



TSKgel REVERSED PHASE COLUMNS

APC
REVERSED
PHASE
CHROMATO
GRAPHY

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➤ **TOSOH HISTORY**

- 1935 FOUNDING OF TOYO SODA MANUFACTURING CO., LTD.
- 1936 OPERATION OF NANYO MANUFACTURING COMPLEX BEGINS
- 1971 SCIENTIFIC INSTRUMENTS DIVISION FORMED, FIRST GPC COLUMN USING TSKgel DEVELOPED BY TOSOH
- 1974 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY COLUMN PLANT IS COMPLETED
- 1979 TOSOH DEVELOPS TOYOPEARL MEDIA
- 1983 TOSOH DEVELOPS HYDROPHOBIC INTERACTION MEDIA
- 1987 TOSOHAS US OPERATIONS FORMED IN MONTGOMERYVILLE
- 1989 TOSOHAS GMBH OPERATIONS FORMED IN STUTTGART
- 1995 TOSOH NANYO GEL FACILITY RECEIVES ISO 9001
- 2000 IN NOVEMBER FORMER TOSOHAS US OPERATIONS BECOMES TOSOH BIOSEP LLC, A 100% SUBSIDIARY OF TOSOH CORPORATION
- 2001 IN JANUARY FORMER TOSOHAS GMBH EUROPEAN OPERATIONS BECOMES TOSOH BIOSEP GMBH, A 100% SUBSIDIARY OF TOSOH CORPORATION
- 2002/ TOSOH CORPORATION ANNOUNCES THAT ALL TOSOH AFFILIATED SCIENTIFIC AND DIAGNOSTIC SYSTEM
- 2003 RELATED COMPANIES IN EUROPE, WILL BE UNIFIED UNDER THE NEW NAME TOSOH BIOSCIENCE.
- 2008 ECOSEC , THE 7TH GENERATION GPC SYSTEM IS INTRODUCED GLOBALLY
- 2009 TOSOH BIOSCIENCE GMBH CELEBRATES ITS 20TH ANNIVERSARY IN STUTTGART
- 2010 TOSOH CELEBRATES ITS 75TH YEAR IN BUSINESS WITH THE OPENING OF FIVE NEW PLANTS, AND CONTINUED RAPID EXPANSION IN CHINA
- 2011 AFTER DEVELOPING THE FIRST TSKgel GPC COLUMN IN 1971, TOSOH NOW LOOKS BACK ON 40 YEARS EXPERIENCE IN SUCCESSFUL TECHNOLOGY IN SIZE EXCLUSION AND GEL PERMEATION CHROMATOGRAPHY

RPC REVERSED PHASE CHROMATOGRAPHY



Reversed Phase (RP) Chromatography is one of the most frequently used chromatographic modes for analytical separations. Starting in the mid 1970s RPC has become the standard technique to analyze small molecular weight compounds in industrial, academic and governmental laboratories. Applications range from neutral polar and non-polar solutes to acidic, basic, and amphoteric compounds and from small molecular weight compounds to biomolecules. 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.

Tosoh Bioscience offers analytical and semi preparative reversed phase (RP) HPLC columns packed with silica or polymer based porous or nonporous beads. They are well suited for a broad range of applications in R&D, quality control or reaction monitoring.

TSKgel ODS-80, ODS-100 and ODS-120 silica based RPC columns offer high resolution power for various applications. For high-speed separations we recommend the porous, silica-based TSKgel Super and ODS-140HTP series or the nonporous, polymeric NPR columns. For better stability at high pH, or to benefit from alternative selectivity and a large pore size, we recommend polymer-based TSKgel columns. For very polar solutes, which are difficult to retain in RP mode, we offer a selection of silica based HILIC columns, which are described in a separate brochure.

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. TSKgel reversed phase columns enable the chromatographer to solve the most complex separation problems.





RPC HOW IT WORKS

Reversed phase (RP) chromatography retains molecules based on their hydrophobic character on a non-polar stationary phase. In an aqueous, moderately polar solvent the hydrophobic patches of the analyte molecule bind to an immobilized hydrophobic ligand. A mobile phase of increasing hydrophobicity is used to release the bound molecule at a point at which the interaction between the exposed patches and the matrix is less favorable than the interaction between the molecule and the solvent. The molecule releases from the matrix and elutes.

Retention can be decreased by adding less polar solvents (methanol, acetonitrile) to the mobile phase. Elution can be performed either in isocratic or gradient mode. Isocratic elution is easy to realize, less expensive and allows solvent recycling. Gradient elution – the continuous reduction of polarity of the aqueous mobile phase by increasing percentage of organic solvent - delivers sharper peaks and faster separation.

The binding of the analyte to the stationary phase is proportional to its hydrophobic surface area. Structural properties of the analyte therefore play an important role for reversed phase retention.

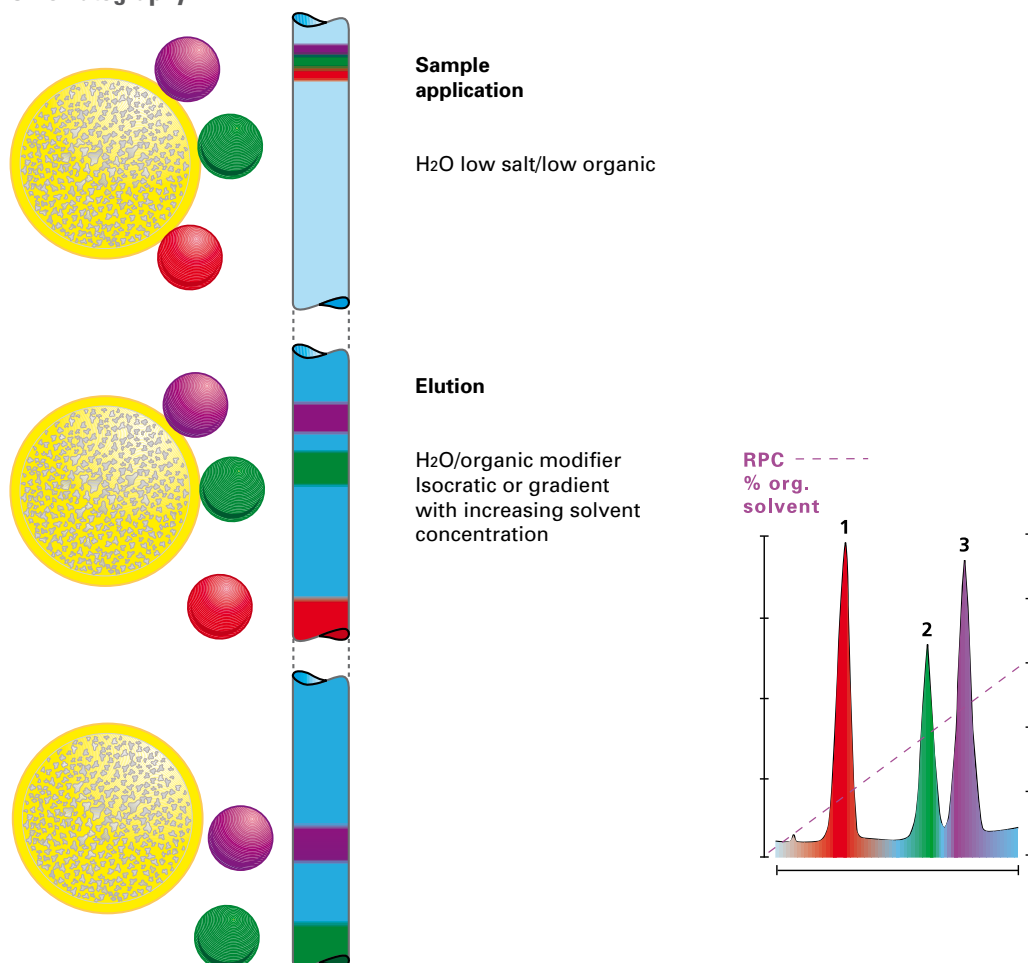
Large hydrophobic surface areas increase retention whereas polar groups reduce retention. Branched chain compounds elute more rapidly than their corresponding linear isomers because the overall surface area is decreased. Very large molecules can show incomplete interaction between the large analyte surface and the alkyl chains of the stationary phase and might have problems entering the pores of the stationary phase.

RP separation of peptides and proteins is usually performed by adding the volatile ionic modifier trifluoroacetic acid (TFA) to the mobile phase for ion pairing. Addition of TFA overcomes peak broadening and asymmetry (tailing) that are believed to result from interactions of peptides and proteins having a variety of polar, ionic, and hydrophobic sites with residual polar silica surfaces. For RP LC/MS analysis formic acid or ammonium formate are the most common modifiers.

For polar compounds, which are not retained on standard RP columns, HILIC could be the solution. A separate HILIC brochure is covering the features of TSKgel HILIC columns.

➤ **FIGURE 1**

Reversed Phase Chromatography



RPC

STATIONARY PHASES



Silica Based Stationary Phases

Silica particles functionalized with straight chain alkyl groups such as C18, C8 or C4 or with aromatic groups such as phenyl are the most widely used RP stationary phases. Their high mechanical stability, monodisperse particles, high surface area, and easily tailored pore size distributions are the advantages of silica phases.

Silica bonding chemistry also allows for a variety of stationary phases with different selectivities. Surface functionalization of silica can be performed in a monomeric or a polymeric reaction. Nowadays, residual silanol groups of the matrix are usually 'endcapped' with covalently-bound small organic silanes.

Polymer Based Stationary Phases

Polymer based RP columns are compatible with a wide range of mobile phase conditions. Since these columns are chemically stable from pH 2-12, they allow robust, reproducible operation at basic pH where silica-based columns have limited chemical stability.

Reversed Phase Selectivity

Subtle differences in the surface chemistries of different RP stationary phases lead to changes in selectivity. A broad range of RP phases is available to meet specific separation needs. TABLE 1 gives a quick overview of some of the features and benefits of silica- and polymer-based TSKgel RPC columns.

➤ TABLE 1

Properties of Silica- and Polymer based TSKgel RPC Columns

TSKgel SILICA	Bonding phase	Functional group	Endcapping	Particle size (µm)	Carbon load	Pore size (Å)	Excl. limit (kDa)	Application/ Features
ODS-100V	monomeric	C18	complete	5	15%	100	8	Higher surface polarity, compatible to 100% aqueous eluents, higher retention of polar compounds
ODS-100Z	monomeric	C18	complete	5	20%	100	8	More hydrophobic than ODS-100V; stronger retention and higher selectivity for non-polar compounds; higher steric selectivity
ODS-140HTP	polymeric	C18	complete	2.3	8%	140	20	High-throughput analyses of hydrophilic or hydrophobic peptides, tryptic digests/ peptide mapping, low MW pharmaceuticals, purines and pyrimidines, nucleosides, nucleotides
Super-ODS	polymeric	C18	complete	2.3	8%	110	20	
Super-Octyl	polymeric	C8	complete	2.3	5%	110	20	
Super-Phenyl	polymeric	C6H5	complete	2.3	3%	110	20	
OligoDNA RP	monomeric	C18	none	5	12%	250	165	Specialty column for analysis and preparative purification of oligonucleotides, RNA and DNA-fragments
TMS-250	monomeric	C1	complete	10	5%	250	200	Specialty column for protein separations
POLYMER								
Octadecyl-2PW	monomeric	C18	-	5	-	125	8	Low MW peptides and pharmaceuticals unstable at low pH
Octadecyl-4PW	monomeric	C18	-	7	-	500	200	Medium and high MW peptides and proteins especially if unstable at low pH
Phenyl-5PW RP	monomeric	C6H5	-	10	-	1,000	1,000	High MW peptides and proteins. Phenyl group modifies selectivity
Octadecyl-NPR	monomeric	C18	-	2.5	-	Nonporous	> 1,000	Rapid separation of high MW peptides and proteins



RPC TSKgel REVERSED PHASE COLUMNS

Tosoh Bioscience offers 15 distinct reversed phase column types, either based on silica or methacrylate particles. For the development of new methods we recommend TSKgel ODS-100 or fast LC columns such as TSKgel ODS-140HTP. Traditional, silica based TSKgel ODS-80 and ODS-120 columns are not described in detail in this brochure.

Particle Design

Tosoh manufactures porous and nonporous spherical packing materials for liquid chromatography covering silica as well as polymer-based particles from as small as 2 μm to as large as 200 μm .

The nomenclature for TSKgel RPC columns is based on the bonded phase characteristics or on the application the column was designed for.

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 with trimethylsilyl groups. Phenyl, Octyl, CN indicate stationary phases containing phenyl, octyl, or cyano groups.

Specialty Phases

OligoDNA RP is designed for analysis of oligo-nucleotides and DNA fragments.

The "Super" and the ODS-140HTP series of small particle size columns are designed for fast analysis and high throughput screening.

NPR columns are packed with 2.5 μm nonporous resin (NPR) suitable for high speed analysis of biopolymers.

TABLE 2
Column Selection for TSKgel Reversed Phase Columns

Sample Solubility	Sample type	Example	Column	Comment	
Organic Soluble	Lipophilic	Steroids, fat soluble vitamins	CN-80TS, ODS-100V		
		Polyaromatic hydrocarbons	ODS-120A	EPA method 610	
Water Soluble	Low MW	Nonionic	Water soluble vitamins	ODS-80TS, ODS-100V/Z	
		Ionic, pH > 2	Sulfonic acids	ODS-80TM, ODS-100V	
	Ionic, pH < 9	Purines, pyrimidines nucleosides, nucleotides	ODS-80TS, ODS-100V		
		Basic drugs	ODS-80TS, ODS-100V		
		Pharmaceuticals	Octadecyl-2PW	Polymer based	
	Medium MW	Oligomers	Oligosaccharides	ODS-80TS, ODS-100V	Aqueous mobile phase
			Peptides	ODS-80TM, ODS-100V	100 – 6,000 Da
				Octadecyl-2PW	100 – 8,000 Da
ODS-120T				100 – 10,000 Da	
High MW		Proteins	Super-ODS/Octyl/Phenyl, ODS-140HTP	100 – 20,000 Da	
			Octadecyl-NPR	1,000 – 1,000,000 Da	
			TMS-250	100 – 200,000 Da	
			Octadecyl-4PW	1,000 – 200,000 Da	
			Phenyl-5PW RP	10,000 – 1,000,000 Da	
		Oligonucleotides	OligoDNA RP	Up to 165,000 Da	

RPC TSKgel ODS-100 UNIVERSAL RP COLUMNS



TSKgel ODS-100V and TSKgel ODS-100Z columns incorporate best-in-class surface properties to limit secondary interactions of basic, acidic and chelating compounds. They offer high efficiency and symmetrical peak shapes. The ultra high purity Type B base silica combined with monomeric bonding chemistry makes the best general purpose RP columns. TSKgel ODS-100 columns are suitable for demanding separations in quality control as well as in R & D.

TSKgel ODS-100V

TSKgel ODS-100V columns are general purpose columns providing strong retention and high selectivity for polar solutes. Based on unique, highly efficient bonding and endcapping procedures, secondary interactions of basic, acidic, and chelating compounds are limited. Monomeric bonded phase chemistry provides complete wetting and retention stability in 100% aqueous mobile phase.

The bonded phase is prepared by an incomplete first reaction with a difunctional octadecylsilane reagent, followed by endcapping with a mixture of two difunctional dialkylsilane reagents (Figure 2).

TSKgel ODS-100Z

TSKgel ODS-100Z RP columns are a great choice when a change of selectivity from TSKgel ODS-100V columns is needed.

They contain a high density monomeric C18 bonded phase (Figure 3) for maximum retention and selectivity of small molecular weight compounds. Exhaustive endcapping prevents secondary interaction with residual silanol groups. The TSKgel ODS-100Z phase is prepared by a first reaction with a difunctional octadecylsilane reagent, followed by repeated endcapping with monofunctional trimethylsilane reagent.

Containing a high carbon content of 20 %, TSKgel ODS-100Z columns exhibit a high stability at low pH. They provide longer retention for non-polar compounds than TSKgel ODS-100V columns. Steric selectivity is higher as well for ODS-100Z.

TSKgel ODS-100 columns are available in 3 μm and 5 μm particle size. 3 μm columns, providing fast and highly efficient separation, are well suited for LC/MS applications.

FIGURE 2 Structure of TSKgel ODS-100V

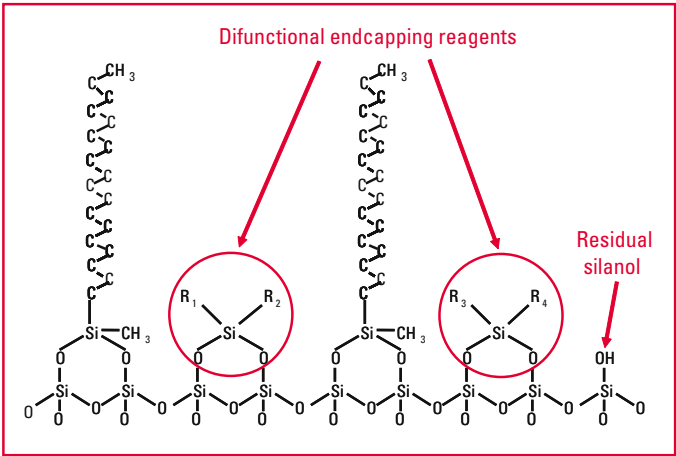
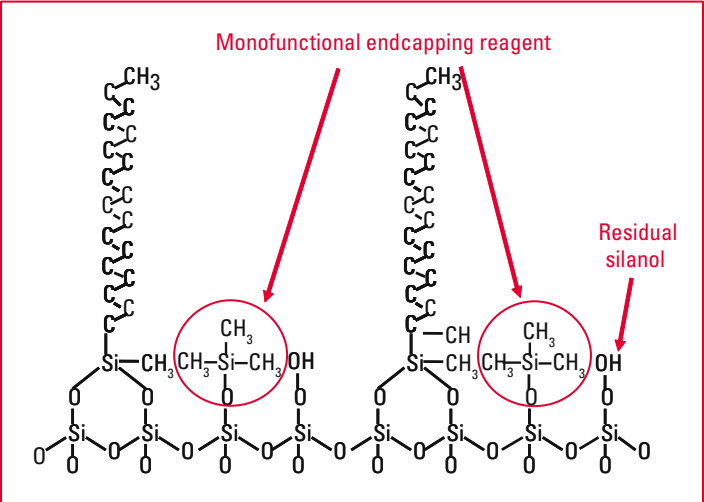


FIGURE 3 Structure of TSKgel ODS-100Z





RPC TSKgel ODS-100 V/Z FEATURES

Bonded Phase Characterization

Standard Reference Material SRM 870 was developed by NIST (National Institute of Standards and Technology) as a means to classify reversed phase columns into closely related groups. Amitriptyline, a tertiary amine, and quinizarin, a strong chelating compound, are in SRM 870, together with other compounds. As shown in **Figure 4**, symmetrical peaks are obtained on both ODS-100 columns for all compounds of SRM 870. Note the good peak shape for quinizarine (peak 4) and for the basic amitriptyline (peak 5).

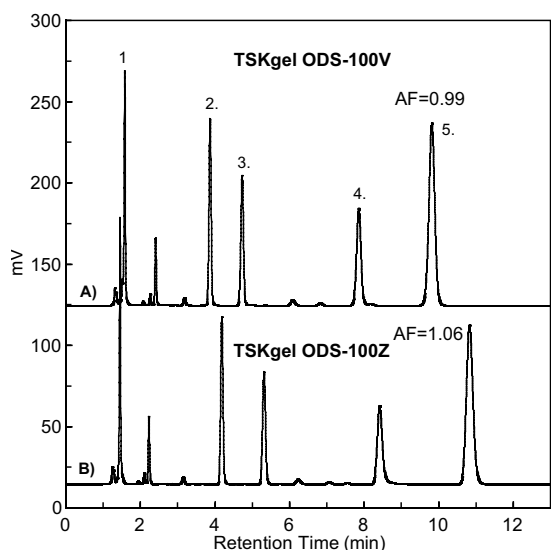
These results indicate the very low amount of non specific interactions of TSKgel ODS-100 columns with chelating compounds and organic bases, respectively.

Lot-to-Lot Reproducibility

Figure 5 shows the separation of 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 minimal lot-to-lot variability and high consistency of the manufactured packing material.

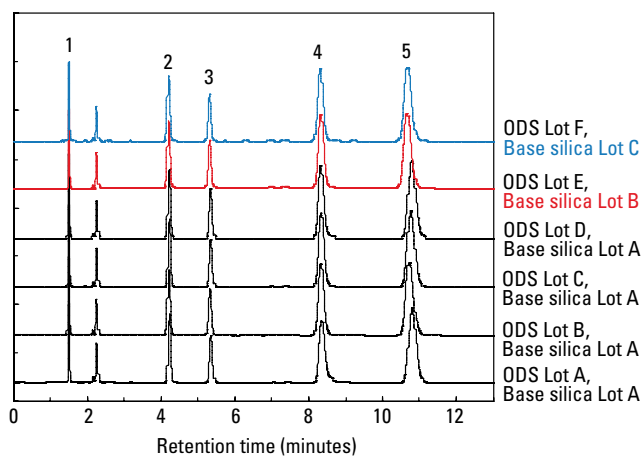
	TSKgel ODS-100V	TSKgel ODS-100Z
Matrix	ultra-pure silica	ultra-pure silica
Particle size	3 µm, 5 µm	3 µm, 5 µm
Pore size	100 Å	100 Å
Specific surface area	450 m ² /g	450 m ² /g
Functional group	C18	C18
Carbon content	15%	20%
Bonding phase	monomeric	monomeric
Endcapping	yes	yes
Sample type	polar	hydrophobic
MW limit	10,000 Da	10,000 Da
pH stability	2 - 7.5	2 - 7.5
Temperature range	10 - 50°C	10 - 50°C
Preferred sample type	Polar, basic, acidic and chelating	Non-polar

FIGURE 4
Separation of SRM 870



Columns: (A) TSKgel ODS-100V 3 µm (4.6 mm ID x 15 cm L)
(B) TSKgel ODS-100Z 3 µm (4.6 mm ID x 15 cm L)
Eluent: 20 mmol/L Phosphate buffer (pH 7.0) /MeOH (20/80)
Flow rate: 1.0 mL/min
Detection: UV @ 254 nm
Temp: 40°C
Inj. volume: 10 µL
Sample: 1. Uracil, 2. Toluene, 3. Ethyl benzene, 4. Quinizarin, 5. Amitriptyline

FIGURE 5
TSKgel ODS-100Z Lot-to-lot Variability



TSKgel ODS-100Z, 5 µm, 4.6 mm ID x 15 cm L

Eluent: 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

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TSKgel ODS-100V APPLICATION DATA

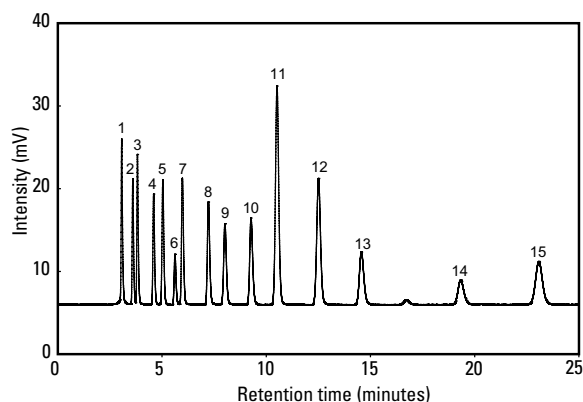


Separation of Organic Acids

Organic acids play an important role in many metabolic processes, fermentation, and food products. TSKgel ODS-100V columns can be operated at low pH conditions in 100% aqueous mobile phases. These conditions are ideal for the separation of a broad range of organic acids. Figure 6 shows a baseline separation (UV@ 210 nm) of 15 organic acids on TSKgel ODS-100V in less than 25 minutes using a simple 0.1% phosphoric acid mobile phase.

▶ FIGURE 6

Separation of Organic Acids



TSKgel ODS-100V, 5 μm, 4.6 mm ID × 25 cm L

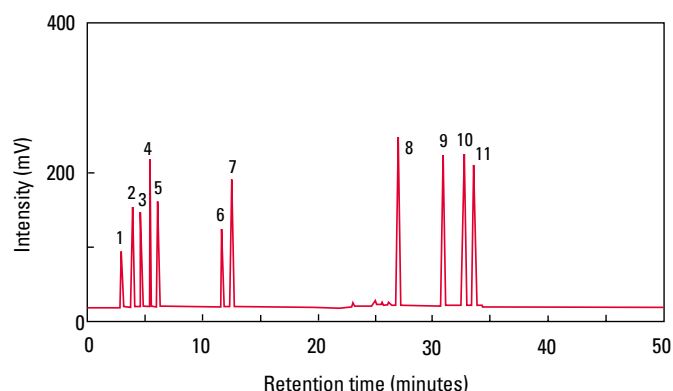
Eluent: 0.1% H₃PO₄, pH 2.3
 Flow rate: 1.0 mL/min
 Temperature: 40°C
 Injection vol.: 10 μL
 Detection: UV @ 210 nm
 Samples:
 1. oxalic acid (0.1 mg/mL)
 2. L-tartaric acid (0.5 mg/mL)
 3. formic acid (1.0 mg/mL)
 4. L-malic acid (1.0 mg/mL)
 5. L-ascorbic acid (0.1 mg/mL)
 6. lactic acid (1.0 mg/mL)
 7. acetic acid (1.0 mg/mL)
 8. maleic acid (0.01 mg/mL)
 9. citric acid (1.0 mg/mL)
 10. succinic acid (1.0 mg/mL)
 11. fumaric acid (0.025 mg/mL)
 12. acrylic acid (0.1 mg/mL)
 13. propionic acid (2.0 mg/mL)
 14. glutaric acid (1.0 mg/mL)
 15. itaconic acid (0.025 mg/mL)

Separation of Vitamins

Water and lipid soluble vitamins were separated in a single run on a TSKgel ODS-100V column as demonstrated in Figure 7. The sample is a mixture of vitamins ranging from the very polar water-soluble vitamin ascorbic acid to the very hydrophobic tocopherol derivatives. 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. The TSKgel ODS-100V column provides high resolution for polar compounds, while at the same time delivers short analysis time for late eluting non-polar compounds.

▶ FIGURE 7

Separation of Vitamins



TSKgel ODS-100V, 5 μm, 4.6 mm ID x 15 cm L

Eluent: A) 0.1% TFA in H₂O
 B) 0.1% TFA in ACN
 Flow rate: 1.0 mL/min
 Detection: UV @ 280 nm
 Temperature: 40°C
 Injection vol.: 5 μL
 Gradient: 0 min (0%B), 20 min (40%B),
 22 min (100%B), 50 min (100%B)
 Samples:
 1. L-ascorbic acid
 2. nicotinic acid
 3. thiamine
 4. pyridoxal
 5. pyridoxine
 6. caffeine
 7. riboflavin
 8. retinol
 9. δ-tocopherol
 10. α-tocopherol
 11. α-tocopherol acetate



RPC TSKgel ODS-100V APPLICATION DATA

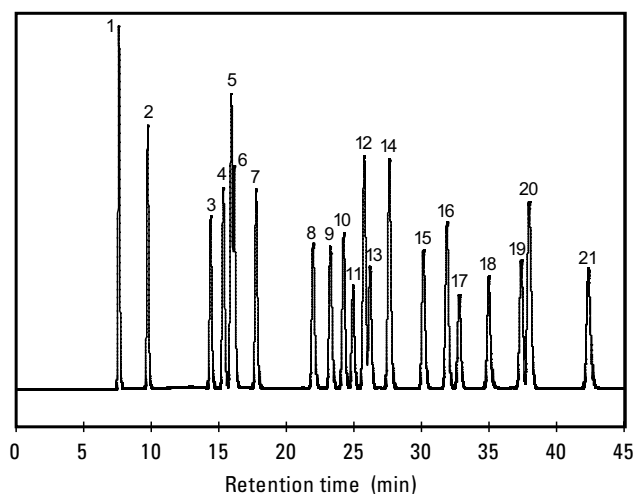
Separation of Nucleotides

The separation of mono-, di-, and triphosphorylated nucleotides on a TSKgel ODS-100V column is shown in **Figure 8**. The separation is accomplished by adding a short chain ion pairing agent, *t*-butylamine, and adjusting the mobile phase pH to pH 6.8.

Separation of Tryptic Peptides

The rapid identification of 20 peptides using a TSKgel ODS-100V column is detailed in **Figure 9**. 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.

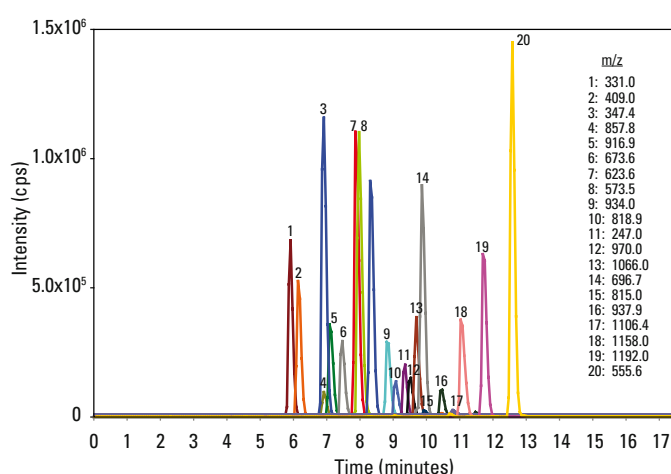
FIGURE 8
Separation of Nucleotides



TSKgel ODS-100V (4.6 mm ID × 25 cm L)

Mobile phases: A) 20 mmol/L *t*-butylamine + H₃PO₄ (pH 6.8)
 B) A/MeOH (90/10)
 Gradient: 0 min (B: 0%) -- 35 min (B: 100%)
 Flow rate: 1.0 mL/min
 Temp: 25°C
 Detection: UV @ 260 nm
 Inj. Volume: 2 µL
 Concentration: 0.3 g/L each
 Samples: 1. CMP, 2. UMP, 3. CDP, 4. dUMP, 5. GMP, 6. IMP, 7. UDP, 8. CTP, 9. TMP, 10. GDP, 11. IDP, 12. AMP, 13. UTP, 14. dGMP, 15. TDP, 16. GTP, 17. ITP, 18. ADP, 19. TTP, 20. dAMP, 21. ATP

FIGURE 9
Separation of Peptides by LC/MS



TSKgel ODS-100V, 3 µm , 2.0 mm ID x 15 cm L

Eluent: A: 0.1% TFA in H₂O,
 B: 0.1% TFA in ACN
 Flow rate: 0.2 mL/min
 Injection vol.: 2 µL
 Gradient: 0 min (10%B), 15 min (70%B),
 17 min (70%B)
 Sample: β-lactoglobulin tryptic digest
 Instrument: Q TRAP, ESI+

RPC

TSKgel ODS-100Z APPLICATION DATA



Separation of Polyphenols

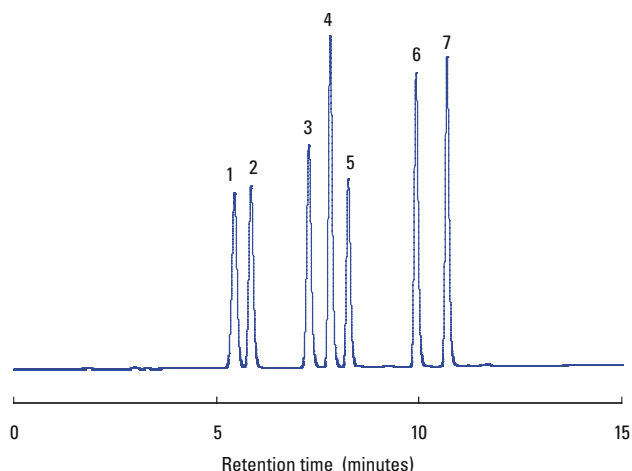
Catechins, which are found in large quantities in tea, are polyphenols. Catechins have been extensively studied for their antioxidant properties. **Figure 10** demonstrates the baseline separation of six catechins in the presence of caffeine on a 15 cm TSKgel ODS-100Z column.

Separation of 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 11**, a TSKgel ODS-100Z column provides superior resolution for oxytetracycline (peak 2) and tetracycline (peak 3) within the mixture.

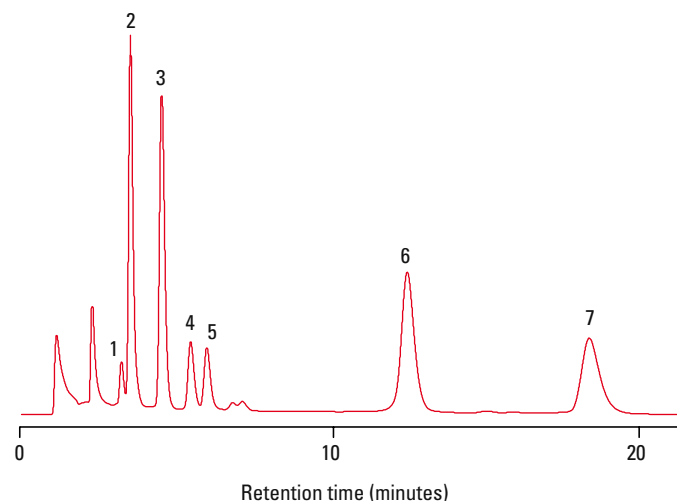
FIGURE 10
Separation of Catechins



TSKgel ODS-100Z, 5 μ m, 4.6 mm ID x 15 cm L

Eluent: A: 10 mmol/L KH_2PO_4 (pH2.5)
B: CH_3OH
Flow rate: 1.0 mL/min
Detection: UV @ 270 nm
Temperature: 40°
Gradient: 0 min (18%B), 15 min (60%B)
Injection vol.: 5 μ L
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)

FIGURE 11
Separation of Tetracycline



TSKgel ODS-100Z, 5 μ m, 4.6 mm ID x 15 cm L

Eluent: 10 mmol/L formic acid/acetonitrile=825/175
Flow rate: 1.0 ml/min
Detection: UV @ 254 nm
Temperature: 10°
Injection vol.: 20 μ L
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)



RPC

SUB-3 μm , HIGH THROUGHPUT RP COLUMNS

TSKgel ODS-140HTP and TSKgel Super Series reversed phase columns are based on small 2.3 μm silica particles. They provide high resolution and short analysis times at moderately high pressures.

TSKgel ODS-140HTP

TSKgel ODS-140HTP columns were designed for use with either UHPLC or conventional HPLC systems. The backpressure of a TSKgel ODS-140HTP column is less than half of the pressure of a 1.7 μm particle size column of the same dimensions.

The polylayer bonding chemistry of TSKgel ODS-140HTP columns results in highly efficient and physically stable columns when operated at high flow rates under high pressure. High efficiency and shorter retention make these columns an optimal fit for high throughput separations including drug discovery, pharmacokinetics and peptide digest separations.

TSKgel Super Series

TSKgel Super-ODS, Super-Octyl and Super-Phenyl RP columns are based on monodisperse spherical 2.3 μm silica particles bonded with, respectively, C18, C8, and phenyl functional groups. The bonded phases have a polymeric structure. An exhaustive endcapping minimizes the presence of residual silanol groups.

TSKgel Super series RP columns are recommended for small molecular weight compounds (<10 kDa) such as peptides, amino acids, nucleotides, and small organic molecules.

In order to fully exploit all benefits of small particle size stationary phases and to achieve optimum resolution it is highly recommended to optimize the HPLC system with respect to low dead volume, fast detector response and high sampling rates.

TABLE 3

Properties of TSKgel sub-3 μm Reversed Phase Columns

	TSKgel ODS-140HTP	TSKgel Super ODS	TSKgel SuperOctyl	TSKgel SuperPhenyl
Matrix	silica	silica	silica	silica
Particle size	2.3 μm	2.3 μm	2.3 μm	2.3 μm
Pore size	140 Å	110 Å	110 Å	110 Å
Endcapping	yes	yes	yes	yes
Functional group	C18	C18	C8	Phenyl
% Carbon	8	8	5	3
pH stability	2 - 7.5	2 - 7.5	2 - 7.5	2 - 7.5
Max. pressure	600 kg/cm ²	250/300 kg/cm ²	250/300 kg/cm ²	250/300 kg/cm ²
Temperature range	10 - 50°C	10 - 50°C	10 - 50°C	10 - 50°C



RPC

TSKgel ODS-140HTP APPLICATION DATA

System Requirements

Sub-3 μm columns can be used on a regular HPLC system if the dead volume is minimized. The following recommendations help the user to achieve optimum results with sub-3 μm columns:

- Use guard filters to reduce particulate contaminations.
- Injection volume should be as low as possible ($\leq 10 \mu\text{l}$).
- To ensure minimal extra-column volume, keep tubing ($\leq 0.1 \text{ mm ID}$) as short as possible.
- Use the smallest detector time constant and highest sampling rate.
- Use semi-micro or micro detector flow cell ($\leq 2 \mu\text{l}$ volume)

Performance Data

TSKgel ODS-140HTP columns operate at lower pressure than competitive sub-2 μm columns (Figure 12). The pressure drop of a 5 cm TSKgel ODS-140HTP column at 25 cm/min is less than half of the pressure of smaller particle size competitive columns. Not surprisingly, the pressure drop over a 10 cm TSKgel ODS-140HTP column was still lower than any of the competitive sub-2 μm 5 cm columns.

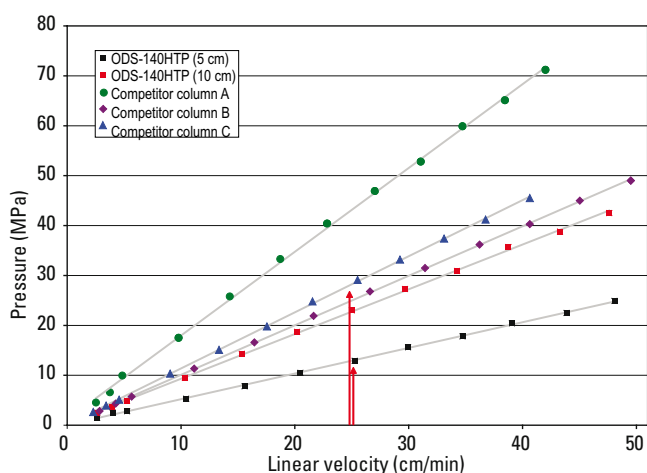
Separation of Herbal Extracts

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 13, the TSKgel ODS-140HTP column is an excellent choice for plant extract separations.

➤ FIGURE 12

Comparison of Pressure Drop

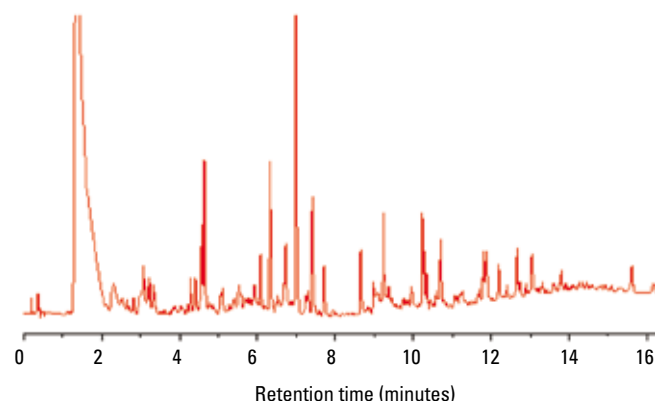


TSKgel ODS-140HTP, 2.3 μm , 2.0 mm ID x 5 cm L
 TSKgel ODS-140HTP, 2.3 μm , 2.0 mm ID x 10 cm L
 Competitor column A, 1.7 μm , 2.1 mm ID x 5 cm L
 Competitor column B, 1.8 μm , 2.1 mm ID x 5 cm L
 Competitor column C, 1.9 μm , 2.1 mm ID x 5 cm L

Eluent: $\text{H}_2\text{O}/\text{ACN}=50/50$
 Detection: UV @ 254 nm
 Temperature: 25°C
 Injection vol.: 2 μL
 Sample: naphthalene

➤ FIGURE 13

Separation of *Crinum Latifolium* L



TSKgel ODS-140HTP, 2.3 μm , 2.1 mm ID x 10 cm L

Instrument: Acquity UPLC System with TUV detector
 Eluent: A: H_2O B: ACN
 Flow rate: 0.523 mL/min
 Detection: UV @ 220 nm
 Temperature: 35°C
 Injection vol.: 2 μL
 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)
 Sampling rate: 80 Hz
 Sample: 50 g/L extract of *Crinum latifolium* L
 by 95% ethanol



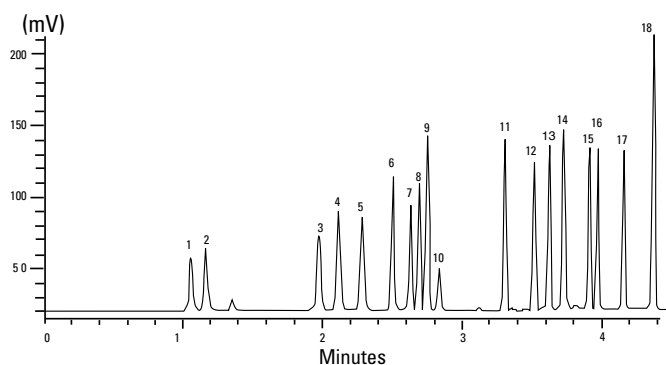
RPC TSKgel SUPER-SERIES APPLICATION DATA

Separation of Amino Acids

The baseline separation of 18 PTC-derivatized amino acids in 5 minutes on a TSKgel Super-ODS column is shown in **Figure 14**.

➤ **FIGURE 14**

Separation of PTC Amino Acids



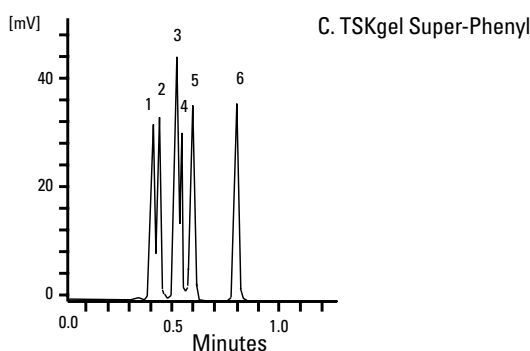
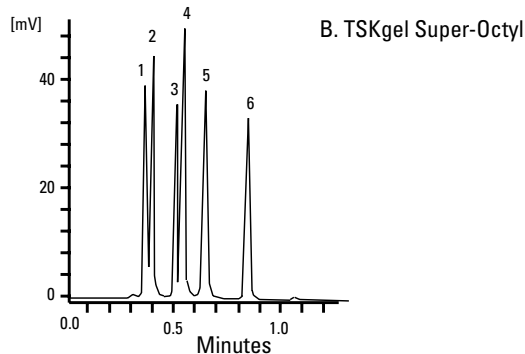
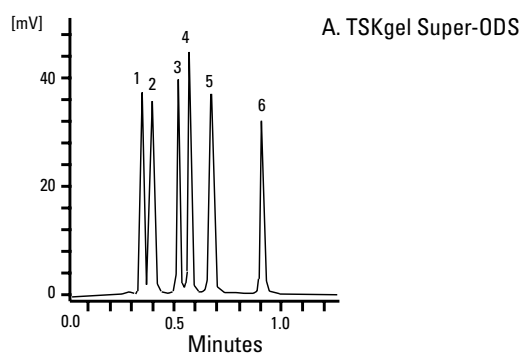
Column: TSKgel Super-ODS (4.6 mm ID x 10 cm L)
 Sample: 1. Asp, 2. Glu, 3. Ser, 4. Gly, 5. His, 6. Arg, 7. Thr, 8. Ala, 9. Pro, 10. PTC-NH₂, 11. Try, 12. Val, 13. Met, 14. Cys, 15. Ile, 16. Leu, 17. Phe, 18. Lys
 Elution: a. ACN/50 mM acetate buffer (pH 6.0)=3/97
 b. ACN/H₂O=60/40
 Flow Rate: 1.5 mL/min
 Detection: UV @ 254 nm
 Injection: 5 μL (250 pmol)
 Temperature: ambient

Comparison of Selectivity

The different selectivity of Super-ODS, Super-Phenyl and Super-Octyl RPC columns is illustrated in **Figure 15** for the analysis of a mixture of six neuropeptides in less than one minute.

➤ **FIGURE 15**

Comparison of Selectivity



Column: Each 4.6 mm ID x 5 cm L
 Sample: 1. oxytocin; 2. α-endorphin; 3. bombesin; 4. leu-enkephalin; 5. γ-endorphin; 6. somatostatin
 Elution: Buffer A. 13 mM HClO₄; Buffer B. 13 mM HClO₄/CH₃ CN = 20/80; 35% B to 80% B in a 3 min linear gradient
 Flow rate: 2.0 mL/min
 Detection: UV @ 220 nm

RPC

TSKgel RP COLUMNS FOR PROTEIN ANALYSIS



Silica Based

TSKgel TMS-250 reversed phase columns are based on 5 μm , 250 \AA pore size silica particles functionalized with trimethylsilyl groups. It allows unhindered access by large biomolecules and is therefore ideally suited for the analysis of proteins. 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 C8 or C18 columns (Figure 16).

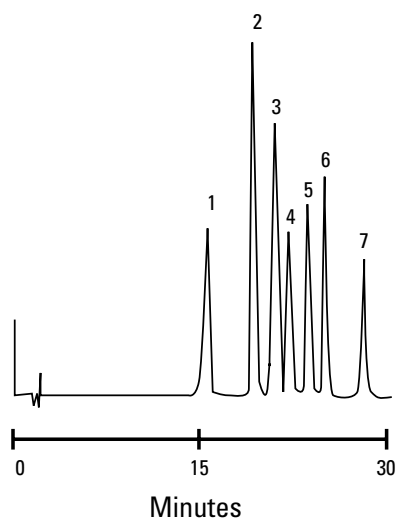
Although standard nomenclature designates the bonded phase of TSKgel TMS-250 as C1, its degree of hydrophobicity and selectivity is similar to those C4 reversed phase columns, frequently used for protein separations.

Polymer Based

TSKgel Octadecyl-4PW is based on 7 or 13 μm particle size, polymeric resin with 500 \AA pores. The highly cross-linked polymethacrylate base material provides excellent stability in high pH buffers and can withstand rigorous cleaning with either acid or base. The 500 \AA pore allows for the analysis of proteins up to 200 kDa while the particle size offerings allow for analytical and semi-preparative scale separations.

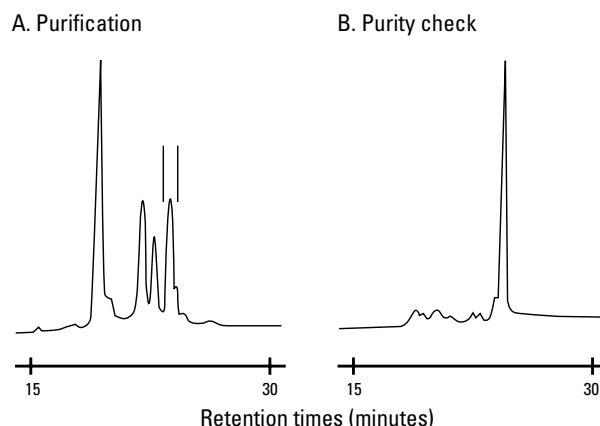
TSKgel Phenyl-5PW is based on 10 μm particle size polymethacrylate resin with 1000 \AA pores. The 1000 \AA pore size accommodates globular protein samples up to 1,000,000 Da (Figure 17).

FIGURE 16
High Resolution Protein Separation on TSKgel TMS-250



TSKgel TMS-250, 4.6 mm ID x 7.5 cm L
 Sample: 5 μg 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 17
Purification and Purity Check of Proteins



TSKgel Phenyl-5PW RP, 10 μm , 4.6 mm ID x 7.5 cm L

Flow rate: 1.0 mL/min
 Detection: UV @ 220 nm
 Elution: 2 min linear gradient from 5% to 20% ACN in 0.05% TFA, followed by (A - 48 min/B - 32 min) linear gradient to (A - 80%/B - 60%) ACN in 0.05% TFA
 Sample: lactate dehydrogenase (700 kDa)
 A. 40 μg in 100 μl
 B. purity check of fraction collected in part A



RPC TSKgel POLYMER BASED RP COLUMNS

Polymer-based reversed phase columns offer the best solution for high pH separations. They are chemically stable from pH 2 to 12, allowing operation at basic pH, where silica-based columns have limited chemical stability. The wider pH range also allows many basic compounds to be analyzed in their uncharged form, thus reducing secondary adsorption and improving peak shape. TSKgel polymer-based columns deliver improved recovery for peptides and proteins due to reduced secondary interactions.

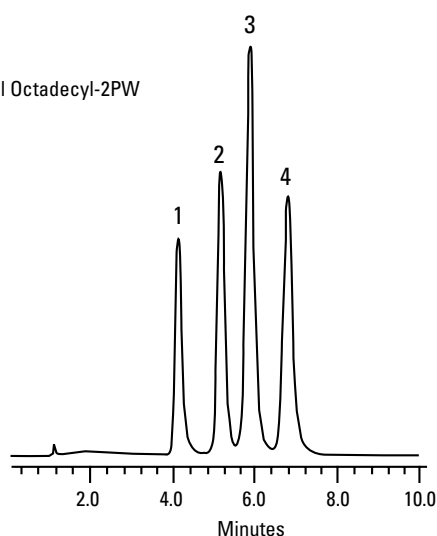
TSKgel Octadecyl-2PW

The highly cross-linked polymethacrylate base material of TSKgel Octadecyl-2PW columns provides excellent stability in high pH buffer systems (Figure 18). They can withstand rigorous cleaning with either acid or base. The 125 Å pores allow for analysis of peptides up to 8,000 Da.

FIGURE 18

Separation of Tricyclic Antidepressant Drugs on TSKgel Octadecyl-2PW

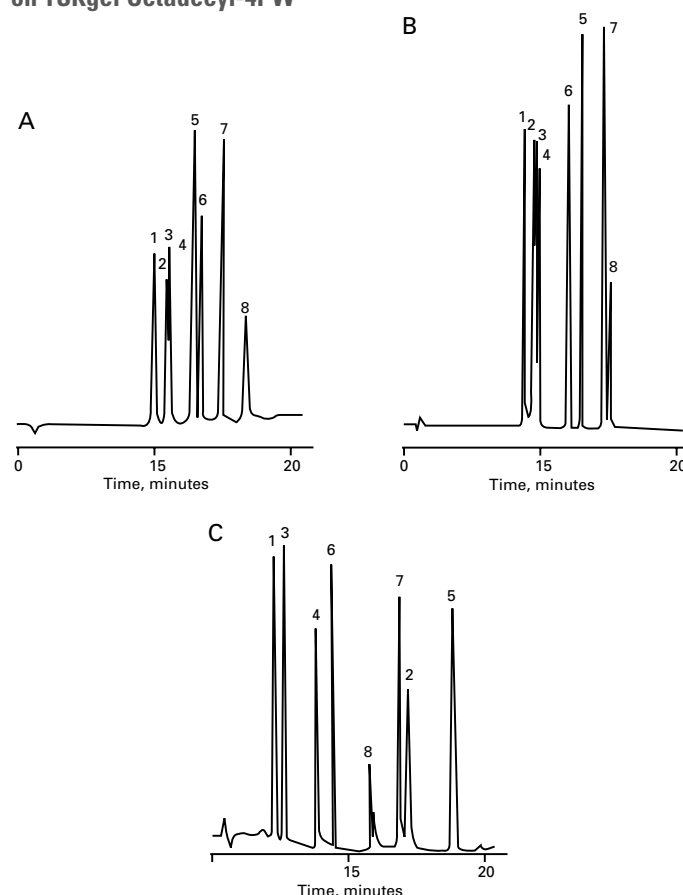
TSKgel Octadecyl-2PW



Column: TSKgel Octadecyl-2PW, 4.6 mm ID x 15 cm L
 Sample: 1. desipramine, 2. imipramine,
 3. amitriptyline, 4. trimipramine
 Elution: 20mM phosphate buffer (pH 11.0)/
 acetonitrile, 40/60
 Flow rate: A. 0.5 mL/min, B. 1.0 mL/min
 Detection: UV @ 254 nm
 Temp.: Ambient

FIGURE 19

Separation of Peptides at Acidic, Neutral or Basic pH on TSKgel Octadecyl-4PW



Column: TSKgel Octadecyl-4PW, 4.6 mm ID x 15 cm L
 Sample: 5-10 µg each of: 1. Met-Enkephalin, 2. Bradykinin,
 3. Leu-Enkephalin, 4. Neurotensin, 5. Bombesin,
 6. Angiotensin, 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

RPC

ORDERING INFORMATION



ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle Size (µm)	Number Theoretical Plates	Flow Rate (mL/min)		Maximum Pressure Drop (kg/cm ²)
						Range	Max.	
Stainless steel TSKgel columns								
21838	ODS-100V, 100 Å	1.0	3.5	3	≈ 2,900	0.02 - 0.05	0.22	150
21839	ODS-100V, 100 Å	1.0	5.0	3	≈ 4,500	0.02 - 0.05	0.22	150
21814	ODS-100V, 100 Å, pk 3*	2.0	1.0	3	≈ 500		0.22	300
22700	ODS-100V, 100 Å	2.0	2.0	3	≈ 1,500			120
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	210
21938	ODS-100V, 100 Å	2.0	10.0	3	≈ 11,500	0.15 - 0.18	0.22	240
21810	ODS-100V, 100 Å	2.0	15.0	3	≈ 17,500	0.15 - 0.18	0.22	240
22701	ODS-100V, 100 Å	2.0	25.0	3	≈ 28,000			300
22702	ODS-100V, 100 Å	3.0	2.0	3	≈ 2,000			120
22703	ODS-100V, 100 Å	3.0	3.5	3	≈ 4,000			120
21842	ODS-100V, 100 Å	3.0	5.0	3	≈ 6,000			150
21843	ODS-100V, 100 Å	3.0	7.5	3	≈ 9,000			210
21939	ODS-100V, 100 Å	3.0	10.0	3	≈ 12,000			240
21844	ODS-100V, 100 Å	3.0	15.0	3	≈ 18,000			240
22704	ODS-100V, 100 Å	3.0	25.0	3	≈ 29,000			300
22705	ODS-100V, 100 Å	4.6	2.0	3	≈ 2,500			120
22706	ODS-100V, 100 Å	4.6	3.5	3	≈ 4,500			120
21831	ODS-100V, 100 Å	4.6	5.0	3	≈ 6,500	0.7 - 1.0	1.2	150
21830	ODS-100V, 100 Å	4.6	7.5	3	≈ 9,750	0.7 - 1.0	1.2	210
21940	ODS-100V, 100 Å	4.6	10.0	3	≈ 13,500	0.7 - 1.0	1.2	240
21829	ODS-100V, 100 Å	4.6	15.0	3	≈ 19,500	0.7 - 1.0	1.2	240
22707	ODS-100V, 100 Å	4.6	25.0	3	≈ 30,000			300
21457	ODS-100V, 100 Å	2.0	5.0	5	≈ 3,000	0.15 - 0.18	0.22	180
22708	ODS-100V, 100 Å, pk 3*	2.0	1.0	5	> 300			280
22709	ODS-100V, 100 Å	2.0	2.0	5	> 1,000			90
22710	ODS-100V, 100 Å	2.0	3.5	5	> 2,500			90
22711	ODS-100V, 100 Å	2.0	7.5	5	≈ 5,500			180
22712	ODS-100V, 100 Å	2.0	10.0	5	≈ 7,000			180
21458	ODS-100V, 100 Å	2.0	15.0	5	≈ 11,000	0.15 - 0.18	0.22	180
22713	ODS-100V, 100 Å	2.0	25.0	5	≈ 18,000			180
22714	ODS-100V, 100 Å	3.0	2.0	5	≈ 1,000			90
22715	ODS-100V, 100 Å	3.0	3.5	5	≈ 3,000			90
22716	ODS-100V, 100 Å	3.0	5.0	5	≈ 4,000			120
22717	ODS-100V, 100 Å	3.0	7.5	5	≈ 6,000			180
22718	ODS-100V, 100 Å	3.0	10.0	5	≈ 8,500			180
22719	ODS-100V, 100 Å	3.0	15.0	5	≈ 13,000			180
22720	ODS-100V, 100 Å	3.0	25.0	5	≈ 21,000			180
22721	ODS-100V, 100 Å	4.6	2.0	5	≈ 1,500			90
22722	ODS-100V, 100 Å	4.6	3.5	5	≈ 3,000			90
22723	ODS-100V, 100 Å	4.6	5.0	5	≈ 4,500			120
22724	ODS-100V, 100 Å	4.6	7.5	5	≈ 7,000			180
22725	ODS-100V, 100 Å	4.6	10.0	5	≈ 9,000			180
21455	ODS-100V, 100 Å	4.6	15.0	5	≈ 14,000	0.7 - 1.0	1.2	180
21456	ODS-100V, 100 Å	4.6	25.0	5	≈ 23,000	0.7 - 1.0	1.2	210
22726	ODS-100Z, 100 Å, pk 3*	2.0	1.0	3	≈ 500			300
22727	ODS-100Z, 100 Å	2.0	2.0	3	≈ 1,500			120
22728	ODS-100Z, 100 Å	2.0	3.5	3	≈ 4,000			150
22729	ODS-100Z, 100 Å	2.0	5.0	3	≈ 5,700			150
22730	ODS-100Z, 100 Å	2.0	7.5	3	≈ 8,600			210
22731	ODS-100Z, 100 Å	2.0	10.0	3	≈ 11,500			240
22732	ODS-100Z, 100 Å	2.0	15.0	3	≈ 17,500			240
22733	ODS-100Z, 100 Å	2.0	25.0	3	≈ 28,000			300
22734	ODS-100Z, 100 Å	3.0	2.0	3	≈ 2,000			120
22735	ODS-100Z, 100 Å	3.0	3.5	3	≈ 4,000			120
22736	ODS-100Z, 100 Å	3.0	5.0	3	≈ 6,000			150
22737	ODS-100Z, 100 Å	3.0	7.5	3	≈ 9,000			210
22738	ODS-100Z, 100 Å	3.0	10.0	3	≈ 12,000			240
22739	ODS-100Z, 100 Å	3.0	15.0	3	≈ 18,000			240
22740	ODS-100Z, 100 Å	3.0	25.0	3	≈ 29,000			300
22741	ODS-100Z, 100 Å	4.6	2.0	3	≈ 2,500			120
22742	ODS-100Z, 100 Å	4.6	3.5	3	≈ 4,500			120

*needs cartridge holder



RPC ORDERING INFORMATION

► ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle Size (µm)	Number Theoretical Plates	Flow Rate (mL/min) Range	Max.	Maximum Pressure Drop (kg/cm ²)
22743	ODS-100Z, 100 Å	4.6	5.0	3	≥ 6,500			150
22744	ODS-100Z, 100 Å	4.6	7.5	3	≥ 9,750			210
22745	ODS-100Z, 100 Å	4.6	10.0	3	≥ 13,500			240
22746	ODS-100Z, 100 Å	4.6	15.0	3	≥ 19,500			240
22747	ODS-100Z, 100 Å	4.6	25.0	3	≥ 30,000			300
22748	ODS-100Z, 100 Å, pk 3*	2.0	1.0	5	≥ 300			280
22749	ODS-100Z, 100 Å	2.0	2.0	5	≥ 1,000			90
22750	ODS-100Z, 100 Å	2.0	3.5	5	≥ 2,500			90
21460	ODS-100Z, 100 Å	2.0	5.0	5	≥ 3,000	0.15 - 0.18	0.22	180
22751	ODS-100Z, 100 Å	2.0	7.5	5	≥ 5,500			180
22752	ODS-100Z, 100 Å	2.0	10.0	5	≥ 7,000			180
21459	ODS-100Z, 100 Å	2.0	15.0	5	≥ 11,000	0.15 - 0.18	0.22	180
22753	ODS-100Z, 100 Å	2.0	25.0	5	≥ 18,000			180
22754	ODS-100Z, 100 Å	3.0	2.0	5	≥ 1,200			90
22755	ODS-100Z, 100 Å	3.0	3.5	5	≥ 3,000			90
22756	ODS-100Z, 100 Å	3.0	5.0	5	≥ 4,000			120
22757	ODS-100Z, 100 Å	3.0	7.5	5	≥ 6,000			180
22758	ODS-100Z, 100 Å	3.0	10.0	5	≥ 8,500			180
22759	ODS-100Z, 100 Å	3.0	15.0	5	≥ 13,000			180
22760	ODS-100Z, 100 Å	3.0	25.0	5	≥ 21,000			180
22761	ODS-100Z, 100 Å	4.6	2.0	5	≥ 1,500			90
22762	ODS-100Z, 100 Å	4.6	3.5	5	≥ 3,000			90
22763	ODS-100Z, 100 Å	4.6	5.0	5	≥ 4,500			120
22764	ODS-100Z, 100 Å	4.6	7.5	5	≥ 7,000			180
22765	ODS-100Z, 100 Å	4.6	10.0	5	≥ 9,000			180
21461	ODS-100Z, 100 Å	4.6	15.0	5	≥ 14,000	0.7 - 1.0	1.2	180
21462	ODS-100Z, 100 Å	4.6	25.0	5	≥ 23,000	0.7 - 1.0	1.2	210

Guard column products

21997	ODS-100V, Guardgel Cartridge, pk 3	2.0	1.0	3				For all 3 µm ODS-100V 2 & 3 mm ID columns
21453	ODS-100V Guard Cartridge, pk 3	3.2	1.5	5				For all ODS-100V 4.6 mm ID columns
21841	ODS-100V Guard Cartridge, pk 3	2.0	1.0	5				For all 5 µm ODS-100V 2 & 3 mm ID columns
21454	ODS-100Z Guard Cartridge, pk 3	3.2	1.5	5				For all ODS-100Z 4.6 mm ID columns
21996	ODS-100Z, Guardgel Cartridge, pk 3	2.0	1.0	3				For all 3 µm ODS-100Z 2 & 3 mm ID columns
21995	ODS-100Z, Guardgel Cartridge, pk 3	2.0	1.0	5				For all 5 µm ODS-100Z 2 & 3 mm ID columns

Stainless steel TSKgel columns

21927	TSKgel ODS-140HTP	2.1	5.0	2.3	≥ 7,000			600
21928	TSKgel ODS-140HTP	2.1	10.0	2.3	≥ 14,000			600
18150	ODS-80TS, 80 Å	2.0	15.0	5	≥ 11,000	0.15 - 0.18	0.22	200
18151	ODS-80TS, 80 Å	2.0	25.0	5	≥ 18,000	0.15 - 0.18	0.22	300
17200	ODS-80TS, 80 Å	4.6	7.5	5	≥ 4,500	0.8 - 1.0	1.2	100
17201	ODS-80TS, 80 Å	4.6	15.0	5	≥ 11,000	0.8 - 1.0	1.2	200
17202	ODS-80TS, 80 Å	4.6	25.0	5	≥ 18,000	0.8 - 1.0	1.2	300
17380	ODS-80TS, 80 Å	21.5	30.0	10	≥ 6,000	4.0 - 6.0	12.0	60
16651	ODS-80TM, 80 Å	4.6	7.5	5	≥ 4,500	0.8 - 1.0	1.2	100
08148	ODS-80TM, 80 Å	4.6	15.0	5	≥ 11,000	0.8 - 1.0	1.2	200
08149	ODS-80TM, 80 Å	4.6	25.0	5	≥ 18,000	0.8 - 1.0	1.2	300
14002	ODS-80TM, 80 Å	21.5	30.0	10	≥ 6,000	4.0 - 6.0	12.0	60
17344	Octyl-80TS, 80 Å	4.6	15.0	5	≥ 11,000	0.8 - 1.0	1.2	200
17345	Octyl-80TS, 80 Å	4.6	25.0	5	≥ 18,000	0.8 - 1.0	1.2	300
17348	CN-80TS, 80 Å	4.6	15.0	5	≥ 11,000	0.8 - 1.0	1.2	200
17349	CN-80TS, 80 Å	4.6	25.0	5	≥ 18,000	0.8 - 1.0	1.2	300

Guard column products

19011	ODS-80TS Guard cartridge, pk 3	3.2	1.5	5				For all 4.6 mm ID ODS-80TS columns
19012	Octyl-80TS Guard cartridge, pk 3	3.2	1.5	5				For all 4.6 mm ID Octyl-80TS columns
17385	ODS-80TS Guard column	21.5	7.5	10				For P/N 17380
14098	ODS-80TM Guard column	21.5	7.5	10				For P/N 14002
19004	ODS-80TM Guard cartridge, pk 3	3.2	1.5	5				For 4.6 mm ID ODS-80TM columns
19013	CN-80TS Guard cartridge, pk 3	3.2	1.5	5				For 4.6 mm ID CN-80TS columns

*needs cartridge holder

