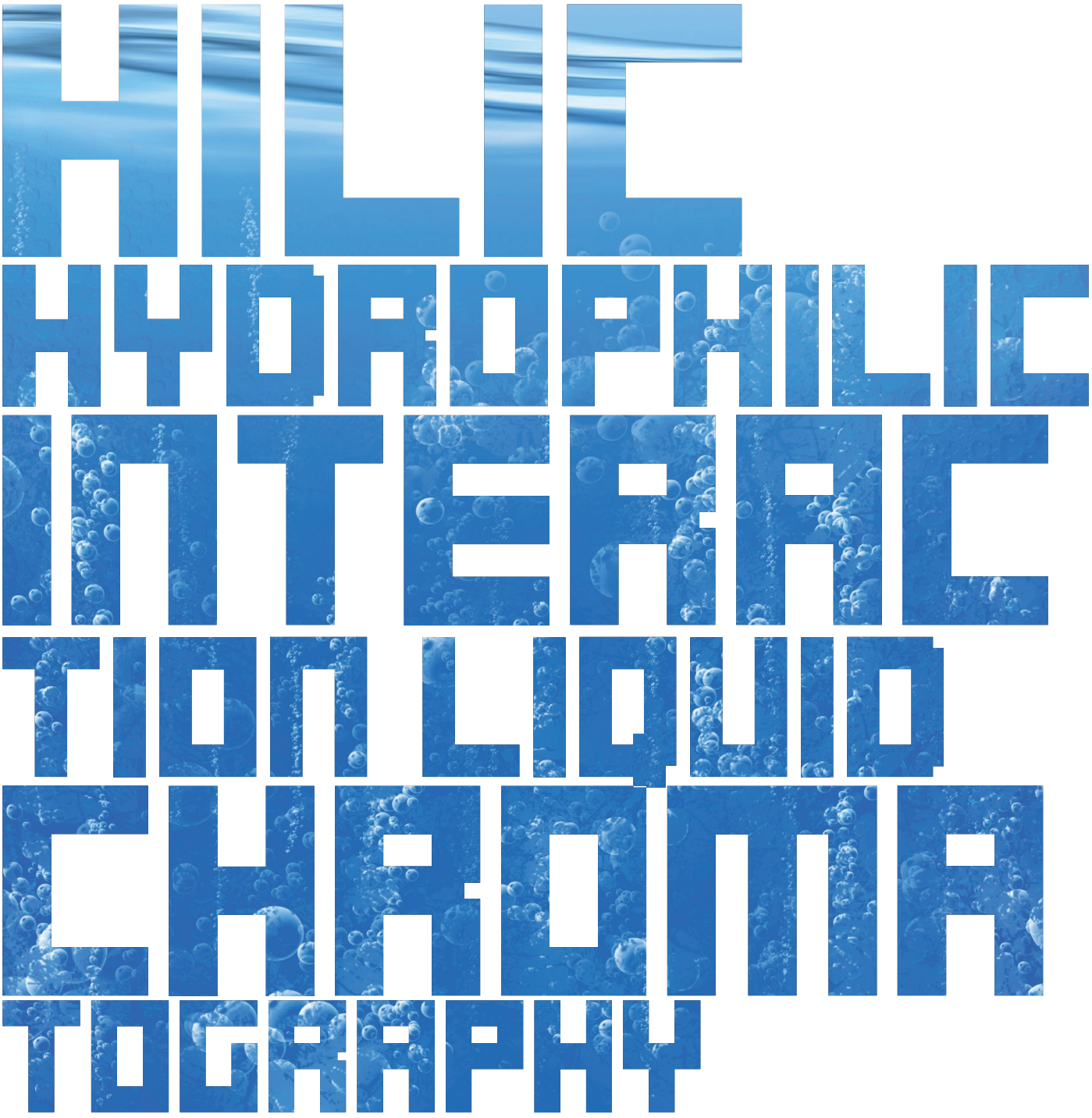
TOSOH

TSKgel HILIC COLUMNS



TOSOH BIOSCIENCE

1
TOSOH BIOSCIENCE GMBH
IM LEUSCHNERPARK 4
64347 GRIESHEIM
GERMANY

T + 49 (0) 6155 70437 00
F + 49 (0) 6155 83579 00
INFO.TBG@TOSOH.COM
WWW.TOSOHBIOSCIENCE.DE

2
TOSOH BIOSCIENCE LLC
3604 HORIZON DRIVE,
SUITE 100
KING OF PRUSSIA, PA 19406, USA

T +1 484 805 1219
F +1 610 272 3028
INFO.TBL@TOSOH.COM
WWW.SEPARATIONS.US.TOSOHBIOSCIENCE.COM

3
TOSOH CORPORATION
3-8-2 SHIBA, MINATO-KU
TOKYO 105-8623
JAPAN

T +81 3 5427 5118
F +81 3 5427 5198
INFO@TOSOH.CO.JP
WWW.TOSOHBIOSCIENCE.COM

4
TOSOH BIOSCIENCE SHANGHAI CO. LTD.
ROOM 301, PLAZA B,
NO. 1289 YI SHAN ROAD
XU HUI DISTRICT
SHANGHAI, 200233, CHINA
T +86 21 3461 0856
F +86 21 3461 0858
INFO@TOSOH.COM.CN
WWW.SEPARATIONS.ASIA.TOSOHBIOSCIENCE.COM

5
TOSOH ASIA PTE. LTD.
63 MARKET STREET #10-03
BANK OF SINGAPORE CENTRE
SINGAPORE 048942, SINGAPORE

T +65 6226 5106
F +65 6226 5215
INFO.TSAS@TOSOH.COM
WWW.SEPARATIONS.ASIA.TOSOHBIOSCIENCE.COM

TOSOH HISTORY

1935	FOUNDING OF TOYO SODA MANUFACTURING CO., LTD.
1936	OPERATION OF NANYO MANUFACTURING COMPLEX BEGINS
1971	SCIENTIFIC INSTRUMENTS DIVISION FORMED, FIRST GPC COLUMN USING TSKgel DEVELOPED BY TOSOH
1974	HIGH PERFORMANCE LIQUID CHROMATOGRAPHY COLUMN PLANT IS COMPLETED
1979	TOSOH DEVELOPS TOYOPEARL MEDIA
1983	TOSOH DEVELOPS HYDROPHOBIC INTERACTION MEDIA
1987	TOSOHAAS US OPERATIONS FORMED IN MONTGOMERYVILLE
1989	TOSOHAAS GMBH OPERATIONS FORMED IN STUTTGART
1995	TOSOH NANYO GEL FACILITY RECEIVES ISO 9001
2002/2003	ALL TOSOH AFFILIATED SCIENTIFIC & DIAGNOSTIC SYSTEM RELATED COMPANIES IN EUROPE ARE UNIFIED UNDER THE NAME TOSOH BIOSCIENCE.
2008	EcoSEC, THE 7TH GENERATION GPC SYSTEM IS INTRODUCED GLOBALLY
2010	TOSOH CELEBRATES ITS 75TH YEAR IN BUSINESS WITH THE OPENING OF FIVE NEW PLANTS, AND CONTINUED RAPID EXPANSION IN CHINA
2011	TOSOH BIOSCIENCE CELEBRATES 40 YEARS OF OPERATION
2012	TOSOH RELEASES FIRST TOYOPEARL MIXED-MODE RESIN TOYOPEARL MX-Trp-650M
2013	TOSOH RELEASES A HIGH CAPACITY PROTEIN A CHROMATOGRAPHY RESIN
2014	TOSOH BIOSCIENCE GMBH CELEBRATES ITS 25TH ANNIVERSARY IN STUTTGART
2015	TOSOH BIOSCIENCE SUCCESSFULLY MOVES ITS SALES & MARKETING OFFICES TO GRIESHEIM, DARMSTADT

HILIC
HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY

Hydrophilic interaction liquid chromatography (HILIC) is used primarily for the separation of polar and hydrophilic compounds. HILIC stationary phases are polar, similar to normal phase chromatography (NPC), but mobile phases are similar to reversed phase chromatography (RPC). Typical mobile phases are aqueous buffers with organic modifiers - primarily acetonitrile - applied in isocratic or gradient mode. In contrast to RPC, water has the highest elution power in HILIC mode. Therefore HILIC gradients usually start with a high percentage of acetonitrile. Typical HILIC stationary phases are silica or polymer particles carrying polar functional groups, e.g. hydroxyl, carbamoyl, amino or zwitterionic groups.

Analysis of glycans, carbohydrates, peptides, polar drugs and metabolites, vitamins and other hydrophilic compounds are typical HILIC applications. HILIC is ideally suited for mass spectrometric analysis of water soluble polar compounds, because the high organic content in the mobile phase increases MS detection sensitivity. While using similar eluent systems HILIC and reversed phase can also be combined for two-dimensional liquid chromatography (2D-LC).

Tosoh Corporation employs state-of-the-art manufacturing techniques that result in uniformly bonded packing materials with narrow pore size distributions and well-defined particle sizes to ensure high performance. Silica based TSK-GEL Amide-80 and NH2-100 HILIC columns enable the user to solve the most complex separation problems.

HIGHLIGHTS

- HILIC offers orthogonal selectivity to reversed phase chromatography
- Covalently bonded carbamoyl and amino phases expand selectivity options
- Novel TSKgel NH2-100 columns show superior stability compared to conventional amino phases
- TSKgel Amide-80 columns provide unique retention mechanism for saccharide analysis
- Superior resolution and sensitivity with 3 µm particle size

HILIC
HOW IT WORKS

It is commonly believed that in HILIC the aqueous content of the mobile phase creates a water rich layer on the surface of the stationary phase. This allows for partitioning of solutes between the more organic mobile phase and the aqueous layer. Hydrogen bonding and dipole-dipole interactions have been supposed to be the dominating retention mechanisms in HILIC mode (Figure 1).

The number of polar groups, as well as the conformation and solubility of the sample in the mobile phase determine the elution order. Since the retention is also related to the type of functional groups of the stationary phase, it varies between different HILIC phases. Compared to RPC the elution order in HILIC mode is inversed for most compounds. Figure 2 gives an example for the differences in selectivity of HILIC and RPC. Peptides were separated by C18 and HILIC columns of the same dimensions using the same eluents but almost inverse gradients.

At low acetonitrile concentrations HILIC columns show a reversed phase mode of retention. The HILIC mode can only be executed when starting at high acetonitrile concentrations.

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.

In method development HILIC is an option as soon as polar compounds have to be analyzed and retention on reversed phase columns is too low. Since common RPC solvents can be used, TSK-GEL HILIC columns can be implemented in method development systems using automated column selection. A range of reversed phase columns differing in hydrophobicity or carrying polar embedded groups and one of the TSK-GEL HILIC column types should deliver an indication for the right direction of method development.

TSK-GEL HILIC columns are available in various dimensions and particles sizes, functionalized with carbamoyl- or amino-groups. This enables the user to perfectly match HILIC selectivity to specific separation needs.

FIGURE 1

HILIC principles

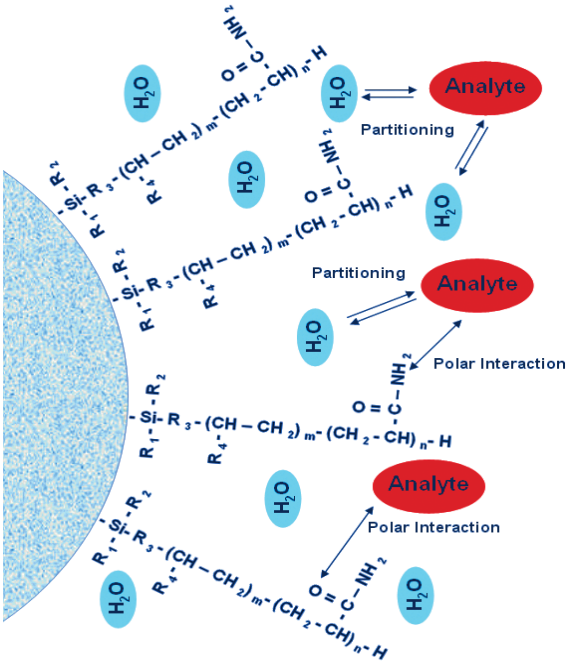
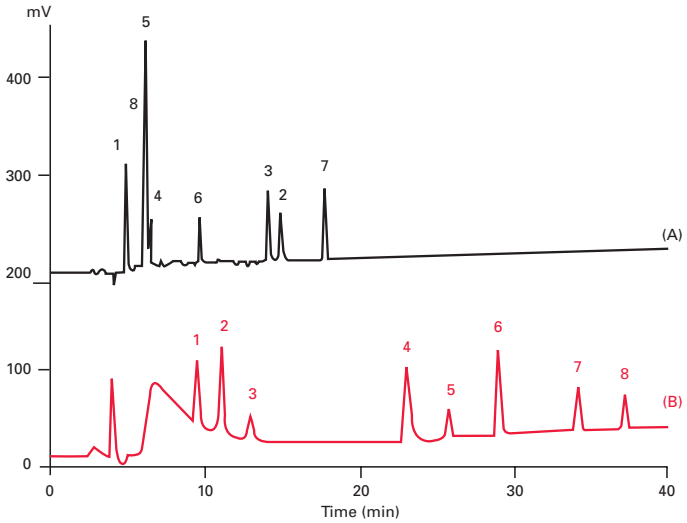


FIGURE 2

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

HILIC
TSKgel Amide-80

TSKgel Amide-80 columns with small particle size (3 µm) and a new high resolution type of TSKgel Amide-80 5 µm columns are the latest additions to the well-known TSKgel Amide-80 series. For years TSKgel Amide-80 columns are used successfully for HILIC separations of polar compounds, documented in more than 250 scientific publications. Packed with spherical silica particles that are covalently bonded with non-ionic carbamoyl groups (Figure 3), they provide higher stability than conventional amino-phases and a unique selectivity. TSKgel Amide-80 3 µm columns reduce analysis time and improve peak capacity and sensitivity for both, HPLC and LC-MS analysis.

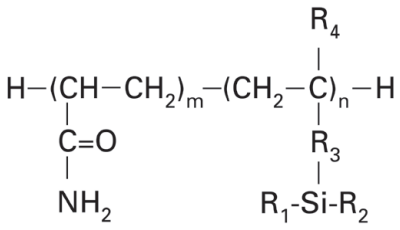
An additional benefit of TSKgel Amide-80 for mass spectrometric as well as for evaporative light scattering detection is the virtual absence of column bleeding due to the covalently bonded functional groups.

Separation of polar compounds

Figure 4 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.

FIGURE 3

Structure of TSKgel Amide-80



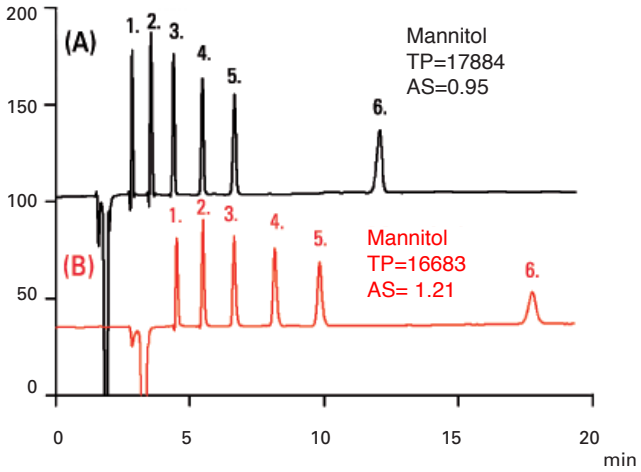
Comparison of the retention between mannitol and inositol, each with 6 hydroxyl groups, shows that inositol, which has a cyclic structure and lower solubility in the mobile phase is retained longer. Overall the 3 µm column provides better resolution at reduced analysis time when compared to the 5 µm TSKgel Amide-80 column.

TSKgel Amide-80 long term stability

The high stability of TSKgel Amide-80 columns is demonstrated in Figure 5 showing the same analysis after 330, 660 and more than 1000 runs compared to the first injection. Only 5% reduction of column performance (theoretical plates) is observed after more than 1000 injections.

FIGURE 4

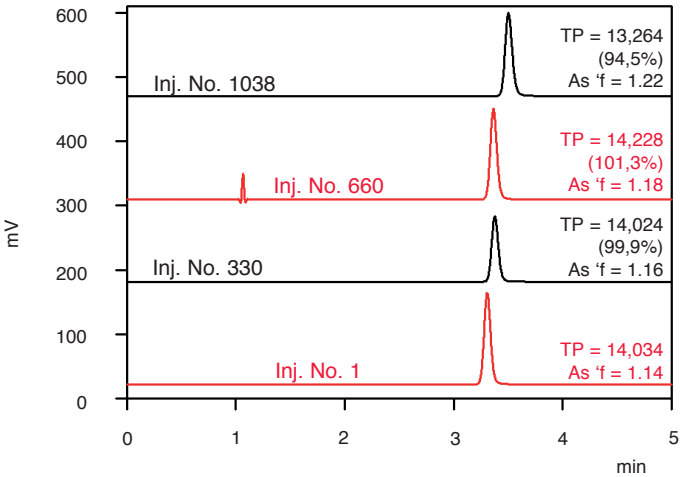
Separation of polyalcohols on TSKgel Amide-80 3 µm and 5 µm



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	
Sample:	1. Ethyleneglycol	2. Glycerin
	3. Erythritol	4. Xylitol
	5. Mannitol	6. Inositol

FIGURE 5

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 °C
Inj. volume :	2 µL
Sample:	Uracil (37 mg/L)

HILIC
TSKgel NH2-100

TSKgel NH2-100 3 µm columns are the latest addition to the HILIC column family. They expand the selectivity range of TSK-GEL HILIC solutions by a new, robust amino-phase. In contrast to conventional silica-based amino phases the new column offers expanded stability under HILIC conditions. It is well suited for the analysis of all types of hydrophilic compounds like carbohydrates, peptides, vitamins, polar drugs or metabolites.

The NH2-100 HILIC phase is based on a silica particle with 3 µm particle and 100 Å pore size, treated with a special endcapping procedure. Amino groups are introduced step wisely after endcapping (Figure 6). The amino groups act as HILIC functional groups without any peak splits. Due to their high ligand density and large surface area TSKgel NH2-100 3µm columns show high retention for very polar compounds.

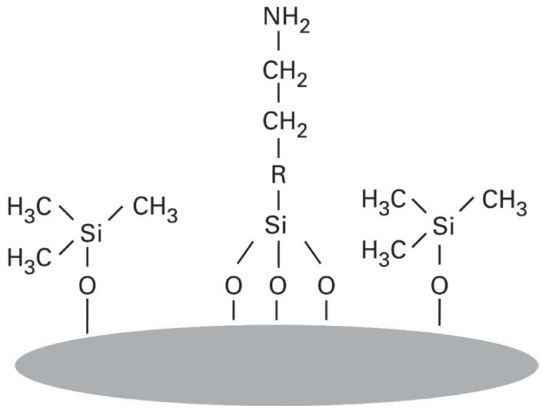
Separation of polar compounds

Figure 7 shows the separation of a standard solution of water soluble vitamins on a TSKgel NH2-100 column compared to a TSKgel Amide-80 column.

Dimension (4.6 mm ID x 15 cm L), particle size (3 µm), flow rate and mobile phase were identical for both columns. The elution order of the compounds changes when applying the same mobile phase to both columns: The TSKgel NH2-100 column shows stronger retention for nicotinic acid, vitamin C, and vitamin B12, while retention of vitamin B1, B2, and pyridoxine is reduced.

FIGURE 6

Structure of TSKgel NH2-100

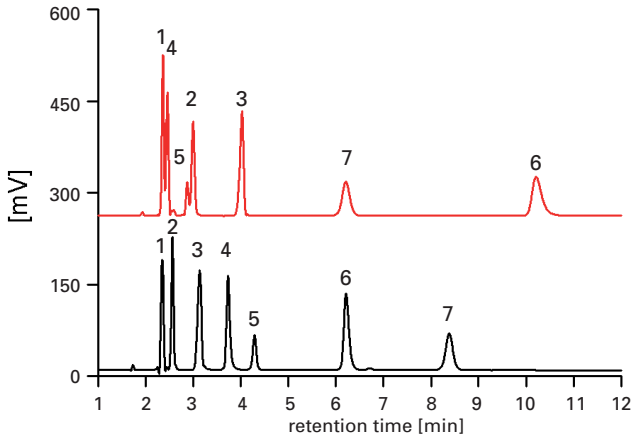


TSKgel NH2-100 long term stability

The high stability of TSKgel NH2-100 columns is demonstrated in Figure 8 showing the change in retention time of inositol after more than 400 hours of flushing with mobile phase compared to the first injection. Only slight reduction of retention time is observed with the TSKgel NH2-100 column compared to a conventional amino-phase.

FIGURE 7

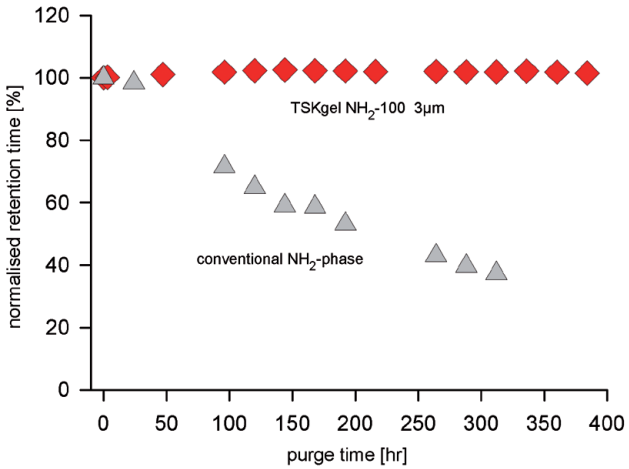
Separation of water soluble vitamins



Columns:	TSKgel Amide-80 3 µm, 4.6 mm ID x 15 cm L
	TSKgel NH2-100 3 µm, 4.6 mm ID x 15 cm L
Eluent:	25 mM phosphate buffer (pH 2.5)/ACN=30/70
Flow rate:	1 mL/min
Temp.:	40°C
Detection:	UV@254 nm
Sample:	Vitamin standard mixture:
	1 = Nicotinamide, 2 = Vitamin B2, 3 = Pyridoxine,
	4 = Nicotinic acid, 5 = Vitamin C, 6 = Vitamin B1,
	7 = Vitamin B12
Injection:	5 µL

FIGURE 8

Long term stability of TSKgel NH2-100 columns



Column :	TSKgel NH2-100 3 µm, 4.6 mm ID x 15 cm L
	Conventional Amino column, 4.6 mm ID.x 25 cm L
Eluent:	H_2O/ACN (25/75)
Flow rate:	1.0 mL/min
Detect:	RI
Temp.:	40 °C
Injection.:	10 µL
Sample:	Inositol

HILIC APPLICATIONS
GLYCAN ANALYSIS

Glycosilation is one of the most common post-translational modifications in eukaryotic cells. Complex N- and O-linked structures composed of repeating sugar moieties form the so called glycans. HILIC with fluorescence detection is the method of choice to effectively separate, identify and quantify glycans after exoglycosidase cleavage and fluorescent labelling. In order to normalize retention times of complex glycan structures a dextran ladder consisting of glucose oligomers is used as calibration reference. The calculated numbers of glucose units (GU) can be used in subsequent database queries (Glycobase, autoGU) to predict the glycan structure.

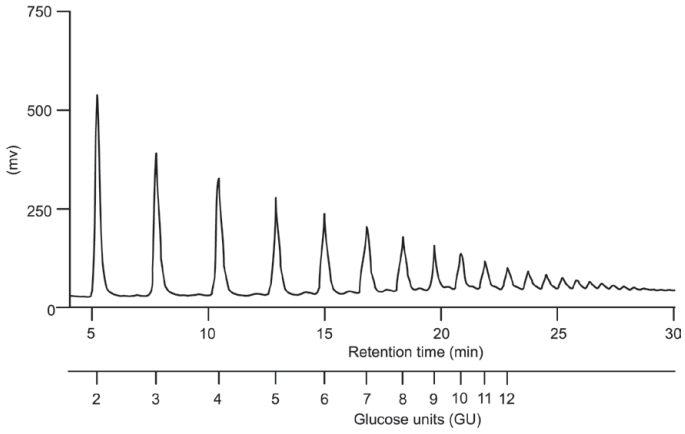
For years TSKgel Amide-80 5 µm columns have been used successfully in glycan analysis. Amide-80 chemistry is ideally suited for the separation of carbohydrate structures. With the new 3 µm particles resolution and sensitivity can be further enhanced. Figure 9 shows the high-resolution separation of a 2-aminobenzamide (2AB) labeled dextran ladder within 30 minutes on a TSKgel Amide-80 3 µm column.

The selectivity of the new TSKgel NH2-100 series differs from TSKgel Amide-80 selectivity as shown in Figure 10. The type of HILIC column should be selected according to the sample type and separation need.

If selectivity or regulatory requirements are not limiting the choice of columns we recommend selecting TSKgel Amide-80 columns instead of amino-phases because they show better long term stability.

FIGURE 9

Separation of a 2AB-labeled Dextran Ladder on TSKgel Amide-80

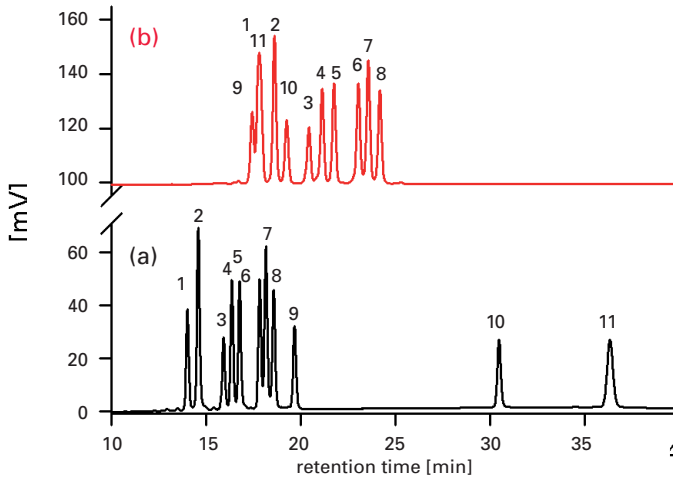


Column:	TSKgel Amide-80 (3 µm, 2.0 mm ID × 15 cm L)
Eluent:	A) 50 mM Ammonium formate (pH 4.3)
	B) Acetonitrile
Gradient:	0-35 min - 75-35 % B
Flow rate:	0.22 mL/min
Detection:	Fluorsecence Ex@360 nm, Em@425 nm
Temperature:	50 °C
Injection vol.:	3 µl
Sample:	CAB-GHP dextran ladder
	(Ludger; ~ 300 fmol for GU2)

* Courtesy of K. Darsow & H. Lange, Institute of Bioprocessing, University of Nürnberg/Erlangen

FIGURE 10

Separation of PA-Glycans on TSKgel NH2-100



Column:	(a): TSKgel NH2-100 3µm, 4.6 mm ID x 15 cm L
	(b): TSKgel Amide-80 3µm, 4.6 mm ID x 15 cm L
Eluent:	(a):
	(A): 0.2 M Triethylamine acetate (pH6.5)/ACN (30/70)
	(B): 0.5 M Triethylamine acetate (pH6.5)/ACN (60/40)
	(b):
	(A): 0.2 M Triethylamine acetate (pH6.5)/ACN (26/74)
	(B): 0.2 M Triethylamine acetate (pH6.5)/ACN (50/50)
Gradient:	0% - 100% B in 30 min, hold at 100% B for 15 min
Flow rate:	1.0 mL/min
Detect.:	Fluorescence Ex@315 nm, Em@380 nm
Temp.:	40 °C
Inj. vol.:	10 µL

HILIC APPLICATION HILIC-MS

High-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) has become a powerful tool when detection sensitivity is an issue. HILIC offers unique advantages for MS 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.

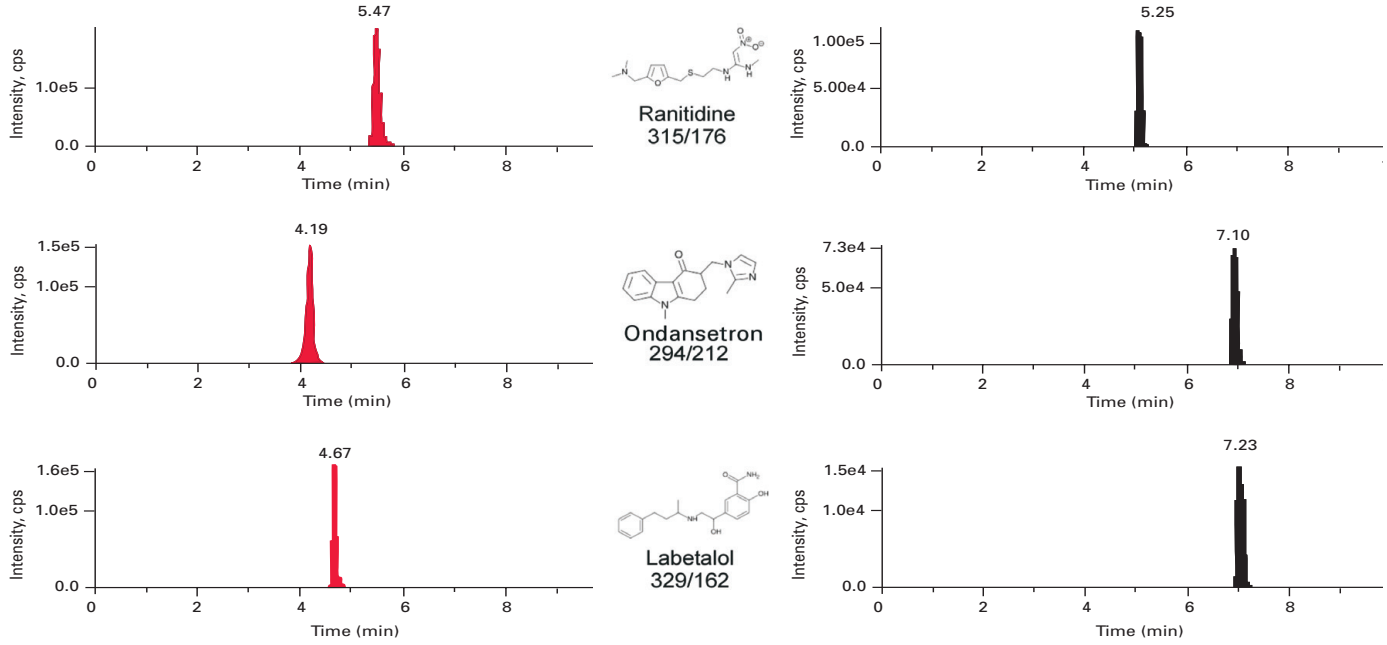
HILIC separations are performed with gradients starting with high percentage of organic solvent and ending with a high portion of aqueous solvent - opposite to typical reversed phase gradients. The elution order of compounds is usually inversed as well. As a result polar compounds are very well separated according to increased polarity in HILIC mode. At the same time the portion of organic solvent in the mobile phase is relatively high.

Figure 11 shows the analysis of basic drug substances using a TSKgel Amide-80 3 µm column compared to the same analysis using a reversed phase TSKgel ODS-100V 3 µm column. Ranitidine, a histamine H2 receptor antagonist, ondansetron, an antiemetic serotonin receptor antagonist, and labetalol, an alpha-1 and beta adrenergic blocker were selected to demonstrate the differences in selectivity and MS-signal response when applying different chromatographic modes.

Ranitidine has the highest number of polar groups among these molecules and as a result shows the highest retention in HILIC and the lowest retention in RPC mode. Signal intensity is almost doubled for ranitidine in HILIC mode. For Labetalol a tenfold increase in signal height can be achieved by using HILIC instead of RPC.

FIGURE 11

LC-MS/MS Analysis of basic drugs in HILIC and RPC mode



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+

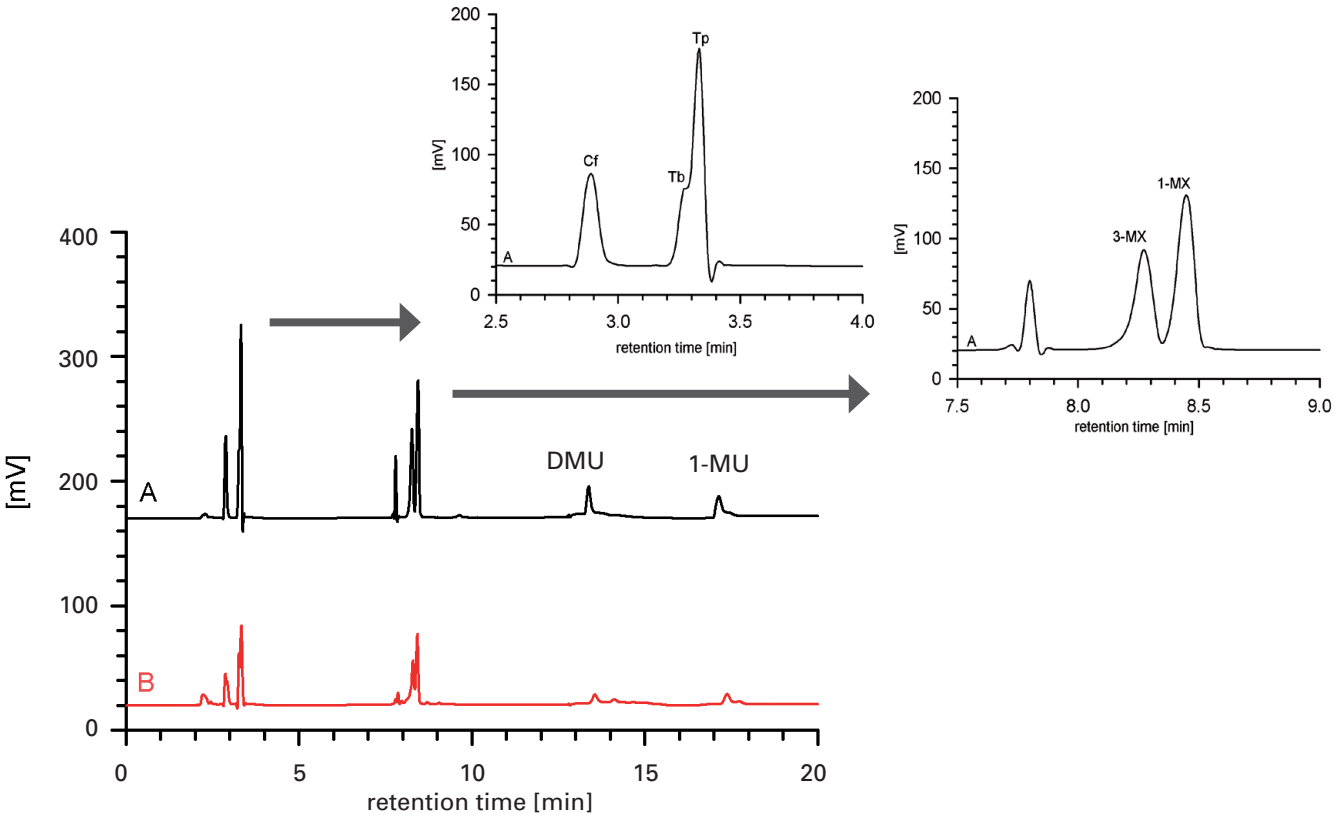
HILIC APPLICATION DRUG METABOLITES

The demand for HILIC separations in the analysis of drug substances is continuously increasing. Combined with tandem or hybrid mass spectrometric detection HILIC is a powerful separation mode for the analysis of polar metabolites in pharmacokinetics or metabolomics studies.

Figure 12 shows the analysis of theophyline and its metabolites in serum after online deproteination, detected by UV absorption. Combining this separation with MS detection would further increase detection sensitivity and facilitate peak identification.

FIGURE 12

Separation of Theophyline and its Metabolites in Serum after online Deproteination



Column:	Analysis: TSKgel NH2-100 3 µm, 4.6 mm ID x 15 cm L
	Deproteination: experimental BSA-ODS-100V precolumn 2.0 mm ID x 1 cm L
Eluent:	Pretreatment; 0.2 M HCO_2NH_4 (pH 3.6) 0 - 0.3 min
	A: ACN
	B: H_2O/ACN=15/85
	C: 0.2 M HCO_2NH_4 (pH 3.6)/ACN=30/70
Step gradient:	0.3 - 2.0 min A, 2.0 - 8.0 min B, 8.0 – 20 min C
Flow rate:	1.0 mL/min, Detection: UV@254 nm,
Temperature:	40 °C
Injection vol.:	5 µL
Sample:	A: Standard
	1. Caffeine (Cf), 2. Theobromine (Tb), 3.Theophyline (TP), 4. 3-Methylxanthine (3-MX), 5. 1-Methylxanthine (1-MX), 6. 1,3-Dimethyluric acid (DMU), 7. 1-Methyluric acid (1-MU) - 50 µg each
	B: Serum spiked with the standard samples

PRODUCT SPECIFICATION

	TSKgel Amide-80	TSKgel NH2-100
Base material	Silica	Silica
Pore size	100 Å	100 Å
Particle size	3 µm, 5 µm & 10 µm	3 µm
Functional group	Carbamoyl	Aminoethyl

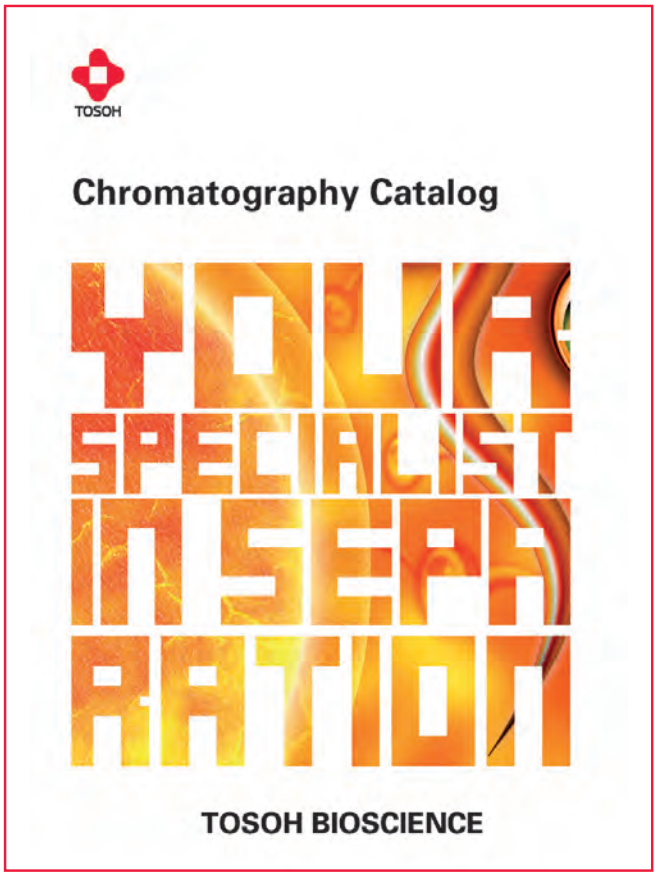
ORDERING INFORMATION

Part #	Description	ID (mm)	Length (cm)	Particle Size (µm)	Number Theoretical Plates	Flow Rate (mL/min) Range	Flow Rate (mL/min) Max.	Maximum Pressure Drop (kg/cm²)
Stainless steel columns								
21864	Amide-80	2.0	5.0	3	≥ 3,500			200
21865	Amide-80	2.0	15.0	3	≥ 13,000			200
21866	Amide-80	4.6	5.0	3	≥ 6,000			200
21867	Amide-80	4.6	15.0	3	≥ 18,500			200
20009	Amide-80	1.0	5.0	5	≥ 300	0.03 - 0.05	0.06	30
20010	Amide-80	1.0	10.0	5	≥ 600	0.03 - 0.05	0.06	60
21486	Amide-80	1.0	15.0	5	≥ 4,000	0.03 - 0.05	0.06	90
21487	Amide-80	1.0	25.0	5	≥ 6,000	0.03 - 0.05	0.06	120
19694	Amide-80	2.0	5.0	5	≥ 1,000	0.15 - 0.20	0.25	40
19695	Amide-80	2.0	10.0	5	≥ 2,000	0.15 - 0.20	0.25	80
19696	Amide-80	2.0	15.0	5	≥ 4,000	0.15 - 0.20	0.25	100
19697	Amide-80	2.0	25.0	5	≥ 6,000	0.15 - 0.20	0.25	150
19532	Amide-80	4.6	5.0	5	≥ 2,500	0.8 - 1.0	1.2	50
19533	Amide-80	4.6	10.0	5	≥ 4,000	0.8 - 1.0	1.2	50
13071	Amide-80	4.6	25.0	5	≥ 8,000	0.8 - 1.0	1.2	150
21982	Amide-80	4.6	25.0	5	≥ 18,000			150
14459	Amide-80	7.8	30.0	10	≥ 5,000	1.0 - 2.0	3.0	70
14460	Amide-80	21.5	30.0	10	≥ 8,000	4.0 - 6.0	8.0	30
21967	NH2-100	2.0	5.0	3	≥ 4,000			150
21968	NH2-100	2.0	15.0	3	≥ 15,000			200
21969	NH2-100	4.6	5.0	3	≥ 6,000			50
21970	NH2-100	4.6	15.0	3	≥ 18,000			150

Guard column products					
21862	Amide-80 Guard cartridge, pk 3	2.0	1.0	3	For 2.0 mm ID columns
21863	Amide-80 Guard cartridge, pk 3	3.2	1.5	3	For 4.6 mm ID columns
21941	Amide-80 Guard cartridge, pk 3	2.0	1.0	5	For all 2.0 mm ID columns
19021	Amide-80 Guard column	4.6	1.0	5	For all 4.6 mm ID columns
19010	Amide-80 Guard cartridge, pk 3	3.2	1.5	5	For all 4.6 mm ID columns
14461	Amide-80 Guard column	21.5	7.5	10	For 21.5 mm ID column
21971	NH2-100 Guard cartridge, pk 3			2.0	For 2.0 mm ID columns
21972	NH2-100 Guard cartridge, pk 3			3.2	For 4.6 mm ID columns
19308	Amide-80 Guard cartridge holder				For 2.0 mm ID x 1.0 cm L guard cartridges
19018	Amide-80 Guard cartridge holder				For 3.2 mm ID x 1.5 cm L guard cartridges

For detailed Toyopearl packing instructions, request our **TOYOPEARL Instruction Manual**.

To get an overview about the whole range of TSKgel columns and small TOYOPEARL and TSKgel bulk media, please request our **Chromatography catalog**.



For a deeper insight into applications and all questions related to the practical use of TSKgel and TOYOPEARL, check out the website **www.tosohbioscience.de** and related catalogs or instruction manuals.

Our technical experts are happy to discuss your specific separation needs by phone: **+49 (0)6155-70437-36** or **techsupport.tbg@tosoh.com**

TOSOH BIOSCIENCE

Im Leuschnerpark 4 64347 Griesheim, Germany
Tel: +49 6155-7043700 Fax: +49 6155-8357900
info.tbg@tosoh.com www.tosohbioscience.de

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