ID x 10 cm L
	B) TSKgel ODS-100V, 5 µm, 2.0 mm ID x 15 cm L
Sample:	1. Uracil, 2. Benzene, 3. Toluene, 4. Naphthalene
Eluent:	H_2O/MeOH (30/70)
Flow rate:	0.20 mL/min
Inj. vol.:	2 µL
Temp.:	25 °C
Detection:	UV @ 254 nm

Figure 5

The difference in behaviour between 5 µm and 3 µm with increasing linear velocity is shown in Figure 6. The right choice of organic modifier in the mobile phase is also essential for a good separation. Comparison between Acetonitrile and Methanol as organic modifier is shown in Figure 7.

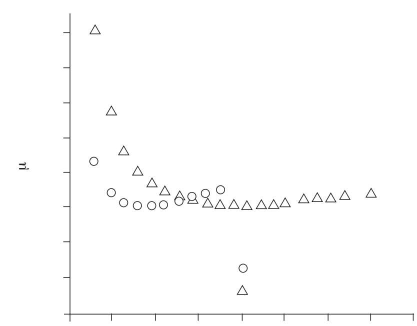
Van Deemter curves for 3 µm and 5 µm TSKgel ODS-100V



Column:	TSKgel ODS-100V, 3 µm, 4.6 mm ID x 15 cm L
	TSKgel ODS-100V, 5 µm, 4.6 mm ID x 15 cm L
Sample:	Naphthalene
Eluent:	H_2O/MeOH (30/70)
Inj. vol.:	10 µL
Temp.:	40 °C
Detection:	UV @ 254 nm

Figure 6

Van Deemter curves for acetonitrile vs. methanol with TSKgel ODS-100V, 3 µm



Column:	TSKgel ODS-100V, 3 µm, 2.0 mm ID x 15 cm L
Sample:	Naphthalene
Eluent:	H_2O/MeOH (30/70)
	H_2O/CH_3CN (40/60)
Inj. vol.:	2 µL
Temp.:	25 °C
Detection:	UV @ 254 nm

Figure 7

Difficult Separations

2. TSK-GEL SuperSeries

HIGHLIGHTS

- The silica particles used in SuperSeries columns are monodisperse spherical 2 µm beads with 110 Å pores.
- TSKgel Super-ODS, Super-Octyl and Super-Phenyl packings are bonded with, respectively, C18, C8 and phenyl functional groups. The bonded phases have a polymeric structure. An exhaustive endcapping reaction minimizes the presence of residual silanol groups.
- 2 µm particles provide superior resolution and speed, as well as improved sensitivity.
- Pressure drop is not excessive due to the monodisperse particle size distribution.
- Stainless steel columns are available with 4.6 mm and 1 mm ID formats.

APPLICATIONS

Super-ODS, Super-Octyl, Super-Phenyl

- Recommended for small molecular weight compounds **(<20,000kDa)** such as peptides, amino acids, tryptic digests, nucleotides, pharmaceutical molecules, and food and beverage samples.
- The baseline separation of 18 PTC amino acids in 5 minutes is shown in Figure 8.
- The ability to perform very fast analyses is shown in Figure 9 for the separation of peptides on Super-ODS using flow rates up to 3 mL/min.
- The different selectivity of ODS, Octyl and Phenyl functional groups is illustrated in Figure 10 for the analysis of a mixture of six neuropeptides in less than one minute.

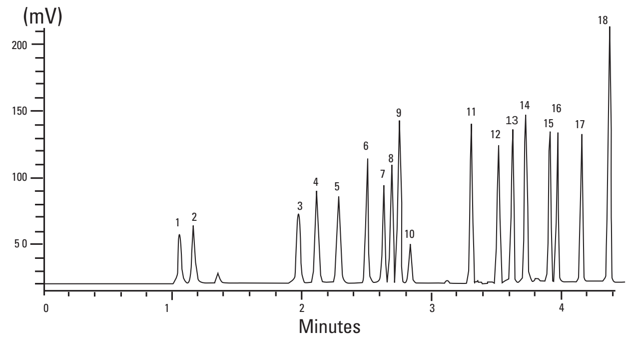
	TSKgel Super-ODS	TSKgel Super-Octyl	TSKgel Super-Phenyl
Matrix	silica	silica	silica
Particle size	2.3 µm	2.3 µm	2.3 µm
Pore size	110 Å	110 Å	110 Å
Specific surface area	96 m^2/g	450 m^2/g	n.a.
Functional group	polymeric C18	polymeric C18	polymeric phenyl
Carbon content	8%	5%	3%
Bonding phase	polymeric	polymeric	polymeric
End-capping	yes	yes	yes
Sample type	hydrophilic/hydrophobic		

Table 2 Physical properties of TSKgel SuperSeries Columns

PRACTICAL ASPECTS

- Applicable under normal HPLC pressure conditions with 2 µm particles.
- Better resolution to detect low concentrations of impurities in pharmaceutical formulations during stability testing.
- TSKgel Super-ODS is a column of high value for method development, particularly for revalidation of old methods, for FDA-registration.

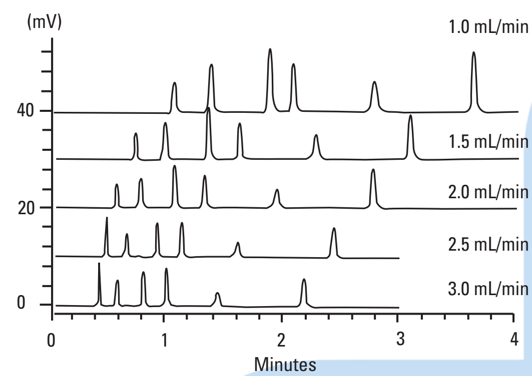
TSKgel Super-ODS for rapid separation of eighteen PTC-derivatized amino acids



Column: TSKgel Super-ODS, 4.6 mm ID x 10 cm L
Sample: 1. ASP, 2. Glu, 3. Ser, 4. Gly, 5. His, 6. Arg, 7. Thr, 8. Ala, 9. Pro, 10. PTC-NH2, 11. Try, 12. Val, 13. Met, 14. Cys, 15. Ile, 16. Leu, 17. Phe, 18. Lys
Elution: a) ACN (50 mM acetate buffer, pH 6.0, 3/97)
b) ACN/H_2O (60/40)
Flow rate: 1.5 mL/min
Detection: UV @ 254 nm
Injection: 5 µl (250 pmol)
Temperature: ambient

Figure 8

Chromatogram of peptide mixture at various flow rates



Column: TSKgel Super-ODS, 4.6 mm ID x 5 cm L
Sample: 1. Oxytocin; 2. Alpha-Endorphin; 3. Bombesin; 4. Leu-Enkephalin; 5. Gamma-Endorphin; 6. Somatostatin
Elution: 13 mM $HClO_4/CH_3CN$, linear gradient of CH_3CN from 23% to 56%
Flow rate: 1.0 to 3.0mL/min
Detection: UV @ 220 nm
Temperature: ambient

Figure 9

Difficult Separations

- 15.000 injections on a TSKgel Super-ODS, 4.6 mm ID x 10 cm L, with real pharmaceutical samples are reported.

MATERIAL DATA

It is evident that, when using small particles, you have the benefit of high linear velocity without loosing separation power.

A critical aspect with small particle sized material is the high pressure increase to be expected with increasing flow rates. As shown in Figure 4, pressure drops are moderate with 2 µm Super RP material. This is achieved by a narrow particle size distribution.

Separation and detection of degradation products 0.1% limit during stability testing.

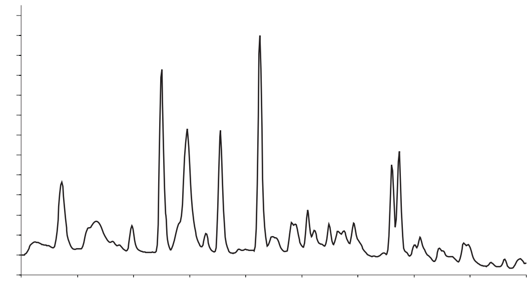
Example: Standard Methylprednisolon in tablets (see Figure 11)

During stability testing with long time storage, more and more problems occur with degradation products with by-products, and pharmaceutical manufacturers are forced to detect an analyse molecular structure when peak area is higher than 0.1% of the main substance peak.

Therefore it makes sense to use modern high resolution column types, to get all peaks resolved with a high resolution, to avoid co-elution of several compounds.

That means, with high resolution all degradation or reaction products are mostly under the 0.1% area counts of the main substance, and analysis of molecular structure of these degradation products is not necessary.

Separation and detection of degradation products in tablets

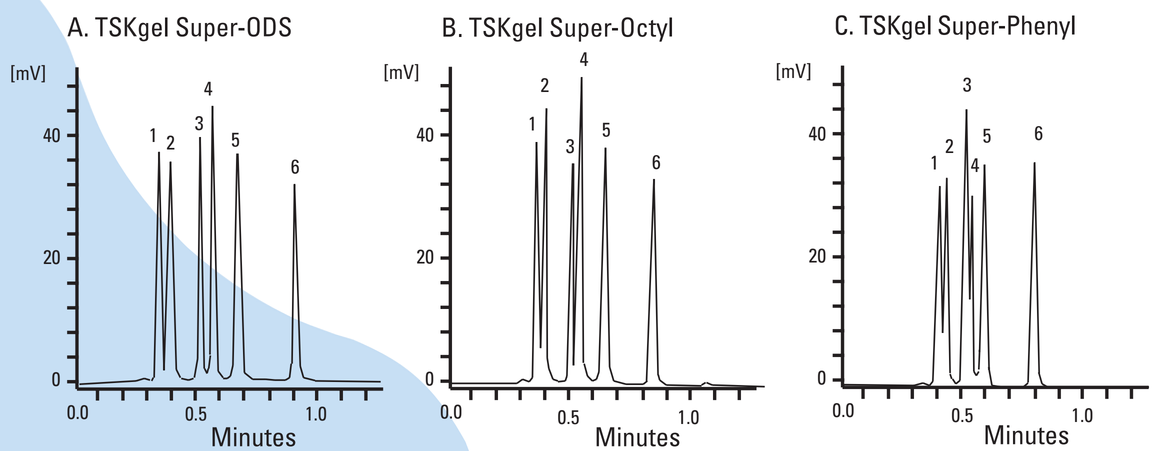


Column:	TSKgel Super-ODS, 4.6 mm ID x 10 cm L
Sample:	Methylprednisolon tablets
Sample conc.:	8 mg Metylprednisolon per 10 ml MeOH/ H_2O 70/30; v/v
Inj. vol.:	10 µl
Flow rate:	1.5 ml/min
Eluent A:	Acetonitrile
Eluent B:	0.05 % H_3PO_4 in H_2O
Gradient prof.:	Start: 85.0% B; 16 min: 77.0% B; 20 min: 72.5% B; 30 min: 36.0% B; 32 min: 36.0% B; 33 min: 85.0% B; 35 min: Stop
Detection:	UV 254 nm
Temperature:	Room temperature

Methylprednisolon, detected with high sensitivity, and high resolution for all degradation products. With permission of H. Rausch, Phytochem, Neu-Ulm

Figure 11

Rapid separation of peptide mixture on TSK-GEL SuperSeries columns



Column:	Each 4.6 mm ID x 5 cm L
Sample:	1. Oxytocin; 2. Alpha-Endorphin; 3. Bombesin; 4. Leu-Enkephalin; 5. Gamma-Endorphin; 6. Somatostatin
Elution:	Buffer A. 13 mM $HClO_4$; Buffer B. 13 mM $HClO_4/CH_3CN$ (20/80); 35% B to 80% B in a 3 min linear gradient
Flow rate:	2.0 mL/min
Detection:	UV @ 220 nm

Figure 10

High pH, Special Applications

Do you have strong basic compounds with pKs > 8.0?

Silica based columns are not stable under these conditions and do not deliver reproducible results?

Our solution:

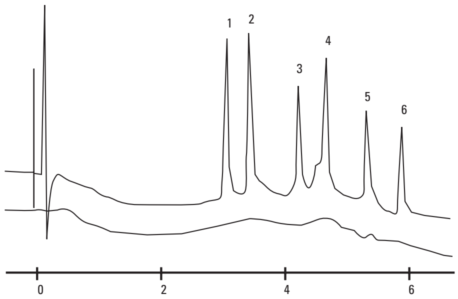
1 .TSK-GEL Polymer based Columns (RP PW-Columns)

The best solution for very high pH!

HIGHLIGHTS

- Polymer-based RPC columns are chemically stable from **pH 2.0 – 12.0.**
- Cleaning and removing of endotoxins by using either strong acid or base.
- Available packed with nonporous resins (NPR) or with porous resins of various pore sizes.
- 2.5 µm particle size TSKgel Octadecyl-NPR resin features fast kinetics, resulting in high column efficiency, and quantitative protein recovery at sub-µg loads.
- TSKgel Octadecyl-2PW is based on 5 µm particle size G2000PW resin, with 125 Å pores.
- TSKgel Octadecyl-4PW is based on 7 µm particle size G4000PW resin, which contains 500 Å pores.
- TSKgel Phenyl-5PW RP is based on 10 µm particle size G5000PW resin, which has an average pore size of 1000 Å. In comparison with Phenyl-5PW packing material used in HIC, the greater level of hydrophobicity in TSKgel Phenyl-5PW RP makes this material more suitable for use in RPC.

Analysis and recovery of nanogram protein samples



Column: TSKgel Octydecyl-NPR, 4.6 mm ID x 3.5 cm L
Sample: 50 ng each of 1. Ribonuclease A, 2. Insulin, 3. Cytochrome C, 4. Lysozyme, 5. Transferrin, 6. Myglobin
Elution: 10 min linear gradient from 15% to 80% CH_3CN in 5 mM $HClO_4$
Flow rate: 1.5 mL/min
Detection: UV @ 220 nm
Note: Blank injection also shown

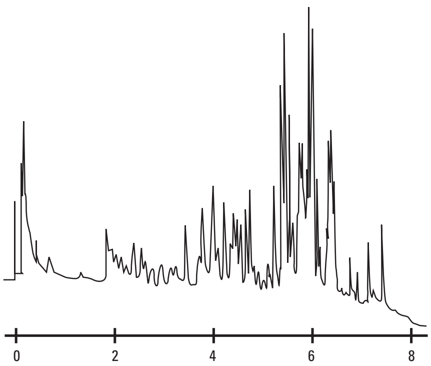
Figure 12

APPLICATIONS

TSKgel Octadecyl-NPR

- High efficiency purification of proteins and peptides at sub-µg loads.
- Nonporous particles are more stable to higher pressures than porous particles.
- Quantitative recovery of a mixture of globular proteins is demonstrated in Figure 12.
- Figure 13 demonstrates the high resolution obtained on a 4.6 mm ID x 3.5 cm L column for a tryptic digest of a bovine serum albumin analog.

Fast, high resolution analysis of tryptic digest



Column: TSKgel Octydecyl-NPR, 4.6 mm ID x 3.5 cm L
Sample: Tryptic digest of reduced and S-carboxymethylated Bovine Serum Albumin, 10 µg
Elution: 10 min linear gradient from 0% to 60% CH_3CN in 0.05 M phosphate buffer, pH 2.8, containing 1 mM sodium dodecyl sulfate
Flow rate: 1.5 mL/min
Detection: UV @ 210 nm

Figure 13

TSKgel Octadecyl-2PW

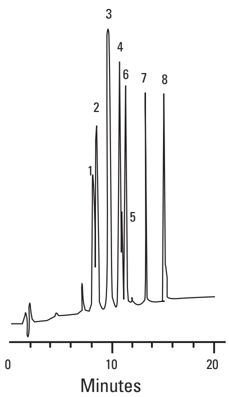
- For analyzing small MW pharmaceutical compounds at basic pH.
- Faster analysis than competitive polymeric reversed phase packings.
- The rapid separation of a mixture of eight peptides is shown in Figure 14.
- Performance comparison of PS-DVB and Octadecyl-PW columns is shown in Figure 15.

TSKgel Octadecyl-4PW

- Synthetic peptides and small proteins
- Changing pH to optimize peptide retention and selectivtiy, depending on different pH-ranges, see Figure 16.

High pH, Special Applications

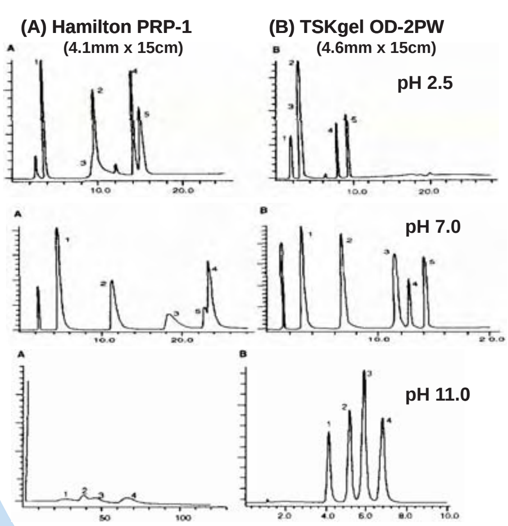
Separation of eight peptides on TSKgel Octadecyl-2PW



Column: TSKgel Octydecyl-2PW, 4.6 mm ID x 15 cm L
Sample: 1) Met-Enkephaln, 2) Bradykinin, 3) Leu-Enkephalin, 4) Neurotensin, 5) Bombesin, 6) Angiotensin I, 7) Somatostatin, 8) Insulin (bovine)
Elution: 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
Temerature: ambient

Figure 14

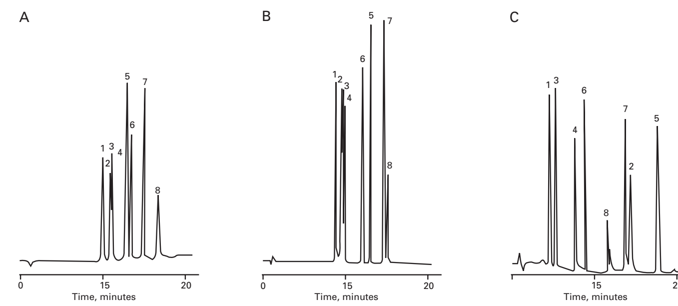
Performance-Comparison: PS-DVB column vs. TSKgel OD-2PW columns



Sample: 1. Sulpiride, 2. Disopyramide, 3. Chlorpheniramine, 4. Ciltiazem, 5. Hydroxizine
Buffer: 20 mM phosphate buffer/ACN
Elution: 30 min linear gradient from 20% ACN to 100% ACN
Flow rate: A) 0.5 mL/min, B) 1.0 mL/min
Detection: UV @ 254 nm
Temperature: ambient

Figure 15

Changing pH to optimize peptide retention and selectivity



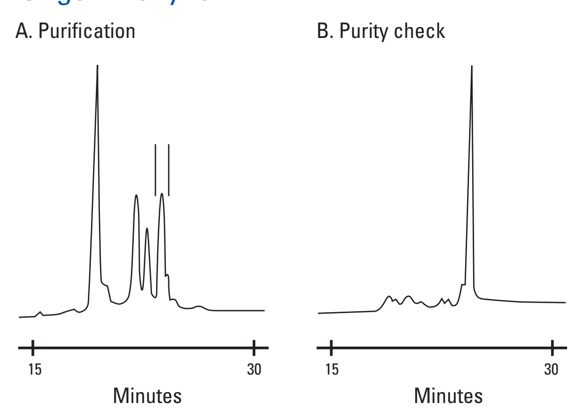
Column: TSKgel Octadecyl-4PW, 4.6 mm ID x 15 cm L
Sample: 5-10 g each of: 1. Met-Enkephalin, 2. Bradykinin, 3. Leu-Enkephalin, 4. Neurotensin, 5. Bombesin, 6. Angiotensin I, 7. Somatostatin 8. Insulin
Solvent progr.: 50 min linear gradient from 0% to 80% acetonitrile in: A: 0.2% TFA (pH 1.9, B: 50 mM sodium phosphate (pH 7.1), C: 200 mM ammonia (pH 10.8)
Flow rate: 1.0 mL/min
Detection: UV @ 215 nm
Temperature: 25°C

Figure 16

TSKgel Phenyl-5PW RP

- Proteins, including high MW.
- High loads (high capacity).
- The use of Phenyl-5PW RP for the purification and subsequent purity check of lactate dehydrogenase is shown in Figure 17.

Protein purification and purity check on TSKgel Phenyl-5PW RP



Column: TSKgel Phenyl-5PW RP, 4.6 mm ID x 7.5 cm L
Sample: Lactate dehydrogenase, A. 40 µg in 100 µl, B. purity check of fraction collected part A
Elution: 2 min linear gradient from 5% to 20% CH3CN in 0.05 TFA, followed by (A - 48 min/B - 32 min)linear gradient to (A - 80%/B - 60%) CH_3CN in 0.05 TFA
Flow rate: 1.0 mL/min
Detection: UV @ 220 nm

Figure 17

High pH, Special Applications

2. Special Application TSK-GEL RPC Columns

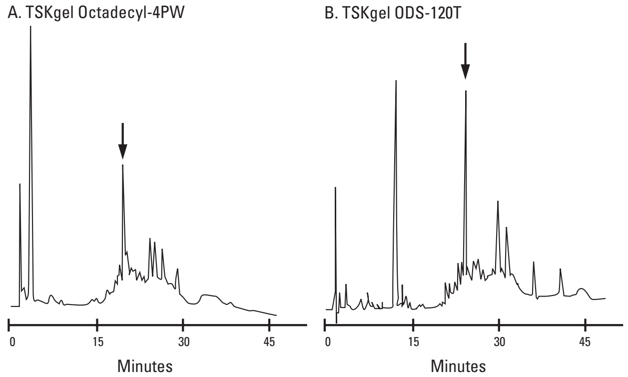
HIGHLIGHTS

- TSKgel OligoDNA RP and TSKgel TMS-250 incorporate 5 µm or 10 µm spherical porous silica with 250 Å pores to allow unhindered access by large oligonucleotides and proteins respectively.
- TSKgel OligoDNA RP contains a monomeric C18 bonded phase that is not endcapped.
- TSKgel TMS-250 is exhaustively and repeatedly reacted with trimethyl silyl groups. Standard nomenclature designates the bonded phase as C1.
- TSKgel OligoDNA RP is available in 4.6 mm ID and 7.8 mm ID (both 15 cm length), while TSKgel TMS-250 is available in 4.6 mm ID x 7.5 cm L.

	Silica matrix	Polymer matrix
Separation power	high	medium
Stability at pH < 2.0 and pH > 8.0 high temperatures	 low low	 high (pH 2.0-12.0) medium
Interaction with polar substances due to metal impurities	 possible possible	 low no
Pressure resistance	high	medium-low

Table 3

Analysis of a synhetic peptide reaction mixture on TSKgel Octadecyl-4PW and TSKgel ODS-120T columns



Column: A. TSKgel Octadecyl-4PW, 4.6 mm ID x 15 cm L ; B. TSKgel ODS-120T, 4.6 mm ID x 15 cm L
Sample: Triacontadipeptide (EAEDLQVGQVELGGGPGAGSLQPLALEGSLQC) indicated by arrow; 50 µg in 50 µl
Elution: 48 min linear gradient from 14% to 50% CH_3CN in 0.1% TFA
Flow rate: 1.0 mL/min
Detection: UV @ 215 nm

Figure 18

APPLICATIONS

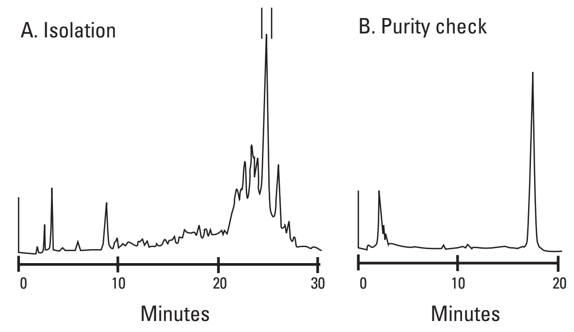
TSKgel Octadecyl-4PW

- As shown in Figure 18, selectivity for a synthetic peptide reaction mixture differs from that on the silica-based ODS-120T column.

TSKgel OligoDNA RP

- Purification and analysis of oligonucleotides (up to 500-mer), RNAs, and DNA fragments.
- The separation shown in Figure 20, illustrates the high-resolving power of TSKgel OligoDNA RP columns for octamers of similar sequence.
- 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 19. The purity of the isolated oligonucleotide was subsequently verified on an analytical 4.6 mm ID TSKgel OligoDNA RP column.

Purification of synthetic 49-mer oligonucleotide

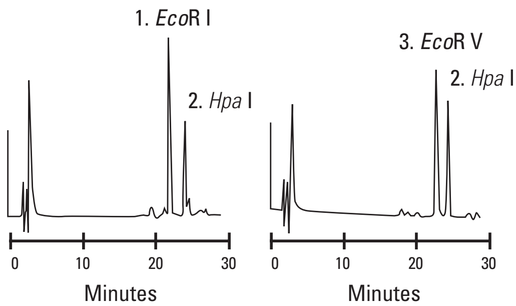


Column: TSKgel OligoDNA RP, 7.8 mm ID x 15 cm L; TSKgel OligoDNA RP, 4.6 mm ID x 15 cm L
Sample: Synthetic 49-mer oligonucleotide, d(AGCTTGGGCTGCAGGTCGTCTCTAGAGGATC CCC GGGCGAGCTCGAATT)
Elution: 120 min linear gradient from 6.25% to 25% CH_3CN for the 7.8 mm ID column, or B. 90 min linear gradient from 7.5% to 25% CH_3CN for the 4.6 mm ID column, both in 0.1 M 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

Figure 19

High pH, Special Applications

Separation of octamers of similar sequence with TSKgel OligoDNA RP



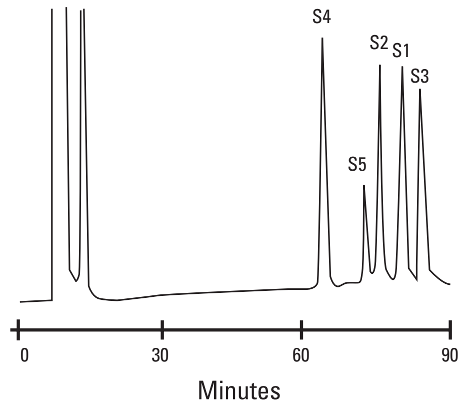
Column:	TSKgel OligoDNA RP, 4.6 mm ID x 15 cm L
Sample:	1. Linker EcoR I, d(CGAATTCG); 2. Hpa I, d (CGTTAACG) ; 3. Linker EcoR V, d(CGATATCG)
Elution:	120 min linear gradient from 5% to 25% CH_3CN in 0.1 M ammonium acetate, pH 7.0
Flow Rate:	1.0 mL/min
Detection:	UV @ 260 nm

Figure 20

TSKgel TMS-250

- The resolution of proteins on TSKgel TMS-250 columns is shown in Figure 20. The “wide-pore” TMS-250 packing can accommodate large proteins, such as aldolase (158,000 Da).
- Figure 22 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 molecular mass ranging from 10,000 to 26,000 Da were resolved without significant cross-contamination using a specially packed 7.5 mm L x 30 cm L column.

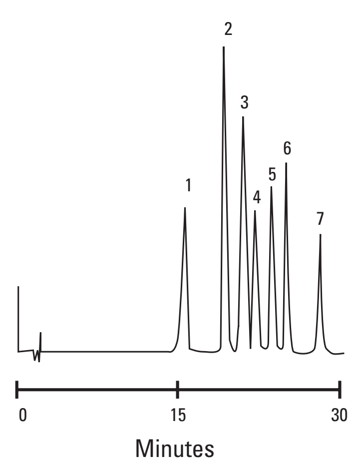
High resolution of protein subunits with TSKgel TMS-250



Column:	TSKgel TMS-250, 7.5 mm ID x 30 cm L
Sample:	Purified Bordetella Pertussis Toxin in 0.1 M phosphate buffer, pH 7.2, with 0.5 M NaCl and 30% glycerol
Injection:	100 µg in 500 µl
Elution:	load and 12 min wash with 34% CH_3CN in 0.1% TFA, followed by a 100 min linear gradient from 34% to 47% pH CH_3CN in 0.1% TFA
Flow rate:	1.5 mL/min
Detection:	UV @ 210 nm and 280 nm (not shown)
Recovery:	Mass recovery of the five subunits was 95.8%

Figure 21

High resolution protein separation on TSKgel TMS-250



Column:	TSKgel TMS-250, 4.6 mm ID x 7.5 cm L
Sample:	5 lg each of: 1. Ribonuclease A, 2. Cytochrome C, 3. Lysozyme, 4. Bovine Serum Albumin, 5. Aldolase, 6. Carbonic Anhydrase, 7. Ovalbumin
Elution:	60 min (TMS-250) 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

Figure 22

No Separation on RPC: HILIC

Hydrophilic compounds like peptides, carbohydrates, polar natural compounds, amino acids, oligonucleotides, organic polar acids and base or even proteins, which can not be separated on RP-columns:

Our solution is HILIC

TSK-GEL Amide-80

HIGHLIGHTS

- Its principal advantage: hydrophilic compounds can be retained on the HILIC column, while nonpolar sample impurities elute unretained in the void volume.
- Spherical silica particles, covalently bonded with carbamoyl groups
- The polar functional groups of the sample hydrogen-bond with the amide functionality of the stationary phase and result in retention.
- Elution order of analytes is determined by the number of hydroxy groups on the sample, the molecular conformation of the compounds, and their relative solubility in the mobile phase.

COLUMN OPERATION AND SPECIFICATIONS

The TSKgel Amide-80 column can be operated over a broad range of mobile phase conditions for use with many sample polarities. Factors to consider when employing this column include:

Sample Loading Capacity: this is dependent upon the polarity of the mobile phase. Loading capacity increases with decreasing mobile phase polarity.

	TSKgel Amide-80
Matrix	silica
Particle size	5 µm, 10 µm
Pore size	80 Å
Specific surface area	n.a.
Functional group	carbamoyl
Carbon content	n.a.
Bonding phase	n.a.
End-capping	no
Sample type	peptides, small compounds

Table 4 Physical properties of TSKgel Amide-80 Columns

For example, the highest loading capacity for mannitol (200 µg) occurs with a mobile phase of 75:25 acetonitrile/water. However, <100 µg of mannitol can be loaded in a mobile phase of 65:35 acetonitrile/water. The maximum sample volume for a 4.6 mm ID x 25 cm L Amide-80 analytical column is 50 µL.

Flow Rate and Pressure Limitations: flow rates between 0.8 and 1.0 mL/min for 4.6 mm ID columns and 0.15 to 0.18 mL/min for 2.0 mm ID are recommended. Column pressure drop varies with mobile phase viscosity. For mobile phases containing high water concentrations, the back-pressure should be: <250 kg/cm^2 (3356 psi) for 2 mm ID columns, <150 kg/cm^2 (2250 psi) for 4.6 mm ID columns, <70 kg/cm^2 (1050 psi) for 7.8 mm ID columns, and <30 kg/cm^2 (450 psi) for 21.5 mm ID columns.

Temperature Range: The TSKgel Amide-80 column can be operated over a temperature range of 4-80°C.

Choice of Mobile Phase: the pH range of the TSKgel Amide-80 column is 2.5-7.5 with a salt concentration of up to 100 mM. The column is stable in 100% organic eluents; however, a combination of aqueous and organic solvents is necessary in order to elute retained compounds. Elution volume can be controlled by the mobile phase polarity. As the mobile phase polarity decreases (higher organic content) the sample is retained longer on the column. For example, oligosaccharides require 40-50% water in the mobile phase in order to elute from the Amide-80 column.

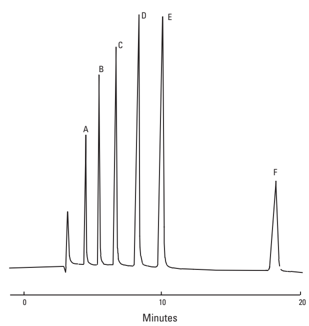
APPLICATIONS

- Polyalcohols can be separated with a mobile phase of organic solvent and water as shown in Figure 23.
- The TSKgel Amide-80 can separate oligosaccharides very rapidly and efficiently. Figure 24 shows a separation of a b-cyclodextrin hydrolysate in less than 10 minutes. The labels indicate the number of base sugars such as glucose in each oligomer.
- Peptides are difficult to solubilize in organic mobile phases. Thus, the use of HILIC in biological applications has been limited. By using an aqueous mobile phase with gradient elution it is feasible to separate peptides on the TSKgel Amide-80 column.

No Separation on RPC: HILIC

- TSKgel Amide-80 is becoming a valuable tool for the analysis of small polar molecule drug candidates that cannot be retained by reverse phase LC columns.

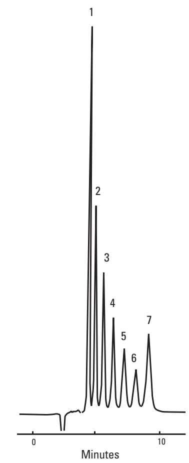
Separation of polyalcohols on TSKgel Amide-80 column



Column: TSKgel Amide-80, 4.6 mm ID x 25 cm L
Sample: A. Ethylene Glycol, 10 µM, 20 µL
B. Glycerine, 10 µM, 20 µL
C. Erythritol, 10 µM, 20 µL
D. Xylitol, 10 µM, 20 µL
E. Mannitol, 10 µM, 20 µL
F. Inositol, 4 µM, 20 µL
Elution: Acetonitrile/H_2O (75/25)
Flow Rate: 1.0 mL/min
Detection: refractive index detector
Temperature: 25°C

Figure 23

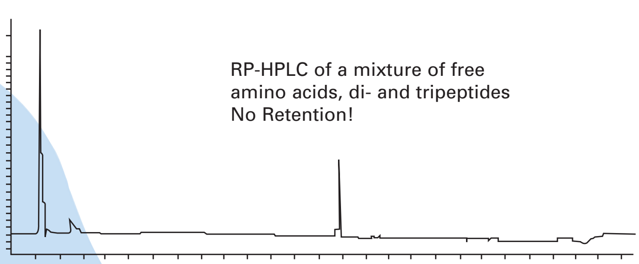
Separation of beta-cyclodextrin on TSKgel Amide-80 column



Column: TSKgel Amide-80, 4.6 mm ID x 25 cm L
Sample: Beta-Cyclodextrin Hydrolysate, 1-7 degrees of polymerization, 4.6 mg/mL, 2 µL
Elution: acetonitrile/water (55/45)
Flow Rate: 1.0 mL/min
Detection: refractive index detector
Temperature: 25°C

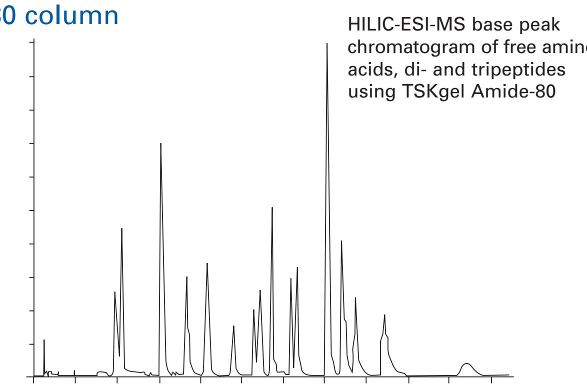
Figure 24

LC-MS Analysis of hydrophilic peptides using TSKgel Amide-80 column



RP-HPLC of a mixture of free amino acids, di- tripeptides

Columns: Grom Sil 120 ODS 4 HE
(3 µm; 4 mm ID x 1 cm L + 4 mm ID x 25 cm L)
Eluent A: 10 mmol/L Ammonium Acetate (pH 6.0) in MilliQ water
Eluent B: 9 mmol/L Ammonium Acetate (pH 6.0) in 60% methanol
Sample: Mix of free amino acids (P,T,S,Q,E,L) and peptides
(ET, TE, SE, EE, KE, EK, KG, EEE, GEG, WGY)
Flow Rate: 0.8 ml/min
Gradient: 0-7 min 0% B
7-30 min 0-70% B
30-35 min 70-100% B
35-43 min 100% B
43-45 min 100-0% B
Temp. 50°C



HILIC-ESI-MS base peak chromatogram of free amino acids, di- and tripeptides

Columns: TSKgel Amide-80, 5 µm; 2 x10 cm + 1.5 x 25 cm (ID x L)
Eluent A: 6.5 mM Ammonium Acetate (pH 5.5) in 90% ACN
Eluent B: 7.2 mM Ammonium Acetate (pH 5.5) in 60% ACN
Sample: Mix of free amino acids (P,T,S,Q,E,L) and peptides
(ET, TE, SE, EE, KE, EK, KG, EEE, GEG, WGY)
Flow Rate: 0.1 ml/min
Gradient: 0-90min 10-100% B
90-95 min 100% B
95-98min 100-10% B
98-115min 10% B

Figure 25 : Comparison of hydrophilic peptides separated on a RPC column and a TSKgel Amide-80 column (HILIC-ESI-MS)
(Ref.: Data reprinted with permission of H. Schlichtherle-Cerny and M. Affolter; Nestlé Research Center Lausanne (2001)

What else do you need?

5. What else?

Use TSKgel guardcolumns to protect your TSK-GEL column and enhance life time.

GUARDING YOUR COLUMN

Good laboratory and manufacturing procedures demand that the separation column be protected by a guard column. Tosoh Bioscience supplies an assortment of packed guard columns, TSK-GEL guardgel kits and guard cartridges. The kits contain the hardware and enough gel packing material to pack and refill several guard columns. Different sizes and types of guardgel kits are available for 4.6 mm, 6.0 mm and 7.8 mm ID stainless steel columns, 5 mm and 8 mm ID glass columns, and semi-preparative 21.5 mm ID stainless steel columns. Packed guard columns are offered for protecting 55 mm ID preparative columns.

How to set the cartridge guardgel

For 3.2 mm ID x 1.5 cm L : Put the guardgel in the inlet holder. SUS filter side is for the inlet and Teflon side for the outlet. Tighten up the holder by hand.

For 2.0 mm ID X 3.2 cm L: Put the guardgel in the holder. Harmonize the direction on the guradgel and the holder. Tighten up the holder by hand.

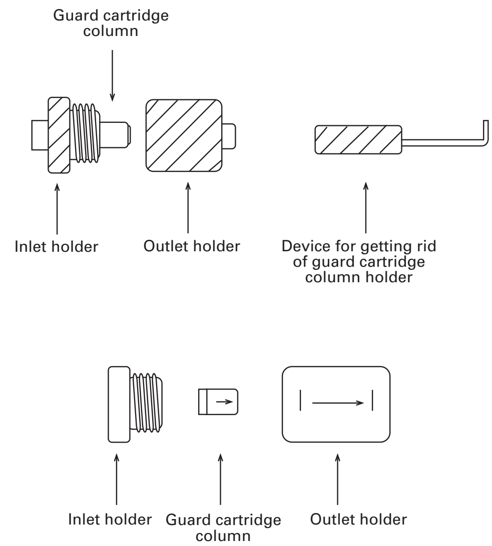


Figure 26

For both guardgels, the incorrect direction setting might cause the insufficient column performance due to the dead volume.

Before connecting to the analytical column, please flow at least 1 mL of the solvent to the guardgel to replace the solvent inside.

In case of 3.2 mm ID X 1.5 cm L, please use the replacement jig for exchange. Hook the jig to the groove on the guardgel and pull.

Trouble Shooting for Reversed-Phase Chromatography

Incident	Cause and Trouble Shooting
I. Unstable baseline	• Caused by organic impurities in water, reagent to produce ghost peak (a) Use ultra pure distilated water, or HPLC grade distilated water, (b) Pass and filtrate eluent on ODS column to remove organic impurities prior to use. • Inadequate degassing
(Appearance of ghost peak)	• Deterioration of TFA in eluent (a) Prepare eluent with TFA just before analysis, and prepare freshly every day.
II. Sample is not retained well on high carbon content ODS column.	• Stationary phase (ODS) does not interact effectively in eluent with low concentration organic modifier less than 50 % (water-rich eluent). (a) Use mono-layer, relatively low carbon content (ca. 15%) ODS. (b) Use hydrophilic interaction chromatography instead.
III. Ionic sample is eluted without reproducibility.	• Eluent pH is close to pKa point of ionic sample. (a) Use pH of eluent more than 1.5 point apart from the pKa of the sample.
IV. Sample is not retained well. (elutes as two peaks which is retained but broad).	• Sample is dissolved with organic modifier to accelerate elution. (a) Dissolve sample with initial buffer or at least the same concentration of organic modifier which sample elutes from column.
V. Peptide sample is not retained well.	• Hydrophobicity of column (matrix) is too weak. (a) Use ODS silica rather than resin-based RPC column. (b) Increase ion-pair reagent like TFA (upto 0.2 %)
(Elution of eluted earlier peaks change.)	• Inadequate re-equilibration of eluent. (a) Equilibration by initial eluent without organic modifier requires at least one hour to obtain reproducible chromatogram even on an analytical column.

For more detailed information, please conact our technical specialists.

Summary of TSK-GEL RPC Columns

Parameters influencing the properties of RPC-Phases

Base Material (Matrix):
- physical and chemical Character (silica or polymer)
- pretreatment, contamination

Method of Modification:
- What is the functional group ?
- monomericor polymeric bonding of functional groups
- carbon content (depending on coverage, distribution and length of functional groups)
- endcapping

Reversed Phase Chromatography is used in research and quality control for the analysis and purification of biopolymers and small molecular weight compounds in the pharmaceutical, biotech and other industries. Tosoh Bioscience offers a broad line of analytical and preparative TSK-GEL columns for the RPC analysis of small molecular weight drugs, amino acids, carbohydrates, lipids, nucleic acid fragments, peptides and proteins. For high-speed separations we recommend the porous, silica-based TSK-GEL SuperSeries and the nonporous, polymeric NPR columns. For greater stability at high pH or to benefit from alternative selectivities, we recommend polymer-based TSK-GEL columns. Tosoh Corporation employs state-of-the-art manufacturing techniques that result in uniformly bonded packing materials with narrow pore size distributions and well-defined particle sizes to ensure high performance at high speed. TSK-GEL reversed phase columns enable the chromatographer to solve the most complex separation problems. The following table gives a quick overview of some of the features and benefits of silica- and polymer-based TSK-GEL RPC columns.

Features and Benefits

Silica-based	Polymer-based
Pure silica in 100V/Z and SuperSeries columns minimize peak tailing and adsorption of basic drugs and chelating agents	Hydrophilic polymeric backbone virtually eliminates secondary interactions
Improved lot-to-lot reproducibility minimizes system downtime	Vary pH from 2-12 to modify retention and selectivity
High recoveries of biomolecules	Columns are compatible with common solvents used in RPC
	Columns are chemically and physically stable

Table 6

COLUMN SELECTION

The majority of the analytical separations performed today are based on RPC columns. Applications range from aqueous to non-aqueous samples, neutral or charged compounds, and molecular weights that vary from less than 100 to 1 million Da. The table on the next page indicates some of the boundaries within which RPC columns (and some polar bonded phase columns) are commonly used.

NOMENCLATURE

The nomenclature for TSK-GEL RPC columns is based either on the characteristics of the individual packing or on the application for which the product was designed. See the individual sections for each product for a complete description. Here are some examples:

Particle Design
- Tosoh Corporation manufactures spherical packing materials for HPLC and Process Chromatography.
- Tosoh produces silica as well as polymer-based (resin) particles from as small as 2 µm to as large as 200 µm.
- Tosoh controls pore size to produce porous silica, pellicular silica, as well as porous and nonporous polymeric resins.

Bonded Phase Chemistry
- ODS stands for octadecylsilyl groups attached to silica particles.
- OD refers to an octadecyl carbon chain attached to a polymer-based resin.
- TMS indicates a primary bonding reaction with trimethylsilyl groups.
- Phenyl, Octyl, CN indicate stationary phases containing phenyl, octyl, or cyano functional groups.

Endcapping

Specialty Phases
- OligoDNA RP is designed for reversed phase analysis of oligonucleotide fragments.
- The “Super” series of columns, based on spherical 2 µm pellicular-type particles, are designed for fast analysis or high throughput screening.
- NPR columns are packed with 2.5 µm nonporous resin (NPR) particles for the high speed analysis of biopolymers.

Properties of TSK-GEL Columns

Properties of silica-based TSK-GEL RPC columns

TSK-GEL	Bonding Phase	Funct. Group	End Capping	Particle Size (µm)	Carbon Load	Pore Size (Å)	Excl. Limit (Da)	Application/ Features
ODS-100V	monomeric	C_{18}	Complete	5	15%	100	8,000	Higher surface polarity, compatible to 100% aqueous eluents, higher retention of polar compounds
ODS-100Z	monomeric	C_{18}	Complete	5	20%	100	8,000	Higher hydrophobicity and higher Optimized retention of middle & hydrophobic compounds
Super-ODS	polymeric	C_{18}	Complete	2	8%	110	20,000	High-throughput analyses of hydrophilic or hydrophobic peptides, tryptic digests/peptide mapping, low MW pharmaceuticals, purines and pyrimidines, nucleosides, nucleotides
Super-Octyl	polymeric	C_8	Complete	2	5%	110	20,000	
Super-Phenyl	polymeric	C_6H_5	Complete	2	3%	110	20,000	
OligoDNA RP	monomeric	C_{18}	None	5	10%	250	165,000	Specialty column for analysis and preparative purification of oligonucleotides, RNA and DNA-fragments
TMS-250	monomeric	C_1	Complete	10	5%	250	200,000	Specialty columns for protein separations

Properties of polymer-based TSK-GEL RPC columns

TSK-GEL	Bonding Phase	Funct. Group	Particle Size (µm)	Pore Size (Å)	Excl. Limit (Da)	Application/ Features
Octadecyl-2PW	monomeric	C_{18}	5	125	8,000	Low MW peptides and pharmaceuticals unstable at low pH
Octadecyl-4PW	monomeric	C_{18}	7	500	200,000	Medium and high MW peptides and proteins especially if unstable at low pH
Phenyl-5PW RP	monomeric	C_6H_5	10	1000	1,000,000	High MW peptides and proteins. Phenyl group modifies selectivity
Octadecyl-NPR	monomeric	C_{18}	2.5	Nonporous	>1,000,000	Rapid separation of high MW peptides and proteins

Properties of TSK-GEL Amide-80 HILIC columns

TSK-GEL	Bonding Phase	Funct. Group	Particle Size (µm)	Pore Size (Å)	Excl. Limit (Da)	Application/ Features
Amide-80	n.a.	Carba-moyl	5, 10	80	6,000	Peptides, small compounds

Table 7

Ordering Information

Part #	Description	ID (mm)	Length (cm)	Particle Size (µm)	Min. Number Theoretical Plates	Flow Rate (mL/min) Range	Max.	Maximum Pressure Drop (kg/cm2)
21813	ODS-100V, 100 Å	2.0	3.5	3	4,000	0.15 – 0.18	0.22	150
21812	ODS-100V, 100 Å	2.0	5.0	3	5,700	0.15 – 0.18	0.22	150
21811	ODS-100V, 100 Å	2.0	7.5	3	8,600	0.15 – 0.18	0.22	200
21810	ODS-100V, 100 Å	2.0	15.0	3	17,500	0.15 – 0.18	0.22	250
21814	ODS-100V, 100 Å, (3/pkg)	2.0	1.0	3	500	0.15 – 0.18	0.22	150
21457	ODS-100V, 100 Å	2.0	5.0	5	3,300	0.15 – 0.18	0.22	50
21458	ODS-100V, 100 Å	2.0	15.0	5	11,000	0.15 – 0.18	0.22	150
21455	ODS-100V, 100 Å	4.6	15.0	5	14,000	0.7 – 1.0	1.2	150
21456	ODS-100V, 100 Å	4.6	25.0	5	23,000	0.7 – 1.0	1.2	200
21460	ODS-100Z, 100 Å	2.0	5.0	5	3,300	0.15 – 0.18	0.22	50
21459	ODS-100Z, 100 Å	2.0	15.0	5	11,000	0.15 – 0.18	0.22	150
21461	ODS-100Z, 100 Å	4.6	15.0	5	14,000	0.7 – 1.0	1.2	150
21462	ODS-100Z, 100 Å	4.6	25.0	5	23,000	0.7 – 1.0	1.2	200
20015	Super-ODS, 110 Å	1.0	5.0	2	1,500	0.03 – 0.05	0.06	150
20016	Super-ODS, 110 Å	1.0	10.0	2	1,500	0.03 – 0.05	0.06	300
19541	Super-ODS, 110 Å	2.0	5.0	2	6,000	0.15 – 0.2	0.25	250
19542	Super-ODS, 110 Å	2.0	10.0	2	12,000	0.15 – 0.2	0.25	250
18154	Super-ODS, 110 Å	4.6	5.0	2	8,000	1.5 – 2.5	4.0	300
18197	Super-ODS, 110 Å	4.6	10.0	2	16,000	1.5 – 2.5	4.0	300
20013	Super-Octyl, 110 Å	2.0	5.0	2	8,000	1.0 – 2.5	4.0	300
20014	Super-Octyl, 110 Å	2.0	10.0	2	8,000	1.0 – 2.5	4.0	300
18275	Super-Octyl, 110 Å	4.6	5.0	2	8,000	1.0 – 2.5	4.0	300
18276	Super-Octyl, 110 Å	4.6	10.0	2	16,000	1.0 – 2.5	4.0	300
20017	Super-Phenyl, 110 Å	2.0	5.0	2	8,000	1.0 – 2.5	4.0	300
20018	Super-Phenyl, 110 Å	2.0	10.0	2	8,000	1.0 – 2.5	4.0	300
18277	Super-Phenyl, 110 Å	4.6	5.0	2	8,000	1.0 – 2.5	4.0	300
18278	Super-Phenyl, 110 Å	4.6	10.0	2	16,000	1.0 – 2.5	4.0	300
13352	OligoDNA RP, 250 Å	4.6	15.0	5	7,000	0.6 – 1.0	1.2	120
13353	OligoDNA RP, 250 Å	7.8	15.0	5	7,000	2.0 – 3.0	3.5	120
07190	TMS-250, 250 Å	4.6	7.5	10	1,500	0.5 – 0.8	1.0	20
14005	Octadecyl-NPR nonporous	4.6	3.5	2.5	1,000	1.0 – 1.5	1.6	200
17500	Octadecyl-2PW, 125 Å	4.6	15.0	5.0	6,000	0.4 – 0.6	1.2	100
17501	Octadecyl-2PW, 125 Å	6.0	15.0	5.0	6,000	0.5 – 1.0	1.5	100
18754	Octadecyl-2PW, 125 Å	2.0	15.0	5.0	>5,000	0.07 – 0.11	0.14	70
13351	Octadecyl-4PW, 500 Å	4.6	15.0	7.0	2,000	0.5 – 1.0	1.2	12
16257	Octadecyl-4PW, 500 Å	21.5	15.0	13.0	2,000	3.0 – 6.0	8.0	35
18755	Octadecyl-4PW, 5000 Å	2.0	15.0	5.0	>2,000	0.08 – 0.17	0.22	100
16258	Octadecyl-4PW, 500 Å	55.0	20.0	20.0	1,700	30.0 – 50.0	60.0	5
18756	Phenyl-5PW RP, 1000 Å	2.0	7.5	10.0	>400	0.05 – 0.1	0.12	10
08043	Phenyl-5PW RP, 1000 Å	4.6	7.5	10.0	700	0.5 – 1.0	1.2	30
16260	Phenyl-5PW RP, 1000 Å	21.5	15.0	13.0	1,000	6.0 – 8.0	8.0	30
16261	Phenyl-5PW RP, 1000 Å	55.0	20.0	20.0	—	30.0 – 40.0	—	—
14006	Phenyl-5PW RP Glass, 1000 Å	5.0	5.0	10.0	500	0.5 – 1.0	1.2	30
14007	Phenyl-5PW RP Glass, 1000 Å	8.0	7.5	10.0	700	1.0 – 2.0	2.5	20

pkg = package

Ordering Information

Part #	Description	ID (mm)	Length (cm)	Particle Size (µm)	Min. Number Theoretical Plates	Flow Rate (mL/min) Range	Max.	Maximum Pressure Drop (kg/cm2)
20009	Amide-80, 80 Å	1.0	5.0	5	n/a			
19694	Amide-80, 80 Å	2.0	5.0	5	n/a	0.15 – 0.18	0.22	250
19695	Amide-80, 80 Å	2.0	10.0	5	n/a	0.15 – 0.18	0.22	250
19696	Amide-80, 80 Å	2.0	15.0	5	n/a	0.15 – 0.18	0.22	250
19697	Amide-80, 80 Å	2.0	25.0	5	n/a	0.15 – 0.18	0.22	250
19532	Amide-80, 80 Å	4.6	5.0	5	2,500	0.8 – 1.0	1.2	150
19533	Amide-80, 80 Å	4.6	10.0	5	4,000	0.8 – 1.0	1.2	150
13071	Amide-80, 80 Å	4.6	25.0	5	8,000	0.8 – 1.0	1.2	150
14459	Amide-80, 80 Å	7.8	30.0	10	5,000	1.0 – 2.0	3.0	70
14460	Amide-80, 80 Å	21.5	30.0	10	5,000	2.0 – 4.0	8.0	30

Guard column products

Part #	Description	ID (mm)	Length (cm)	Particle Size (µm)	
18206	Cartridge holder				For P/N 18207
18207	Guard cartridge	4.0	4.0	2	For 4.6 mm ID and 7.8 mm ID RPC columns
19308	Guard cartridge holder	2.0	1.0		For all 2 mm ID Guard cartridges
14100	Guard cartridge holder	3.2	1.5		For 3.2 mm ID X 1.5 cm L cartridges
19672	Super-ODS Guardcolumn	2.0	1.0	2	For 2 mm ID Super-ODS columns
42159	Phenyl-5PW RP Cartridge, pkg 3	2.0	1.0	5.0	For P/N 18756
14126	Phenyl-5PW RP Cartridge, pkg 3	3.2	1.5		For P/N 08043
14022	Phenyl-5PW RP Guardgel Kit, Glass			20.0	For P/Ns 14006 and 14007
16262	Phenyl-5PW RP Guard column	45.0	5.0		For P/N 16261
42161	Octadecyl-2PW Cartridge	2.0	1.0	5.0	For P/N 18754
17502	Octadecyl-2PW Guard column	4.6	1.0	5.0	For P/N 17500
17503	Octadecyl-2PW Guard column	6.0	1.0	5.0	For P/N 17501
42160	Octadecyl-4PW Cartridge, pkg 3	2.0	1.0	5.0	For P/N 18755
16749	Octadecyl-4PW Prep Guardgel Kit	10.0	2.0	20.0	For P/N 16257
16259	Octadecyl-4PW Prep Guard column	45.0	5.0	20.0	For P/N 16258
19021	Amide-80 Guard column	4.6	1.0	5	For PN 13071 and 14459
14461	Amide-80 Guard column	21.5	7.5	10	For P/N 14460
42140	Amide-80 Guard cartridge, pkg 3	2.0	1.0	5	For all 2 mm ID columns

pkg = package

Table I
Column Selection for TSK-GEL Reversed Phase Columns

Sample Solubility	Sample Type	Example	Column	Comment
Organic Soluble	Lipophilic	Steroids, fat soluble vitamins	CN-80T_S	
		Polyaromatic hydrocarbons	ODS-120A	EPA method 610
Water Soluble				
Low MW	Nonionic	Water soluble vitamins	ODS-80T_S	
		Saccharides, sugars	Amide-80	Reversed Phase Conditions
	Ionic, pH > 2	Sulfonic acids	ODS-80T_M	
		Purines, pyrimidines nucleosides, nucleotides	ODS-80T_S	
	Ionic, pH < 9	Basic drugs	ODS-80T_S	
	Ionic, pH > 9	Pharmaceuticals	Octadecyl-2PW	Polymer based
Medium MW	Oligomers	Oligosaccharides	ODS-80T_S	Aqueous mobile phase
		Peptides	ODS-80T_M Octadecyl-2PW ODS-120T Super-ODS/Octyl/Phenyl Amide-80	100 – 6,000Da 100 – 8,000Da 100 – 10,000Da 100 – 20,000Da Normal phase conditions
High MW		Proteins	Octadecyl-NPR TMS-250 Octadecyl-4PW Phenyl-5PW RP	1,000 – 1,000,000Da 100 – 200,000Da 1,000 – 200,000Da 10,000 – 1,000,000Da
		Oligonucleotides	OligoDNA RP	Up to 165,000Da

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