Newly developed, high performance HILIC columns for use in pharmaceutical analysis by LC/MS

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Introduction

TSK-GEL Amide-80, 3µm hydrophilic interaction liquid chromatography (HILIC) columns were developed for use in LC/MS applications, in particular for the analysis of active pharmaceutical ingredients and their metabolites. HILIC is known as one type of normal phase chromatography to retain hydrophilic compounds; such compounds are difficult to effectively retain by reversed phase chromatography (RPC). HILIC has been one of the essential tools for the separation of oligosaccharides/glycans in glycomics and recently it has become one of the essential tools in pharmaceutical analysis, along with RPC.

Preliminary Results

TSK-GEL Amide-80, 3µm HILIC columns were prepared by reacting carbamoyl-containing silane reagents to the silica surface. The resulting phase retained polar/hydrophilic compounds that were either only moderately retained, or not at all retained, on reversed phase columns. A 4.6mm ID TSKgel Amide-80, 3µm column was found to show higher column efficiency for mannitol, even at higher linear velocity, when compared with a 4.6mm ID TSKgel Amide-80, 5µm column. As expected, using shorter length columns, faster separations of saccharides, along with higher resolution, were possible on the TSKgel Amide-80, 3µm column. In several glycomics applications, a 2mm ID TSKgel Amide-80, 3µm column showed higher resolution and reduced analysis time for sugar chains as a 2mm ID TSKgel Amide-80, 5µm column. Compared with other commercial HILIC columns, the TSK-GEL Amide-80, 3µm columns showed appropriate retention and good separation of sugar alcohols. Good durability with regards to column performance in repetitive injections was shown with these 3µm columns. The behavior of 2mm ID TSK-GEL Amide-80, 3µm HILIC columns in LC/MS applications was studied, including the effects of mobile phase components, buffer concentration and pH on retention, selectivity and column efficiency.

Experimental Conditions

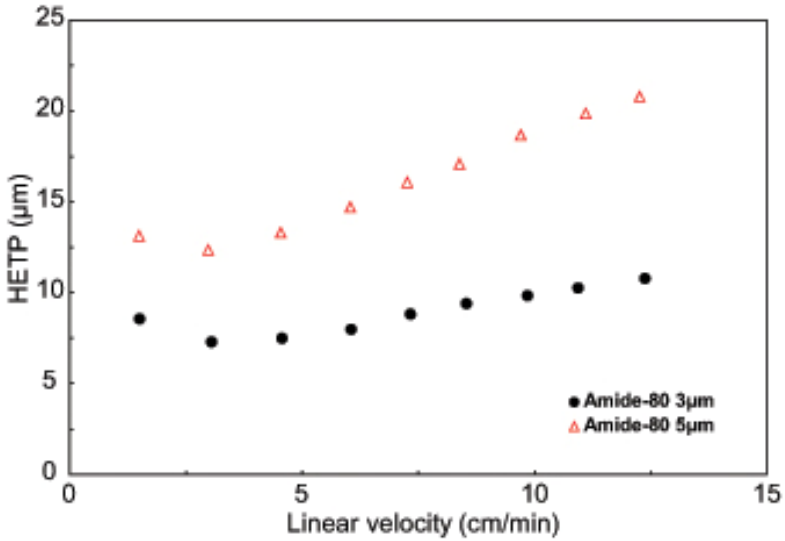
TSK-GEL Amide-80, 3µm HILIC columns were developed by Tosoh Corporation. 4.6mm ID columns were used for fundamental experiments, such as testing for various mobile phase components, buffer concentration and pH.

A Tosoh HLC-8020 series LC system, with a refractive index (RI) detector, was used for all separations. For glycomics and LC/MS applications, 2mm ID columns were used. For LC/MS applications, the columns were coupled to a QTRAP mass spectrometer from Applied Biosystems.

Table 1: Basic Properties of Three Micron TSK-GEL Amide-80 Columns

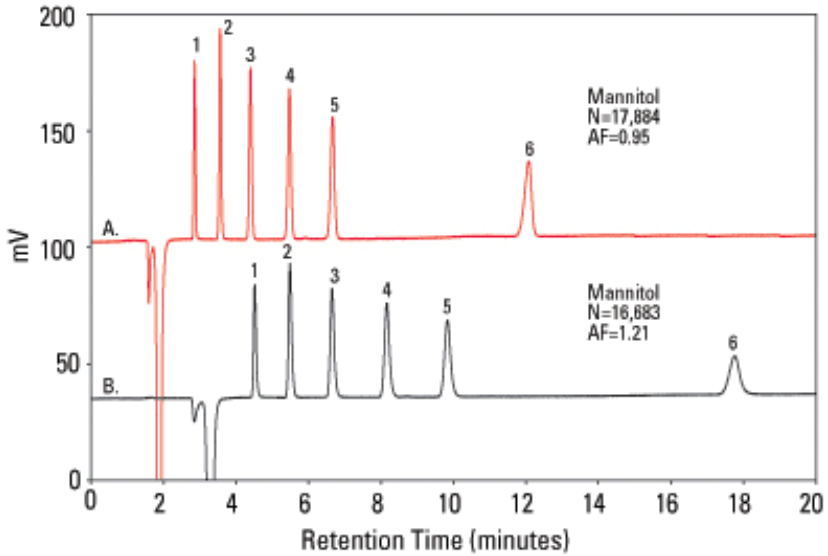
TSK-GEL Amide-80, 3µm	
Particle size (µm)	3
Pore size (nm)	10
Surface area (m^2/g)	450
Functionality	Carbamoyl group

Figure 1: H/u Curves for Three and Five Micron TSK-GEL Amide-80 Columns



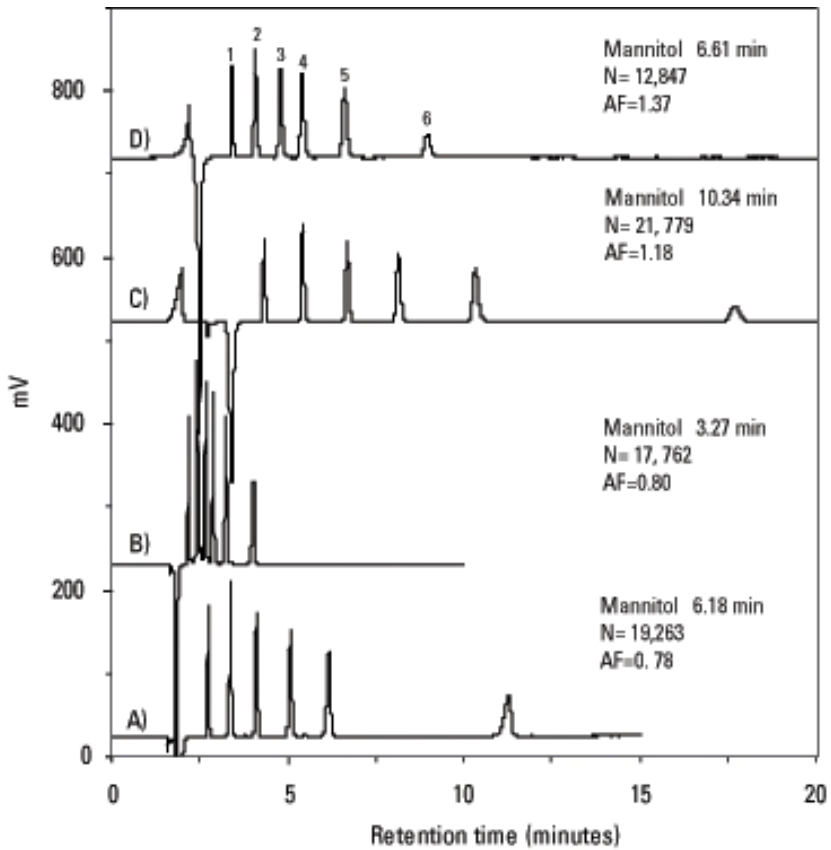
Columns:	TSKgel Amide-80, 3µm (4.6mm ID x 15cm)
	TSKgel Amide-80, 5µm (4.6mm ID x 25cm)
Eluent:	H_2O/ACN=25/75
Flow rate:	1.0mL/min
Detection:	RI
Temperature:	40°C
Inj. volume:	10µL
Sample:	mannitol

Figure 2: Chromatogram Comparison for Sugar Alcohols on TSKgel Amide-80 3µm and 5µm Column



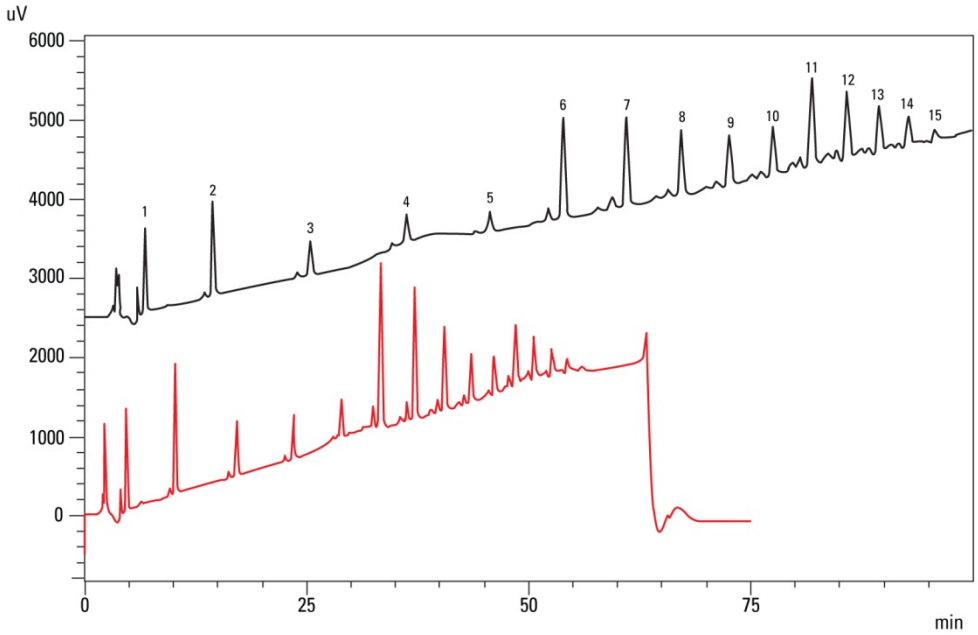
Columns:	A. TSKgel Amide-80, 3µm (4.6mm ID x 15cm) B. TSKgel Amide-80, 5µm (4.6mm ID x 25cm)
Eluent:	H_2O/ACN=25/75
Flow rate:	1.0mL/min
Detection:	RI
Temperature:	25°C
Inj. volume:	10µL
Samples:	1. ethylene glycol 2. glycerin 3. erythritol 4. xylitol 5. mannitol 6. inositol

Figure 3: Comparison of Chromatograms for Sugar Alcohols on TSKgel Amide-80, 3µm and Commercial HILIC Columns



Columns:	A) TSKgel Amide-80, 3µm (4.6mm ID x 15cm)
	B) Luna, 3um NH_2, 100Å (4.6mm ID x 15cm)
	C) Polyamine (4.6mm ID x 25cm)
	D) NH_2P-50 4E (4.6mm ID x 25cm)
Eluent:	H_2O/ACN (25/75)
Flow rate:	1.0ml/min
Detection:	RI
Temperature:	40°C
Inj. volume:	10µL
Samples:	1. ethylene glycol
	2. glycerin
	3. erythritol
	4. xylitol
	5. mannitol
	6. inositol

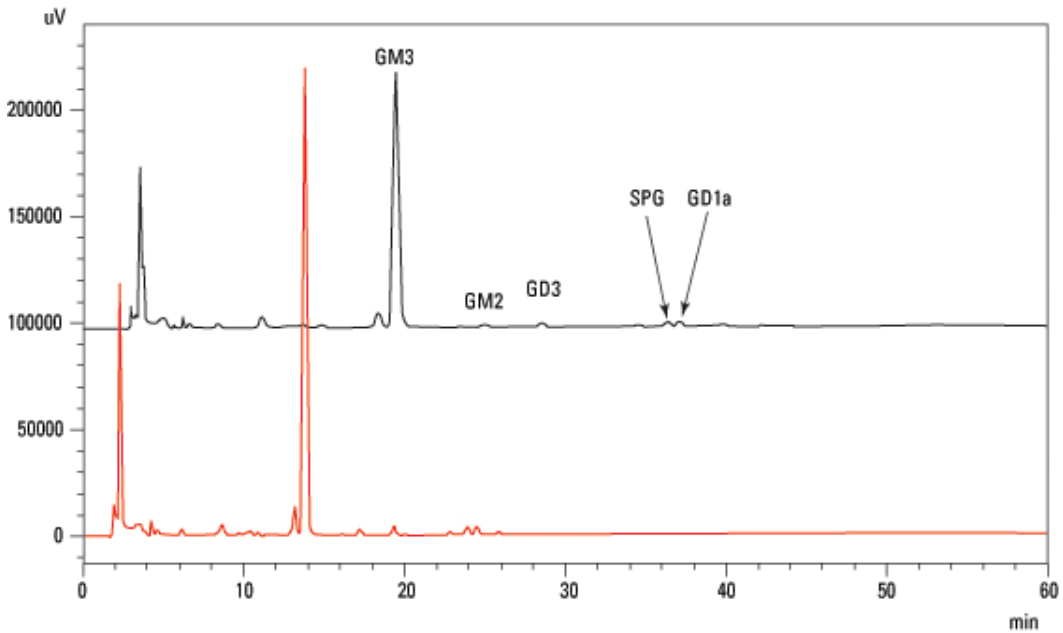
Figure 4: Comparison of Standard PA-Glucose Oligomers* on TSKgel Amide-80, 3µm and 5µm Column



Columns:	TSKgel Amide-80, 5µm (2.0mm ID x 25 cm), upper chromatogram TSKgel Amide-80, 3µm (2.0mm ID x 15 cm), lower chromatogram
Eluent:	Solvent A: ACN/0.5mol/L HAc with 10% ACN, adj. to pH 7.3 with TEA (75:15, v/v) Solvent B: ACN/0.5mol/L HAc with 10% ACN, adj. to pH 7.3 with TEA (40:50, v/v)
Linear gradient:	0 to 100% solvent B in 100min for TSKgel Amide-80, 5µm 0 to 100% solvent B in 60min for TSKgel Amide-80, 3µm
Flow rate:	0.2mL/min
Detection:	fluorescence (exc.: 310 nm, em.: 380 nm)
Temperature:	40°C

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

Figure 5: Comparison of PA-Oligosaccharides of Ganglioside from Serum* on TSKgel Amide-80, 3µm and 5µm Column



Columns:	TSKgel Amide-80, 5µm (2.0mm ID x 25 cm), upper chromatogram TSKgel Amide-80, 3µm (2.0mm ID x 25 cm), lower chromatogram
Eluent:	Solvent A: ACN/0.5mol/L HAc containing 10% ACN, adj. to pH 7.3 with TEA (75:15, v/v) Solvent B: ACN/0.5mol/L HAc containing 10% ACN, adj. to pH 7.3 with TEA (40:50, v/v)
Linear gradient:	0 to 100% solvent B in 100min for TSKgel Amide-80, 5µm 0 to 100% solvent B in 60min for TSKgel Amide-80, 3µm
Flow rate:	0.2mL/min
Detection:	fluorescence (exc.: 310nm, em.: 380nm)
Temperature:	40°C

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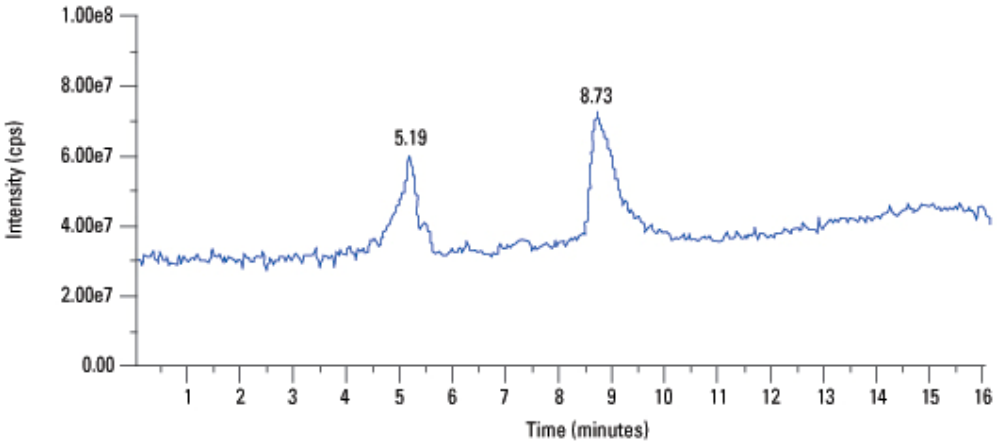
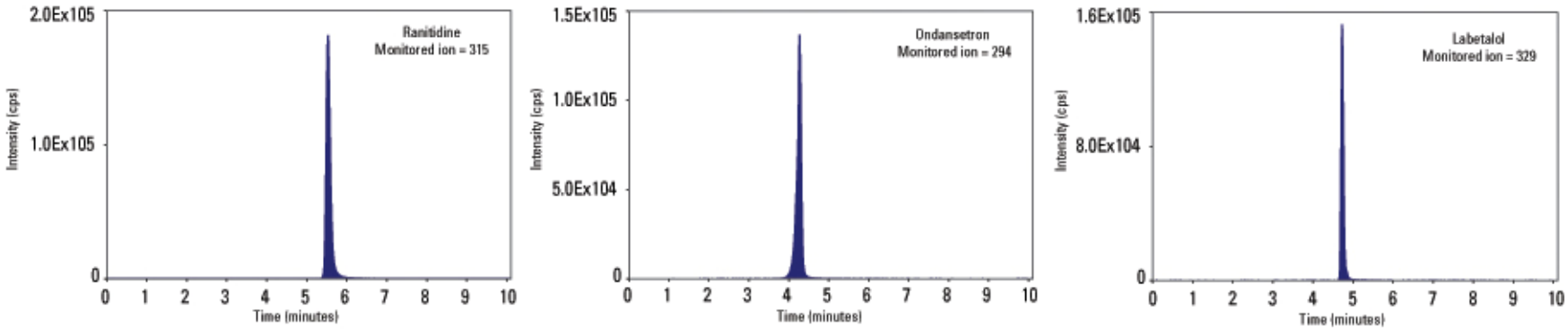
Figure 6: TIC on TSKgel Amide-80, 3µm Column with LC/ESI-MS under Low pH Mobile Phase Conditions



Column:	TSKgel Amide-80, 3µm (2.0mm ID x 15cm)
Eluent:	A: 0.1% HCOOH in H_2O
	B: 0.1% HCOOH in ACN
Gradient:	0min (B 95%)→10min (B 5%)→13min (B 5%)→ 15min (B 95%)
Flow rate:	0.2mL/min
Detector:	ESI+, TIC (Range: 50-1000)

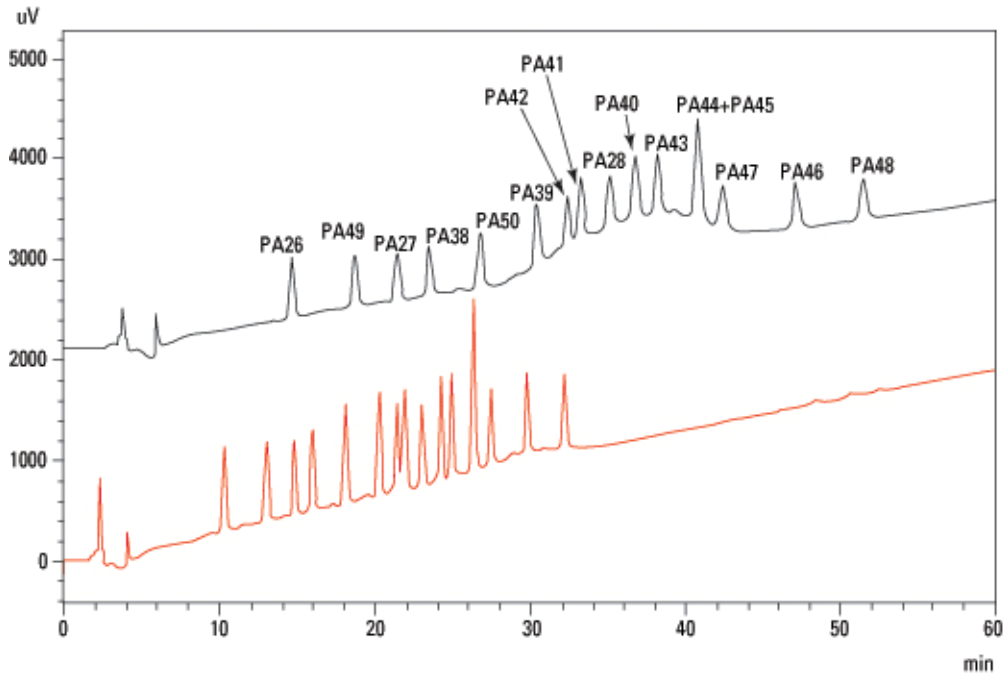
Comment:
Low level of background noise was observed in the total ion chromatogram for LC/ESI-MS using an acidic mobile phase containing 0.1% formic acid with a gradient elution method.
No sample was injected.

Figure 7: Comparing Behavior of Basic Drugs on TSKgel Amide-80, 3µm Column



Column:	TSKgel Amide-80, 3µm (2.0mm ID x 15cm)
Eluent:	A) 10mM $HCOONH_4$ (pH3.75)
	B) ACN
Gradient:	0min (B 90%)→10min (B 40%)→13min (B 40%)
Flow rate:	0.2mL/min
Inj. volume:	5µL
Concentration:	50ng/mL
Instrument:	QTrap (Applied Biosystems), ESI+

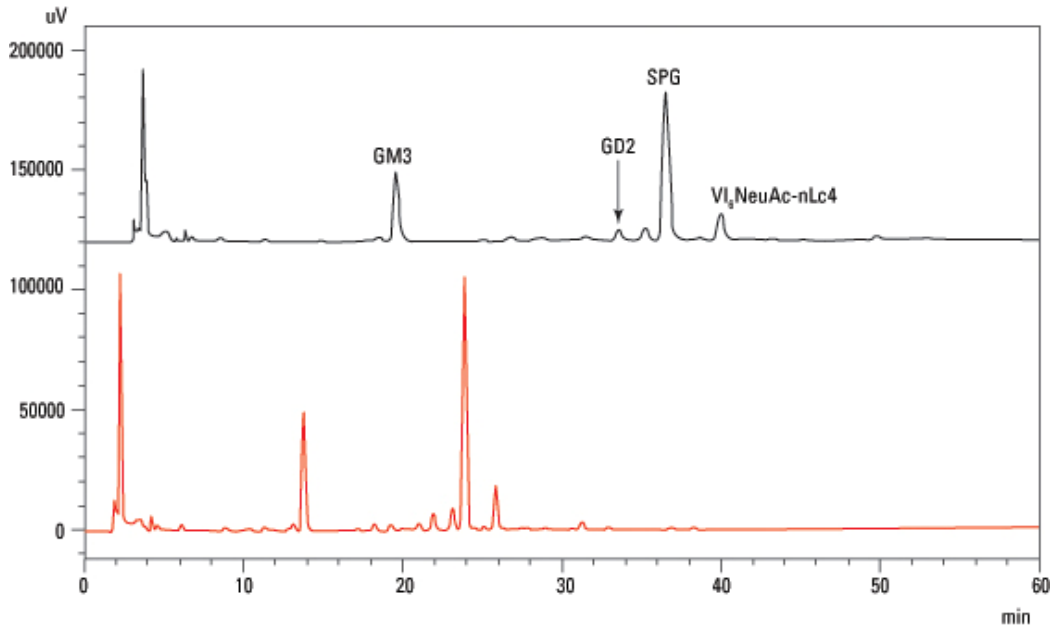
Figure 8: Comparison of Standard PA-Oligosaccharides of Neutral Glycosphingolipid* on TSKgel Amide-80, 3µm and 5µm Column



Column:	TSKgel Amide-80, 5µm (2.0mm ID x 25 cm), upper chromatogram TSKgel Amide-80, 3µm (2.0mm ID x 25 cm), lower chromatogram
Eluent:	Solvent A: ACN/0.5mol/L HAc containing 10% ACN, adj. to pH 7.3 with TEA (75:15, v/v) Solvent B: ACN/0.5mol/L HAc containing 10% ACN, adj. to pH 7.3 with TEA (40:50, v/v)
Linear gradient:	0 to 100% solvent B in 100min for TSKgel Amide-80, 5µm 0 to 100% solvent B in 60min for TSKgel Amide-80, 3µm
Flow rate:	0.2mL/min
Detection:	fluorescence (exc.: 310 nm, em.: 380 nm)
Temperature:	40°C

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

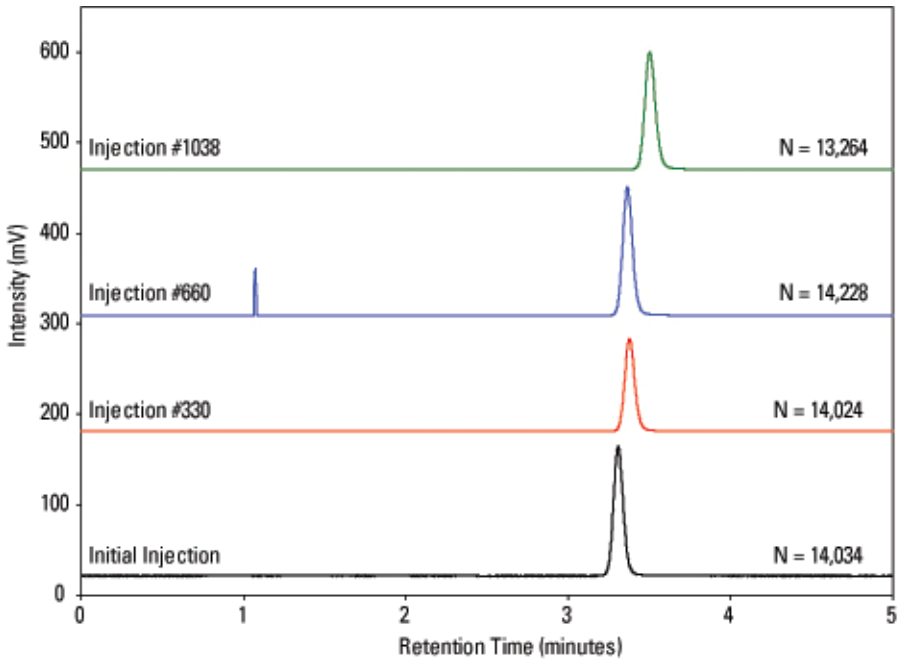
Figure 9: Comparison of PA-Oligosaccharides of Ganglioside from Erythrocyte Membrane* on TSKgel Amide-80, 3µm and 5µm Column



Column:	TSKgel Amide-80, 5µm (2.0mm ID x 25 cm), upper chromatogram TSKgel Amide-80, 3µm (2.0mm ID x 25 cm), lower chromatogram
Eluent:	Solvent A: ACN/0.5mol/L HAc containing 10% ACN, adj. to pH 7.3 with TEA (75:15, v/v) Solvent B: ACN/0.5mol/L HAc containing 10% ACN, adj. to pH 7.3 with TEA (40:50, v/v)
Linear gradient:	0 to 100% solvent B in 100min for TSKgel Amide-80, 5µm 0 to 100% solvent B in 60min for TSKgel Amide-80, 3µm
Flow rate:	0.2mL/min
Detection:	fluorescence (exc.: 310 nm, em.: 380 nm)
Temperature:	40°C

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

Figure 10: Long Term Stability of TSKgel Amide-80, 3µm Column After Repetitive Injections



Inspection conditions

Column:	TSKgel Amide-80, 3µm (2.0mm ID ×15cm)
Eluent:	H_2O/ACN=15/85
Flow rate:	0.2mL/min
Detection:	UV@254nm
Temperature :	25°C
Inj. volume:	2µL
Sample:	uracil (37mg/L)

Running conditions

Eluent:	A: H_2O/ACN(10/90) +0.1% formic acid B: H_2O +0.1% formic acid
Flow rate:	0.2mL/min
Gradient:	0min(B 0%)→12min(B 100%)
Inj. volume:	5µL
Temperature:	40°C
Sample:	uracil (37mg/L)

Conclusions

(1) TSK-GEL Amide-80, 3µm particles are modified with a hydrophilic stationary phase containing carbamoyl functional groups. Therefore, hydrophilic compounds such as saccharides are well retained.

(2) TSK-GEL Amide-80, 3µm columns showed higher column efficiency for mannitol compared with TSK-GEL Amide-80, 5µm columns, while selectivities for saccharides on both columns were very similar.

(3) In glycomics applications, the TSKgel Amide-80, 3µm column showed higher resolution of PA-oligosaccharides, with half the separation time, compared to the TSKgel Amide-80, 5µm column.

(4) The TSKgel Amide-80, 3µm column showed a low background noise in the LC/ESI-MS application.

(5) Good durability with regards to column performance in repetitive injections was shown with TSK-GEL Amide-80, 3µm columns.