HILIC
HYDROPHILIC INTERACTION CHROMATOGRAPHY

HILIC PRODUCTS

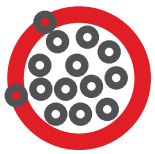
TSKgel Amide-80

NEW

3 µm Particle Size

TOSOH FACT

The first columns used in chromatography were glass, both for liquid-solid chromatography by Tswett in his separation of plant pigments and by James and Martin in their first gas chromatograph. However, as the technique developed and particle size was reduced, the length of the columns in liquid chromatography was decreased. This resulted in the columns having to be operated at higher pressures. To accommodate these higher pressures, stainless steel columns were introduced. Tosoh introduced its first HPLC (GPC) columns in 1971, which were composed of stainless steel. Recently, columns packed in PEEK, a biocompatable fluorocarbon polymer, became available. PEEK can withstand the pressures commonly encountered in HPLC.



INTRODUCTION TO TSK-GEL HILIC COLUMNS

HIGHLIGHTS

- Stable bonding chemistry
- Unique polar phase
- Handles a wide spectrum of sample polarities
- Stable in 100% organic
- Separates many different types of polar molecules
- New 3 µm particle size for LC/MS analysis

Hydrophilic interaction chromatography (HILIC) is used primarily for the separation of polar and hydrophilic compounds. HILIC has similarities with traditional normal phase chromatography, but the mobile phases for HILIC are similar to those known from reversed phase chromatography (RPC). They include polar organic solvents like methanol or acetonitrile and water. Compared to RPC the elution order in HILIC mode is inversed for most substances.

HILIC is often used to separate hydrophilic compounds such as peptides, carbohydrates and small polar drug candidates or metabolites. Hydrophilic compounds are retained on the polar-bonded phase column while nonpolar sample impurities elute unretained in the void volume. In addition it is ideally suited 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.

The TSK-GEL AMIDE-80 column offers an excellent alternative to amino-bonded stationary phases and consists of 3, 5 or 10 µm silica particles in a stainless steel format. Spherical silica particles are covalently bonded with carbamoyl groups. Based on hydrogen bonds the aqueous content of the mobile phase creates a water-rich layer on the particle surface. This allows for partitioning of polar compounds 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. Typical mobile phases consist of acetonitrile buffer mixtures. Samples are eluted from the column by increasing the percentage of the aqueous component.

For years TSKgel Amide-80 columns have been the standard for the analysis of glycans. TSKgel Amide-80 columns packed with 3 µm particles are the newest addition to the TSKgel Amide-80 series. The 3 µm HILIC columns reduce analysis time and improve peak capacity and sensitivity for HPLC and LC-MS analysis.

Column Operation and Specifications

TSKgel Amide-80 columns can be operated over a broad range of mobile phase conditions for use with many sample polarities. Factors to consider when employing this column include:

Sample Loading Capacity: this is dependent upon the polarity of the mobile phase. Loading capacity increases with decreasing mobile phase polarity. For example, the highest loading capacity for mannitol (200 µg) occurs with a mobile phase of 75:25 acetonitrile/water. However, <100 µg of mannitol can be loaded in a mobile phase of 65:35 acetonitrile/water. The maximum sample volume for a 4.6 mm ID x 25 cm L Amide-80 analytical column is 50 µL.

Pressure Limitations: Column pressure drop varies with mobile phase viscosity. For mobile phases containing high water concentrations, the back-pressure should be < 120 kg/cm^2 for 1 mm ID columns, < 150 kg/cm^2 for 2 mm ID columns, <150 kg/cm^2 for 4.6 mm ID columns, < 70 kg/cm^2 (for 7.8 mm ID columns, and < 30 kg/cm^2 for 21.5 mm ID columns.

Temperature Range: the TSKgel Amide-80 column can be operated over a temperature range of 4-80°C (4-40°C for Amide-80 3µm). In general, retention times for carbohydrates decrease with increasing temperature, thereby shortening analysis time. Below certain temperatures some carbohydrates may elute as split peaks. In this case, column heating or addition of triethylamine to the mobile phase is required.

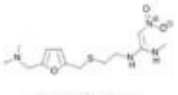
Choice of Mobile Phase: the pH range of the TSKgel Amide-80 column is 2.5-7.5 with a maximum salt concentration of 100 mmol/L. The column is stable in 100% organic; however, a combination of aqueous and organic solvents is necessary in order to create the water-rich surface layer. Elution volume can be controlled by the mobile phase polarity. As the mobile phase polarity decreases (higher organic content) the sample is retained longer on the column. For example, oligosaccharides require 40-50% water in the mobile phase in order to elute from the Amide-80 column.

HILIC

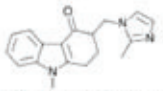
Small polar molecule drugs:

TSKgel Amide-80 is a valuable tool for the analysis of small, polar molecule drugs that cannot be retained very well by reversed phase LC columns.

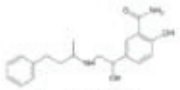
FIGURE 1 compares the separation of polar, drug standards with detection by electrospray ionization mass spectroscopy (ESI-MS) in HILIC mode compared to reversed phase mode. Due to the high organic content of the eluent HILIC analysis provides increased detection sensitivity.



Ranitidine
315/176



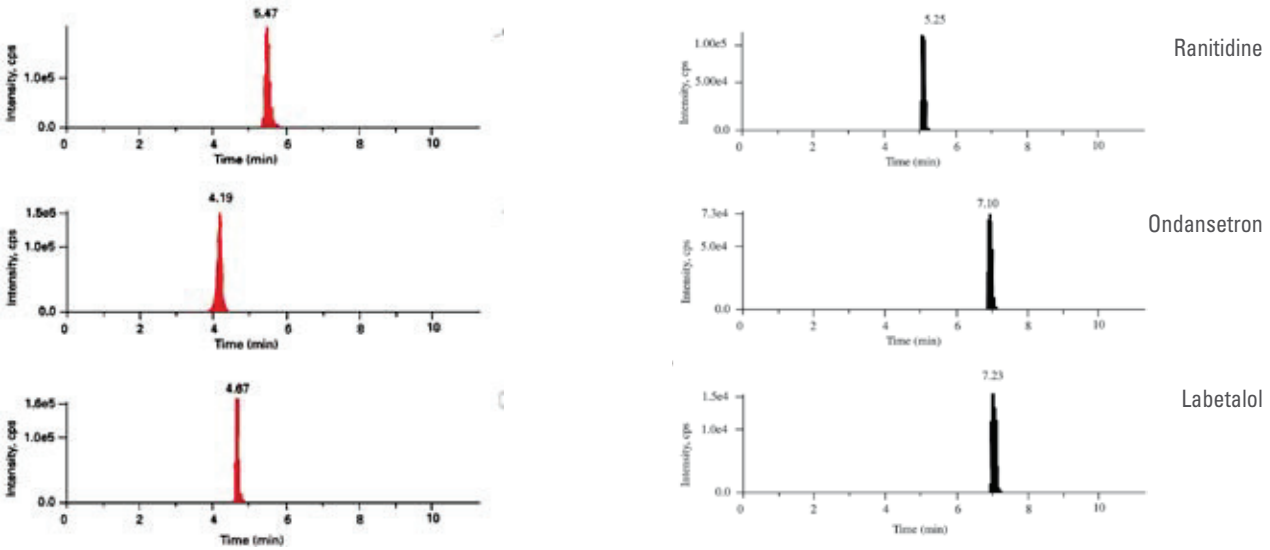
Ondansetron
294/212



Labetalol
329/162

FIGURE 1

ESI-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)	Column:	TSKgel ODS-100V 3 µm (2.0 mm ID x 15 cm L)
Eluent :	A: 10 mM Ammoniumformiate (pH 3.75)	Eluent :	A: 10 mM Ammoniumformiate (pH 3.75)
	B: ACN		B: ACN
Gradient :	0 min (B 90%) -> 10 min (B 40%) ->13 min (B 40%)	Gradient :	0 min (B 0%) -> 10 min (B 80%) ->13 min (B 80%)
Flow rate :	0.2 mL/min	Flow rate :	0.2 mL/min
Inj. volume :	5 µL (50 µg/L)	Inj. volume :	5 µL (50 µg/L)
Detection :	QTrap® LC-MS/MS (Applied Biosystems), ESI+	Detection :	QTrap® LC-MS/MS (Applied Biosystems), ESI+

Polyalcohols

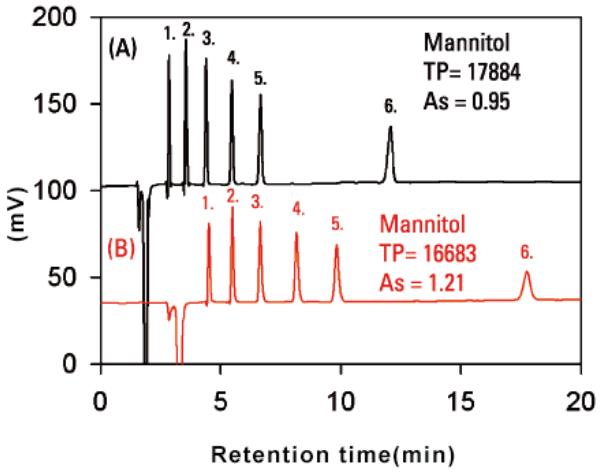
Polyalcohols are typically separated with a mobile phase of organic solvent and water as shown in FIGURE 2 for a 3 µm TSKgel Amide-80 column compared to a 5 µm column.

Oligosaccharides

The TSKgel Amide-80 can separate oligosaccharides very rapidly and efficiently. FIGURE 3 shows a separation of a ß-cyclodextrin hydrolysate in less than 10 minutes. The labels indicate the number of base sugars such as glucose in each oligomer.

FIGURE 2

Separation of polyalcohols on TSGgel Amide-80 3 µm and 5 µm

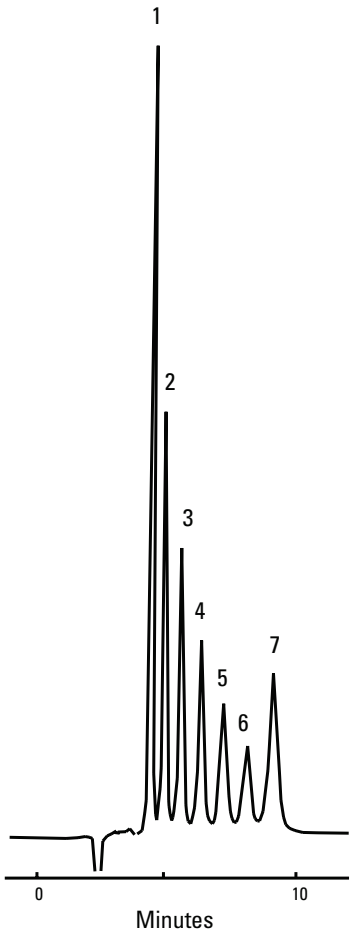


Conditions

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 3

Separation of β-cyclodextrin hydrolysate on TSKgel Amide-80 column



Column:	TSKgel Amide-80, 4.6mm ID x 25cm
Sample:	2µL, β-cyclodextrin hydrolysate, 1-7 degrees of polymerization (4.6mg/mL)
Elution:	ACN/water (55/45)
Flow Rate:	1.0mL/min
Detection:	refractive index detector
Temperature:	25°C

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