TSKgel® Amide-80 Columns for the **Analysis of Hydrophilic Compounds**

Introduction

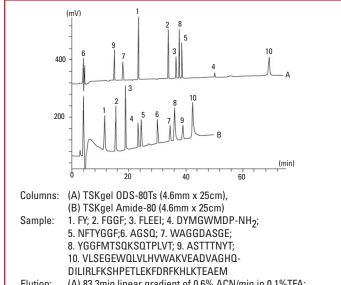
TSKgel Amide-80 columns enable the analysis of hydrophilic biomolecules, such as peptides, carbohydrates, and small molecules for drug discovery, that are either weakly retained or eluted in the void volume of reversed phase chromatography (RPC) columns. Normal phase liquid chromatography (NPLC) and hydrophilic interaction chromatography (HILIC) on TSKgel Amide-80 columns have been employed to effectively separate and analyze these compounds.

The TSKgel Amide-80 columns are packed with 5µm or 10µm spherical silica particles covalently bonded with carbamoyl groups. The polar functional groups of the sample, such as hydroxy groups, form hydrogen bonds with the polar groups (amino groups) of the packing. The number of hydroxy groups, conformation and solubility in the mobile phase determines the order of elution. The TSKgel Amide-80 packing is superior to aminoalkyl silica gel in chemical stability and will not react with reducing sugars in carbohydrate analysis, making them an excellent replacement for amino bonded columns. The superior stability of the TSKgel Amide-80 stationary phase enables detection with Evaporative Light Scattering (ELS) detectors. Aminoalkyl silica gel phases often exhibit leaching which can be detected by ELS instrumentation.

Product Highlights

- Improve resolution of hydrophilic molecules not retained by reversed phase
- Perform NPLC and HILIC analysis for peptides, carbohydrates and small molecules
- Gain superior chemical stability in aqueous and organic solvents — an excellent replacement for amino bonded columns
- Spherical silica particles bonded with carbamoyl groups in 5 and 10 micron particle sizes
- Now in 5cm and 10cm lengths and 2mm IDs for use with LC/MS

Figure 1. Comparison of peptides separated on TSKgel ODS-80Ts by RPC and TSKgel Amide-80 by NPLC1

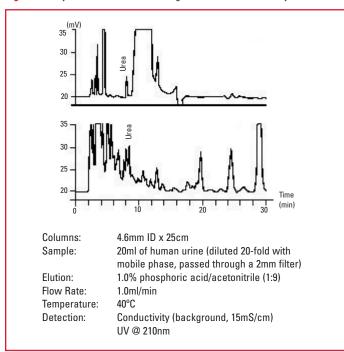


(A) 83.3min linear gradient of 0.6% ACN/min in 0.1%TFA; Elution:

(B) 70min linear gradient of 0.6% water/min in 0.1% TFA/ACN

Flow Rate: 1ml/min Detection: UV @ 215nm

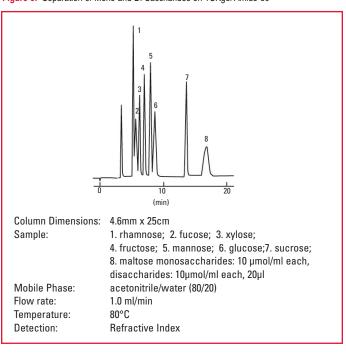
Figure 2. Analysis of Urea in Urine With TSKgel Amide 80 and Conductivity Detection



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Figure 3. Separation of Mono and Di-Saccharides on TSKgel Amide-80



Terreic Acid (MW=154.1) 80 60 40 20 M-H⁻=153.1 0 100 80 60 40 20 Cephalosporin C (MW=415.4) M-H*=414.4 Bialaphos (MW=323.3) M-H+=322.3 Penicillin N (MW=359.4) M+0Ac-H*=417.4 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 Retention Time (min) Column: TSKgel Amide-80 Mobile Phase: 6.5mM ammonium acetate, pH 5.5, 90 min., 10-40% aqueous gradient

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Figure 4. Negative Ion HILIC-ESI-MS Analysis of a Mixture of Four Polar Natural Product Compounds

Ordering Information and Typical Properties for TSKgel Amide-80 Columns

Part #	Description	ID (mm)	Length (cm)	Particle Size (μm)	Pore Size (Å)
42138	Amide-80	2	5	5	80
42131	Amide-80	2	10	5	80
42132	Amide-80	2	15	5	80
42133	Amide-80	2	25	5	80
19532	Amide-80	4.6	5	5	80
19533	Amide-80	4.6	10	5	80
13071	Amide-80	4.6	25	5	80
14459	Amide-80	7.8	30	10	80
14460	Amide-80	21.5	30	10	80
19021	Amide-80 Guard Column for P/N 19532, 19533 & 13071	1	4		
14461	Amide-80 Guard Column for P/N 14460	21.5	7.5		

References:

- Yoshida, T., "Calculation of peptide retention coefficients in normal-phase liquid chromatography," J. Chromatogr. A, 808, 105-112 (1998).
- Strege, M.A., Oral presentation (L-1305) at the 22nd International Symposium on High Performance Liquid Phase Separations and Related Techniques, St. Louis, Missouri, May 2-8, 1998.



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