TOSOH BIOSCIENCE

TSKgel Amide-80 Columns for Hydrophilic Interaction Liquid Chromatography

- *Superior resolution and sensitivity in HILIC mode*
- *Particle sizes of 3, 5 and 10 µm*
- *Covalently bonded carbamoyl functional groups*
- *Unique retention mechanism for saccharide analysis*
- *Ideally suited for the LC-MS analysis of glycans*

HILIC

Hydrophilic interaction chromatography (HILIC) is used primarily for the separation of polar and hydrophilic compounds. HILIC has similarities with traditional normal phase chromatography, but with the important difference that the mobile phases for HILIC are similar to those known from reversed phase chromatography (RPC). They include polar organic solvents such as methanol or acetonitrile and water. Compared to RPC the elution order in HILIC mode is inversed for most substances. While using similar eluent systems Amide-80 and C18 or Phenyl phases can easily be combined for two dimensional liquid chromatography (2D-LC). In addition HILIC is ideal for sensitive LC-MS analysis of water soluble polar compounds because the high organic content in the mobile phase provides rapid evaporation of solvent during electrospray ionization.

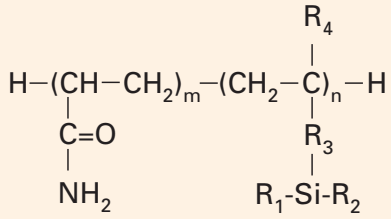


Figure 1

TSKgel Amide-80 stationary phase

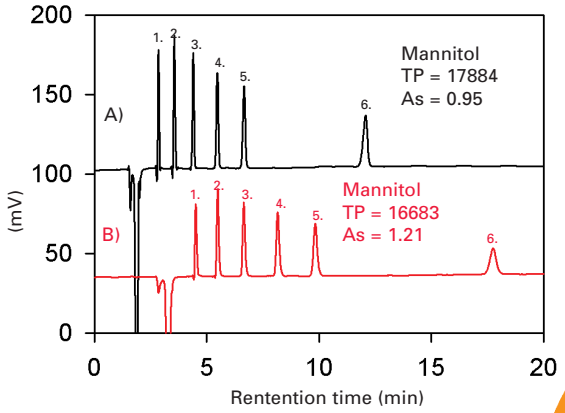
For years TSKgel Amide-80 5 µm columns are used successfully for the separation of polar compounds in HILIC mode. Packed with spherical silica particles that are covalently bonded with non-ionic carbamoyl groups (Figure 1), TSkgel Amide-80 provides higher stability then conventional amino-phases and a unique selectivity.

Based on hydrogen bonds the aqueous content of the mobile phase creates a water-rich layer on the surface of the stationary phase. This allows partitioning of solutes between the more organic mobile phase and the aqueous layer.

The number of polar groups, as well as the conformation and solubility of the sample in the mobile phase determines the elution order. Target applications include the analysis of saccharides, glycosides, oligosaccharides, peptides and hydrophilic drugs.

TSKgel Amide-80 columns packed with 3 µm particles are the newest addition to the well-known TSKgel Amide-80 series. TSKgel Amide-80 3 µm HILIC columns reduce analysis time and improve peak capacity and sensitivity for HPLC and LC-MS analysis.

Separation of sugar alcohols
TSKgel Amide-80 3 µm vs. TSKgel Amide-80 5 µm



Conditions

Column:	A) TSKgel Amide-80 3 µm (4.6 mm ID x 15 cm L)
	B) TSKgel Amide-80 5 µm (4.6 mm ID x 25 cm L)
Eluent:	H_2O/CH_3CN=25/75
Flow rate:	1.0 mL/min
Detection:	Refractive index
Temp.:	25 °C
Inj. volume :	10 µL
Samples:	1. Ethyleneglycol 2. Glycerin
	3. Erythritol 4. Xylitol
	5. Mannitol 6. Inositol

Figure 2

HILIC separation of polar compounds

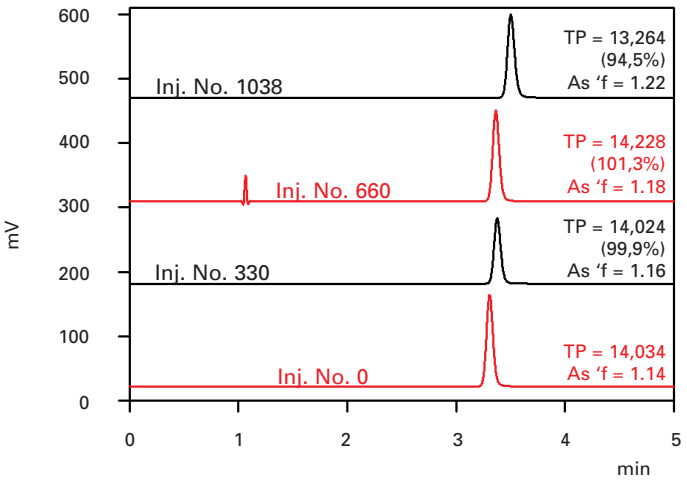
Figure 2 shows the separation of sugar alcohols on a TSKgel Amide-80 3 µm column compared to a TSKgel Amide-80 5 µm column. Basically, the more hydroxyl groups in a compound the more polar it will be and the longer it will be retained on the column. Comparison of the retention between mannitol and inositol, each with 6 hydroxyl groups, shows that inositol, which has a cyclic structure and lower solubility in the mobile phase is retained longer. Overall the 3 µm column provides better resolution at reduced analysis time when compared to the 5 µm TSKgel Amide-80 column.

TSKgel Amide-80 long term stability

The high stability of Amide-80 columns is demonstrated in Figure 3 showing the same analysis after 330, 660 and more than 1,000 runs compared to the first injection. Only 5% reduction of column performance (theoretical plates) is observed after more than 1,000 injections.

Amino phases and pure silica phases, which are used for HILIC mode as well, do not show such long term stability when used with water/acetonitrile eluents.

Durability of TSKgel Amide-80 3 µm



Column:	TSKgel Amide-80 3 µm (2.0 mm ID x 15 cm L)
Eluent :	H_2O/CH_3CN = 15/85
Flow rate :	0.2 mL/min
Detection :	UV @ 254 nm
Temp. :	25 ºC
Inj. volume :	2 µL
Samples:	Uracil (37 mg/L)

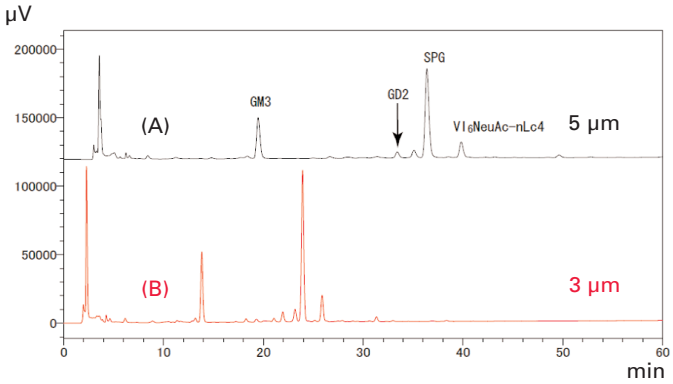
Figure 3

TSKgel Amide-80 in glycomics research

For years TSKgel Amide-80 5 µm columns are used successfully in glycan analysis. With the new 3 µm material resolution and sensitivity can be further enhanced (Figure 4). TSKgel Amide-80 3 µm microbore columns are particularly suited for the LC-MS analysis of glycans in the emerging field of glycomics research.

Separation of PA-glycans from acidic glycolipids in erythrocyte membrane

TSKgel Amide-80 3 µm vs. TSKgel Amide-80 5 µm



Column:	TSKgel Amide-80 5 µm (2.0 mm ID x 25 cm L), TSKgel Amide-80 3 µm (2.0 mm ID x 25 cm L),
Eluent:	
(A)	ACN/0.5 M/L acetic acid containing 10% acetonitrile, pH 7.3 with triethylamine (75:15, v/v)
(B)	ACN/0.5 Ml/L acetic acid containing 10% acetonitrile, pH 7.3 with triethylamine (40:50, v/v)
Gradient:	
(A)	0 to 100% solvent B in 100 min for TSKgel Amide-80 5 µm
(B)	0 to 100% solvent B in 60 min for TSKgel Amide-80 3 µm
Flow rate:	0.2 mL/min
Detection:	fluorescence (Ex.: 310 nm, Em.: 380 nm),
Temp.:	40 °C

* Courtesy of Dr. Yasuhide Miyamoto from Research Institute, Osaka Medical Center for Cancer and Cardiovascular Diseases

Figure 4

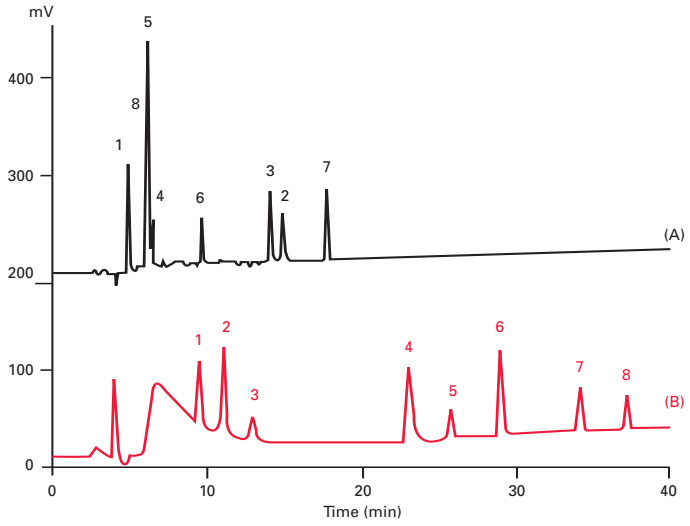
TSKgel Amide-80 in method development

Figure 5 gives an example for the differences in selectivity of HILIC and RPC. Small peptides were separated by ODS and HILIC columns of the same dimensions using the same eluents but almost inverse gradients.

In method development HILIC should be taken into consideration as soon as polar compounds have to be analyzed. Since common RPC solvents can be used, the TSKgel Amide-80 is even suitable for method development systems with automated column selection.

A range of reversed phase columns with different hydrophobicity, columns with polar embedded groups and a TSKgel Amide-80 HILIC column should deliver at least an indication for the right direction.

Selectivity for peptides separated by RP chromatography and HILIC



Columns: (A) TSKgel ODS-80TS, 4.6 mm ID x 25 cm L
(B) TSKgel Amide-80, 4.6 mm ID x 25 cm L
Sample: 1. PG; 2. LG; 3. FG; 4. EHP-NH2; 5. VGSQ; 6. GGYR; 7. WAGGDASGE; 8. DSDPR;
Elution: (A) 0.1 % TFA/ACN, linear gradient of 5 % - 55 % ACN in 83.3 min
(B) 0.1 % TFA/ACN, linear gradient of 97 % - 55 % ACN in 70 min
Flow Rate: 1 mL/min
Detection: UV @ 215 nm

Figure 5

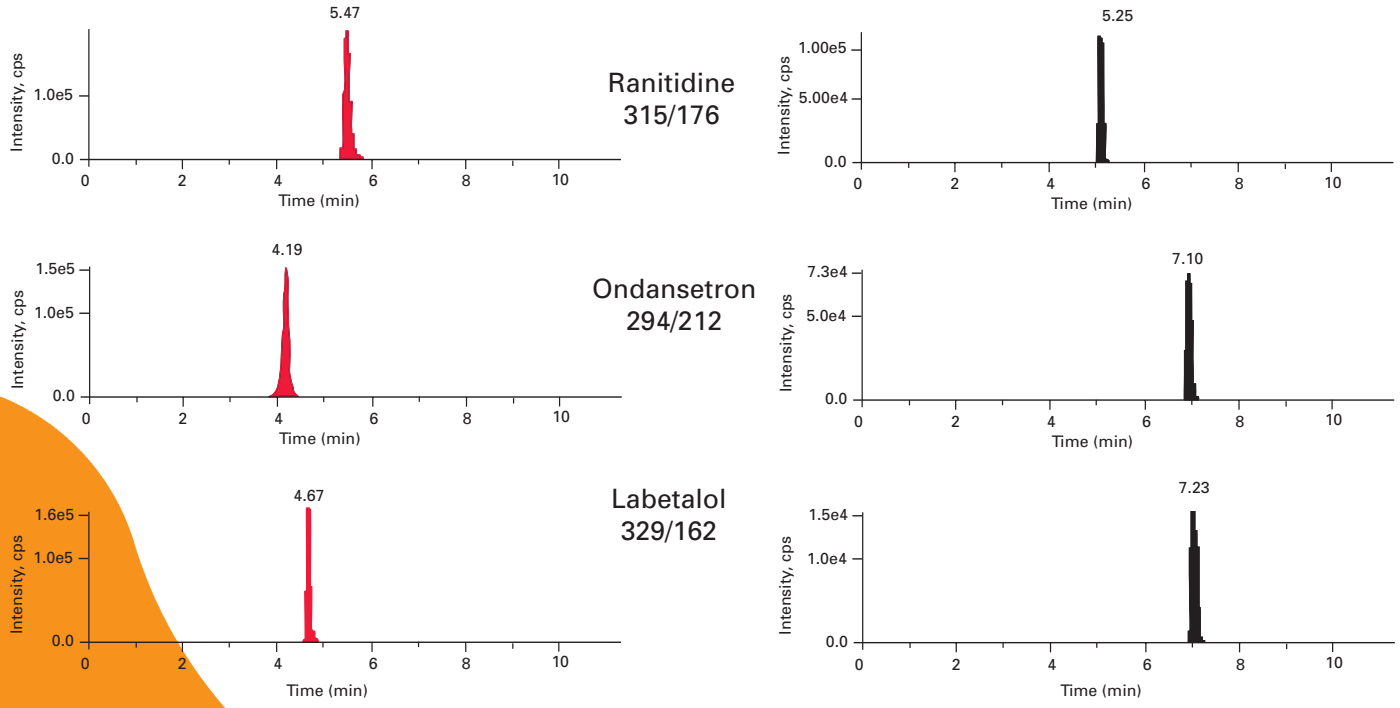
TSKgel Amide-80 for LC-MS

HILIC offers unique advantages for mass spectrometric detection of very polar compounds when compared to reversed phase mode. The higher organic content of the eluent in HILIC mode supports efficient evaporation of the solvent thus enhancing sensitivity and altering ion suppression (Figure 6). An additional benefit of TSKgel Amide-80 for LC-MS use is the virtual absence of column bleeding due to the covalently bonded carbamoyl groups.

Conclusion

With the unique HILIC separation mode polar and hydrophilic compounds are easily separated, using the TSKgel Amide-80 phase. The long column lifetime, chemical stability and outstanding reproducibility makes the TSKgel Amide-80 column a valuable tool for a broad range of applications in industry and research. TSKgel Amide-80 columns are available with particle sizes of 5, 10 and now also 3 µm.

LC-MS analysis of basic drugs on HILIC and Reversed Phase columns with small particles



Column: TSKgel Amide-80 3 µm (2.0 mm ID x 15 cm L)
Eluent : A: 10 mM Ammoniumformiate (pH 3.75)
B: ACN
Gradient : 0 min (B 90%) -> 10 min (B 40%) ->13 min (B 40%)
Flow rate : 0.2 mL/min
Inj. volume : 5 µL (50 µg/L)
Detection : QTrap® LC-MS/MS (Applied Biosystems), ESI+

Column: : TSKgel ODS-100V 3 µm (2.0 mm ID x 15 cm L)
Eluent : A: 10 mM Ammoniumformiate (pH 3.75)
B: ACN
Gradient : 0 min (B 0%) -> 10 min (B 80%) ->13 min (B 80%)
Flow rate : 0.2 mL/min
Inj. volume : 5 µL (50 µg/L)
Detection : QTrap® LC-MS/MS (Applied Biosystems), ESI+

Figure 6

Ordering information

Part-No	Description	Particle size (µm)	ID (mm)	length (cm)
21864	TSKgel Amide-80	3	2.0	5.0
21865	TSKgel Amide-80	3	2.0	15.0
21862	Guard Cartridge for 2 mm ID column, 3 p/pkg	3	2.0	1.0
19308	Guard Cartridge Holder for P/N 21862			
21866	TSKgel Amide-80	3	4.6	5.0
21867	TSKgel Amide-80	3	4.6	15.0
21863	Guard Cartridge for 4,6 mm ID column, 3 p/pkg	3	3.2	1.5
19018	Guard Cartridge Holder for P/N 21863			
20009	TSKgel Amide-80	5	1.0	5.0
20010	TSKgel Amide-80	5	1.0	10.0
21486	TSKgel Amide-80	5	1.0	15.0
21487	TSKgel Amide-80	5	1.0	25.0
19694	TSKgel Amide-80	5	2.0	5.0
19695	TSKgel Amide-80	5	2.0	10.0
19696	TSKgel Amide-80	5	2.0	15.0
19697	TSKgel Amide-80	5	2.0	25.0
21941	Guard Cartridge for 2 mm ID column, 3 p/pkg	5	2.0	1.0
19532	TSKgel Amide-80	5	4.6	5.0
19533	TSKgel Amide-80	5	4.6	10.0
13071	TSKgel Amide-80	5	4.6	25.0
19021	Guard Column for 4.6 + 7.8 mm ID column	5	4.6	1.0
19010	Guard Cartridge for all 4.6 mm ID column, 3 p/pkg	5	3.2	1.0
14459	TSKgel Amide-80	10	7.8	30.0
14460	TSKgel Amide-80	10	21.5	30.0
14461	Guard column for P/N 14460	10	21.5	7.5
19308	Guard Cartridge Holder (2.0 -1.0) for all 2 mm ID x 1 cm L Cartridges			
19018	Guard Cartridge Holder (3.2-1.5), for all 3.2 mm ID x 1.5 cm L Cartridges			

For further details of choice and selection of the TSK-GEL® column that best suits your particular separation needs, please contact us:

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