TSKgel NH_2-100 Columns

A Stable Amino-Based Column Offering Alternate Selectivity for Polar Compounds in Hydrophilic Interaction Liquid Chromatography (HILIC)

Introduction

Hydrophilic interaction liquid chromatography (HILIC) is used primarily to separate polar and hydrophilic compounds that are not sufficiently retained by reversed phase. Target applications for HILIC include the analysis of hydrophilic compounds such as saccharides, glycosides, oligosaccharides, peptides, and drug metabolites.

A new HILIC column, the TSKgel NH_2-100, provides a different selectivity from the well-known TSKgel Amide-80 columns. These novel amino-bonded phase columns *(see Figure 1)* stand out by providing much improved chemical stability, a prerequisite for achieving reproducible and reliable results. Due to a high ligand density and large surface area, these columns show high retention for very polar compounds.

TSKgel NH_2-100 columns are packed with 3µm silica particles containing 100Å pores. A novel bonding strategy improves chemical stability of the bonded phase *(see Figure 2)*. First the silica is reacted with a trimethylsilane endcapping reagent at a low stoichiometric ratio, before reaction of the remaining and accessible silanol groups with trifunctional alkylaminosilane. The resulting bonded phase provides a better safeguard against hydrolysis of the underlying silica.

Product Highlights

- **Alternative Selectivity to TSKgel Amide-80 HILIC Columns**
- **Novel Bonding Strategy Produces High Chemical Stability**
- **High Retention for Very Polar Compounds**
- **Ideally Suited for High Efficiency HPLC and LC/MS**

Figure 1.

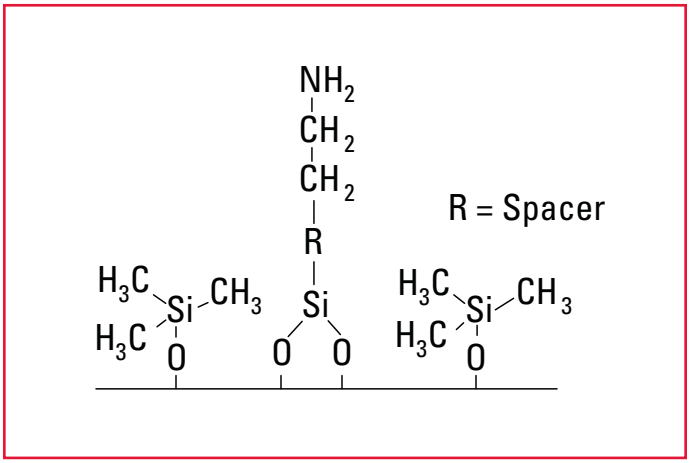
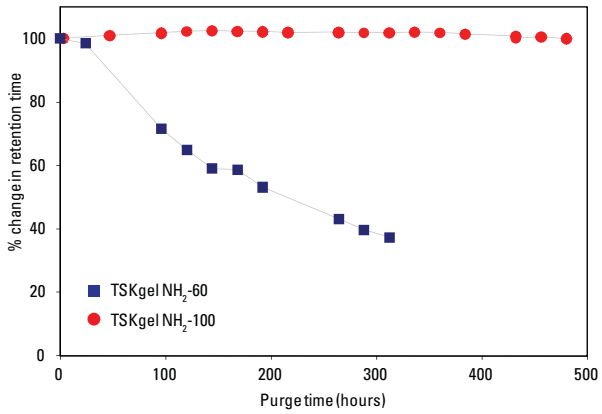


Figure 2.



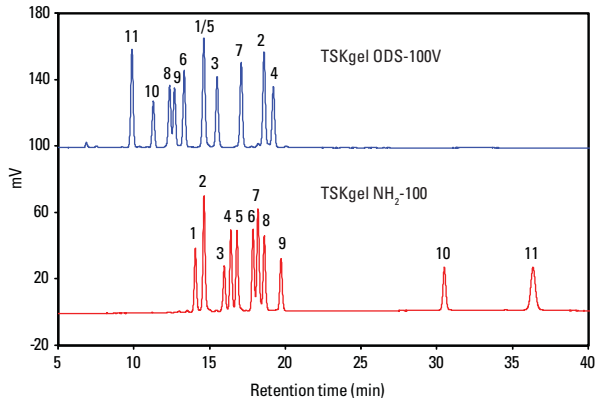
Columns:	A) TSKgel NH_2-100, 3µm, 4.6mm ID x 15cm, B) TSKgel NH_2-60, 4.6mm ID x 25cm
Eluent:	H_2O/ACN (25/75)
Flow rate:	1.0mL/min
Inj. volume:	10µL
Temperature:	40°C
Detection:	RI
Sample:	inositol

Applications

Pyridylaminated Sugars

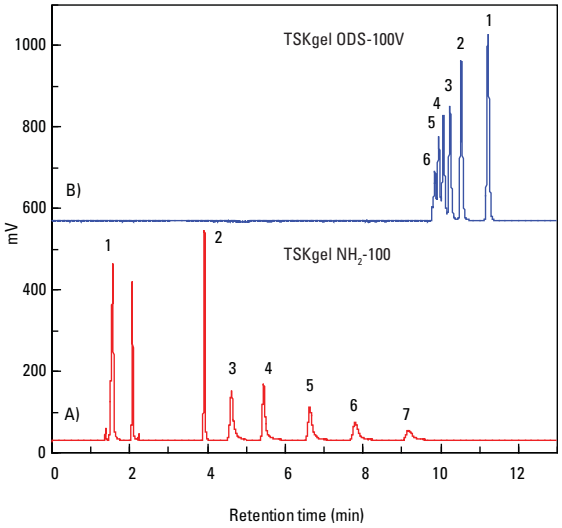
Although good separations of pryridylaminated (PA) sugars *(Figure 3)* can be obtained by reversed phase as well as by HILIC, all components are separated on the TSKgel NH_2-100 column, while two of the components coelute on the TSKgel ODS-100V column. It is believed that all acidic PA sugars were completely separated on the TSKgel NH_2-100 column due to an additional ionic interaction. The fact that the elution order of the acidic PA sugars is almost completely the opposite on the reversed phase and HILIC columns, demonstrates the potential of the TSKgel NH_2-100 column as a second dimension in 2D HPLC.

Figure 3.



Column:	TSKgel NH_2-100, 3µm, 4.6mm ID x 15cm
Eluent:	A: 200mmol/L TEA-Acetic acid (pH6.5)/ACN (30/70) B: 500mmol/L TEA-Acetic acid (pH6.5) /ACN (60/40)
Column:	TSKgel ODS-100V, 3µm, 4.6mm ID x 15cm
Eluent:	A: 50mmol/L HCO_2NH_4/ACN (98/2), B: 50mmol/L HCO_2NH_4/ACN (90/10)
Gradient:	0min (0% B) 30min (100% B) 45min (100% B)
Flow rate:	1.0mL/min
Inj. volume:	10µL
Temperature:	40°C
Detection:	Fs (Ex. 315nm, Em. 380nm)
Samples:	pyridylaminated sugars

Figure 4.



Column:	TSKgel NH_2-100, 3µm, 2.0mm ID x 15cm
Eluent:	A) H_2O/ACN (10/90) + 0.1% TFA B) H_2O + 0.1% TFA
Column:	TSKgel ODS-100V, 3µm, 2.0mm ID x 15cm
Eluent:	A) H_2O/ACN (90/10) + 0.1% TFA B) ACN + 0.1% TFA
Gradient:	0min (0% B) 15min (40% B) 17min (0% B)
Flow rate:	0.20mL/min
Inj. volume:	10µL
Temperature:	40°C
Detection:	UV@313nm
Samples:	1. MTX (MTXPG) 2. $MTXPG_2$ 3. $MTXPG_3$ 4. $MTXPG_4$ 5. $MTXPG_5$ 6. $MTXPG_6$ 7. $MTXPG_7$

MTX and its Derivatives

Polyglutamate metabolites of methotrexate (MTXPG2~7) were separated on TSKgel NH_2-100 and TSKgel ODS-100V narrow bore columns *(Figure 4)*. On the TSKgel NH_2-100 column, MTX polyglutamate metabolites were eluted in the order of the number of glutamate groups in their molecules. Despite the early elution of MTXPG1 and MTXPG2 on the TSKgel NH_2-100 column, the overall separation is superior over what can be accomplished on the C18 column.

Ordering Information

Part #	Description	Matrix	Housing	ID (mm)	Length (cm)
21967	TSKgel NH_2-100, 3µm	Silica	Stainless Steel	2	5
21968	TSKgel NH_2-100, 3µm	Silica	Stainless Steel	2	15
21969	TSKgel NH_2-100, 3µm	Silica	Stainless Steel	4.6	5
21970	TSKgel NH_2-100, 3µm	Silica	Stainless Steel	4.6	15

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