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 67 displays the structure. The 12.5 nm pores allow for analysis of peptides up to 8,000 Da.

Table 22: Product attributes

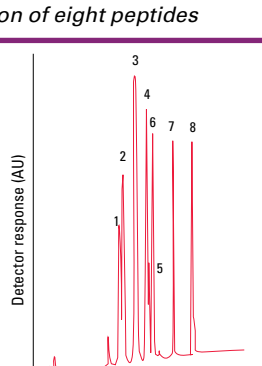
Attribute	Value
Pore size (mean)	12.5 nm
Exclusion limit	8,000 Da
Particle size (mean)	5 µm
pH stability	2.0-12.0
Functional group	C18 (monomeric bonding chemistry)

Figure 67: TSKgel Octadecyl-2PW structure

Neuropeptides

The rapid separation of a mixture of eight peptides using a TSKgel Octadecyl-2PW column is shown in Figure 68. 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 68: Separation of eight peptides

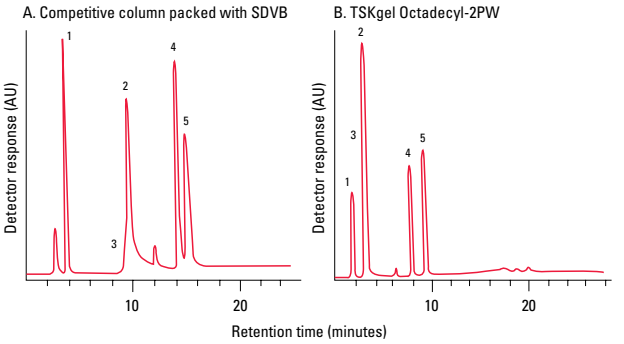


Column:	**TSKgel Octadecyl-2PW, 5 µm, 4.6 mm ID × 15 cm**
Mobile phase:	30 min linear gradient from 0.1% TFA/CH_3CN from 90/10 to 30/70
Flow rate:	1.0 mL/min
Detection:	UV @ 215 nm
Temperature:	ambient
Samples:	1. met-enkephalin 2. bradykinin 3. leu-enkephalin 4. neurotensin 5. bombesin 6. angiotensin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

Common Drugs

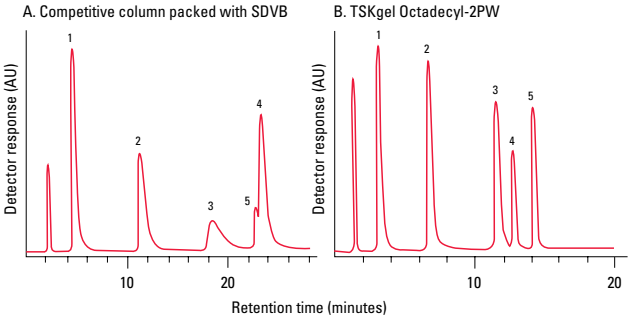
The polymeric backbone of TSKgel Octadecyl-2PW gives this column better pH stability than silica-based columns so the separations can be optimized over a wider pH range, as shown in Figure 69. A pH of 7.0 gives excellent resolution of a mixture of common drugs on the TSKgel Octadecyl-2PW column, while they tail or are unresolved on a competitive PSDVB column.

Figure 69: Comparison over a wide pH range



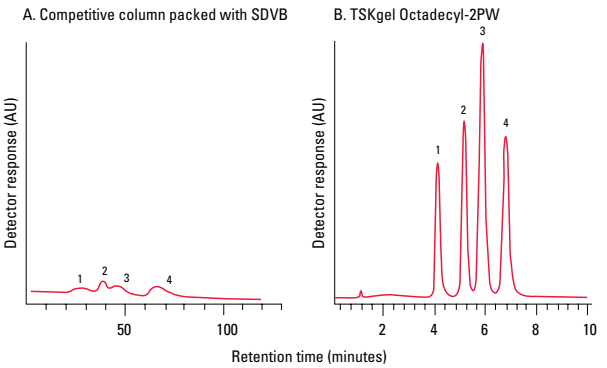
1. pH 2.5

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



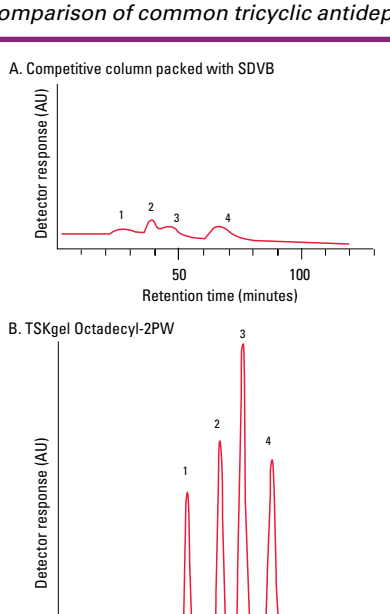
3. pH 11.0

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 70 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 70: 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.

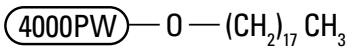
Attributes and Applications

Table 23 lists the attributes of TSKgel Octadecyl-4PW columns, while Figure 71 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)

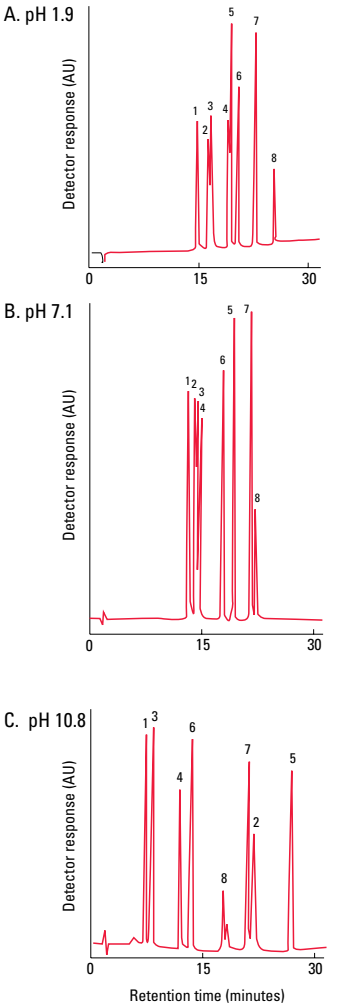
Figure 71: TSKgel Octadecyl-4PW structure



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 72. 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 72: 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 73 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

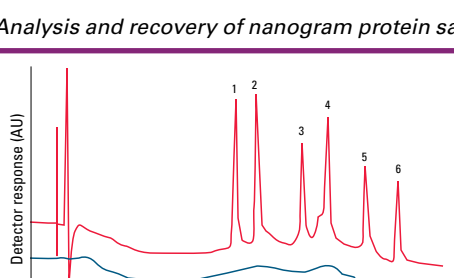
Attribute	Value
Pore size (mean)	nonporous
Exclusion limit	>1.0 × 10^6 Da
Estimated ligand density	1 eq/L
Particle size (mean)	2.5 µm
pH stability	2.0-12.0
Functional group	C18 (monomeric bonding chemistry)

Figure 73: 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 74. 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 74: Analysis and recovery of nanogram protein samples

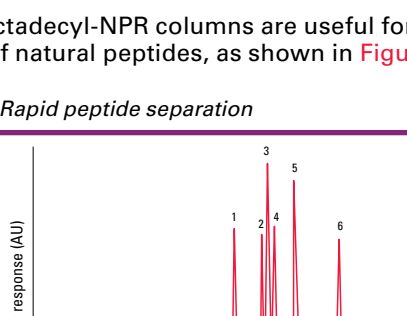


Column:	**TSKgel Octadecyl-NPR, 2.5 µm, 4.6 mm ID × 3.5 cm**
Mobile phase:	10 min linear gradient from 15% to 80% CH_3CN in 5 mmol/L $HClO_4$
Flow rate:	1.5 mL/min
Detection:	UV @ 220 nm
Samples:	50 ng each of 1. ribonuclease A 2. insulin 3. cytochrome C 4. lysozyme 5. transferrin 6. myoglobin
Note: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

Natural Peptides

TSKgel Octadecyl-NPR columns are useful for the rapid analysis of natural peptides, as shown in Figure 75.

Figure 75: Rapid peptide separation

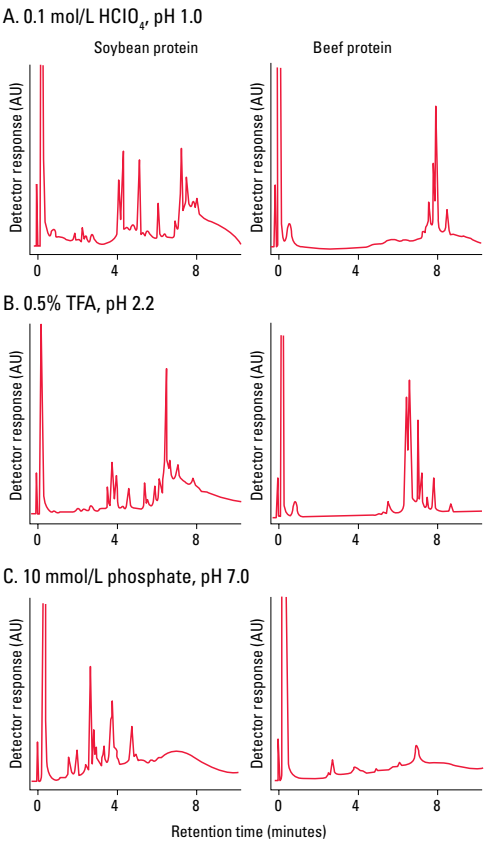


Column:	**TSKgel Octadecyl-NPR, 2.5 µm, 4.6 mm ID × 3.5 cm**
Mobile phase:	10 min linear gradient from 0% to 80% CH_3CN in 0.2% TFA
Flow rate:	1.5 mL/min
Detection:	UV @ 220 nm
Samples:	1. α-endorphin 2. bombasin 3. γ-endorphin 4. angiotensin 5. somatosatin 6. calcitonin

Method Development

Method development is expedient with TSKgel Octadecyl-NPR columns. In Figure 76, two protein extracts were analyzed under three different elution conditions in a relatively short time.

Figure 76: Rapid method development

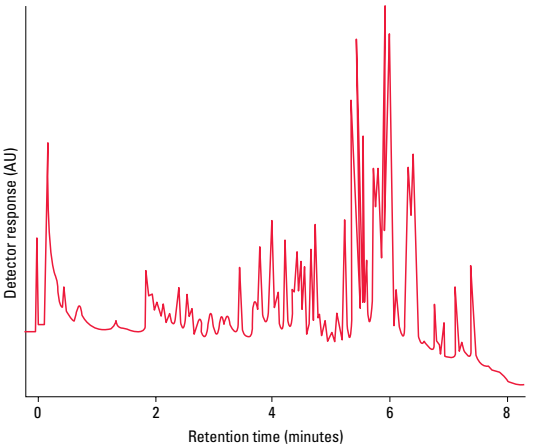


Column:	**TSKgel Octadecyl-NPR, 2.5 µm, 4.6 mm ID × 3.5 cm**
Mobile phase:	A. 10 min linear gradient from 0% to 80% CH_3CN in 0.1 mol/L $HClO_4$ B. 10 min linear gradient from 0% to 80% CH_3CN in 0.05% TFA, pH 2.2 C. 10 min linear gradient from 0% to CH_3CN in 10 mmol/L phosphate buffer to 80% CH_3CN 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 77. 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 77: 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.

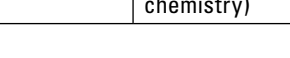
Attributes and Applications

Table 25 lists the attributes of TSKgel Phenyl-5PW RP columns, while Figure 78 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)

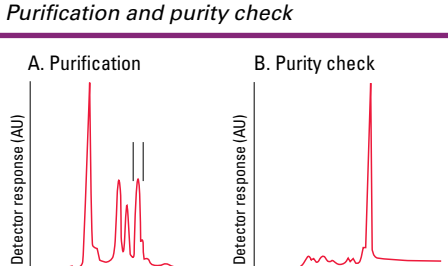
Figure 78: TSKgel Phenyl-5PW RP structure



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 79.

Figure 79: Purification and purity check

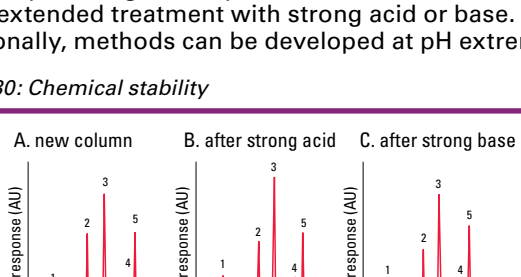


Column:	**TSKgel Phenyl-5PW RP, 10 µm, 4.6 mm ID × 7.5 cm**
Mobile phase:	2 min linear gradient from 5% to 20% CH_3CN in 0.05% TFA, followed by (A - 48 min/B - 32 min) linear gradient to (80%A/60%B) CH_3CN in 0.05% TFA
Flow rate:	1.0 mL/min
Detection:	UV @ 220 nm
Sample:	lactate dehydrogenase A. 40 µg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

Chemical Stability

The chromatograms in Figure 80 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 80: 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