



# TSKgel HILIC COLUMNS

HILIC  
HYDROPHILIC  
INTERACTIONS  
LIQUID  
CHROMATOGRAPHY

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**

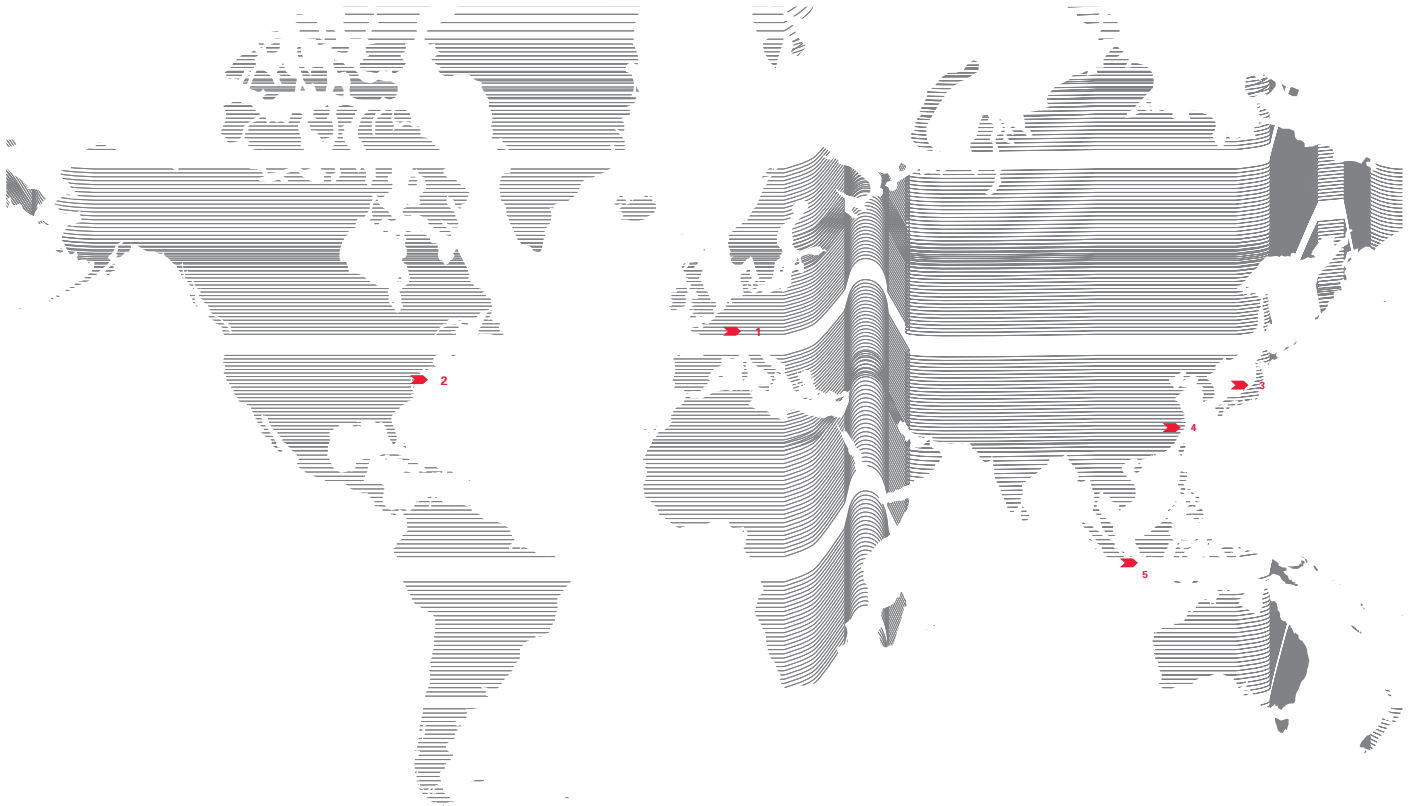
3604 HORIZON DRIVE,  
SUITE 100  
KING OF PRUSSIA, PA 19406, USA

T +1 800 366 4875  
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 First TSKgel GPC column developed
- 1974 HPLC Column Plant starts production
- 1977 First silica based TSKgel SW column for protein analysis
- 1979 Tosoh develops TOYOPEARL media
- 1987 TSKgel SW<sub>XL</sub> for size exclusion chromatography of proteins introduced
- 1995 Tosoh Nanyo gel factory receives ISO9001
- 1997 Reaching the milestone of 10 000 TSKgel G3000SW<sub>XL</sub> columns
- 2008 TSKgel STAT ion exchange columns introduced
- 2013 High capacity TOYOPEARL Protein A resin for antibody purification introduced
- 2014 TOSOH Bioscience GmbH celebrates its 25th anniversary
- 2015 TSKgel UP-SW3000 SE-UHPLC columns allow method transfer from TSKgel SW<sub>XL</sub>
- 2016 Protein A column for fast mAb titer determination
- 2017 Construction of a new R & D laboratory center announced

# 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.

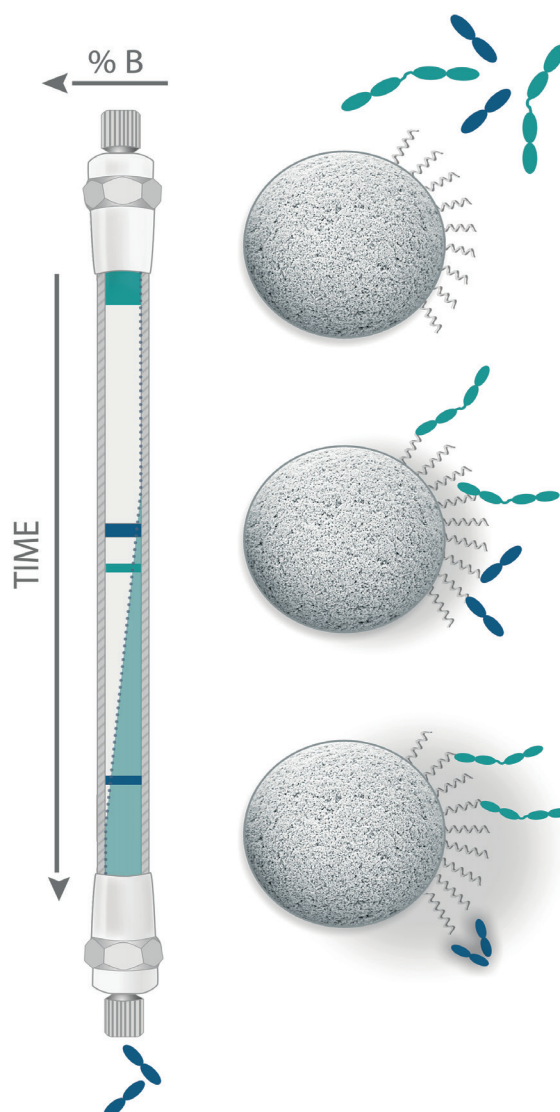
Analysis of glycans, carbohydrates, peptides, polar drugs and metabolites, vitamins and other hydrophilic compounds are typical HILIC applications. HILIC is ideally suited for mass spectrometric analysis of water soluble polar compounds, because the high organic content in the mobile phase increases 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 TSKgel Amide-80 and NH<sub>2</sub>-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
- TSKgel NH<sub>2</sub>-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 for UHPLC and HPLC with 2 μm particle size

### HILIC ILLUSTRATION



➤ MORE INFORMATION ON [bit.ly/TSKgelHILIC](https://bit.ly/TSKgelHILIC)



# 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 inverted 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, TSKgel 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 TSKgel HILIC column types should deliver an indication for the right direction of method development.

TSKgel 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

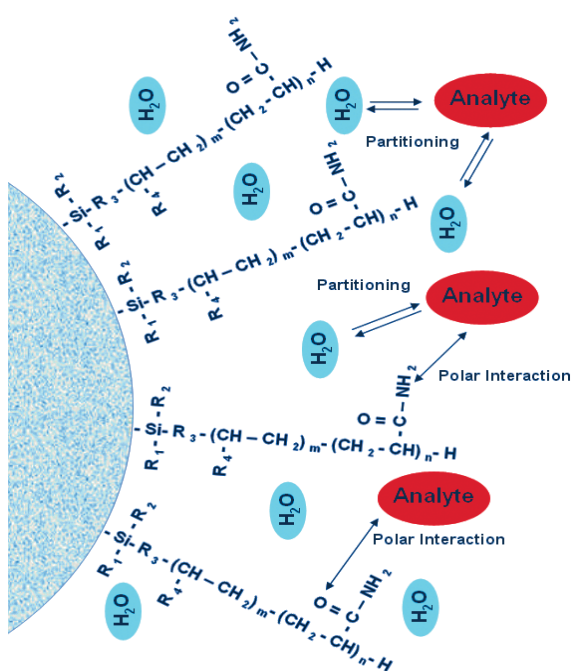
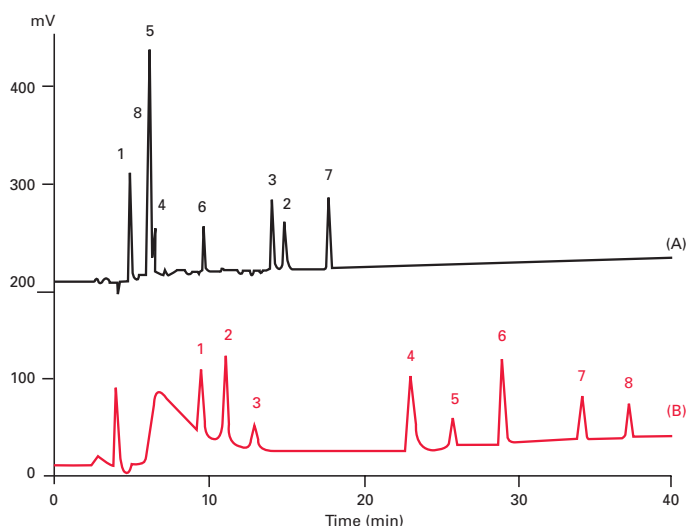


FIGURE 2

## 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-NH<sub>2</sub>; 5. VGSQ;  
6. GGYR; 7. WAGGDASGE; 8. DSDPR;

Mobile phase: (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



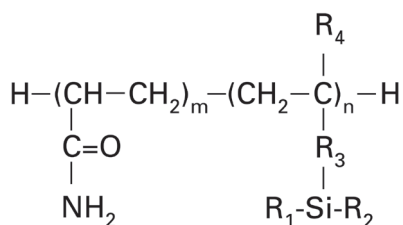
TSKgel Amide-80 stationary phase chemistry uses one of the most popular HILIC ligands, the carbamoyl group. TSKgel Amide-80 columns are packed with spherical silica particles that are covalently bonded with non-ionic carbamoyl groups (Figure 3). They provide a higher stability than conventional amino-phases and a unique selectivity for the analysis of carbohydrates. More than 250 scientific publications prove the successful use of TSKgel Amide-80 columns for the separation of polar compounds with HPLC, UHPLC, and LC-MS.

An important 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.

TSKgel Amide-80 columns with 2 μm particle size suited for both HPLC and UHPLC systems are the latest addition to the Amide-80 series. TSKgel Amide-80 2 μm columns reduce analysis time and improve peak capacity and sensitivity for both, (U)HPLC and LC-MS analysis.

FIGURE 3

STRUCTURE OF TSKgel AMIDE-80



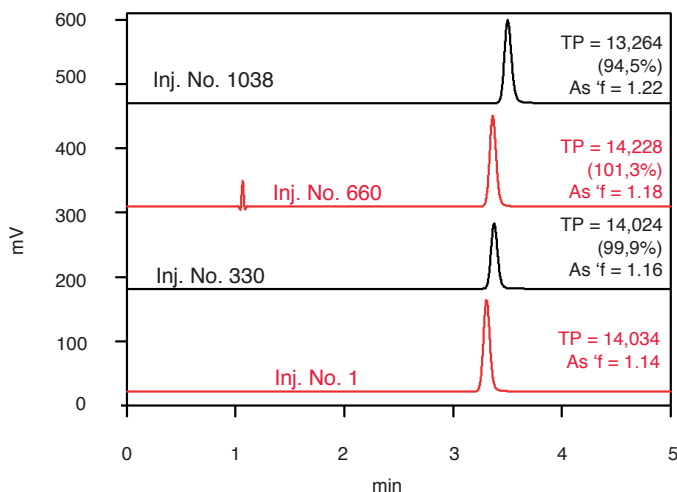
TSKgel Amide-80 LONG TERM STABILITY

The high stability of TSKgel Amide-80 columns is demonstrated in Figure 4 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.

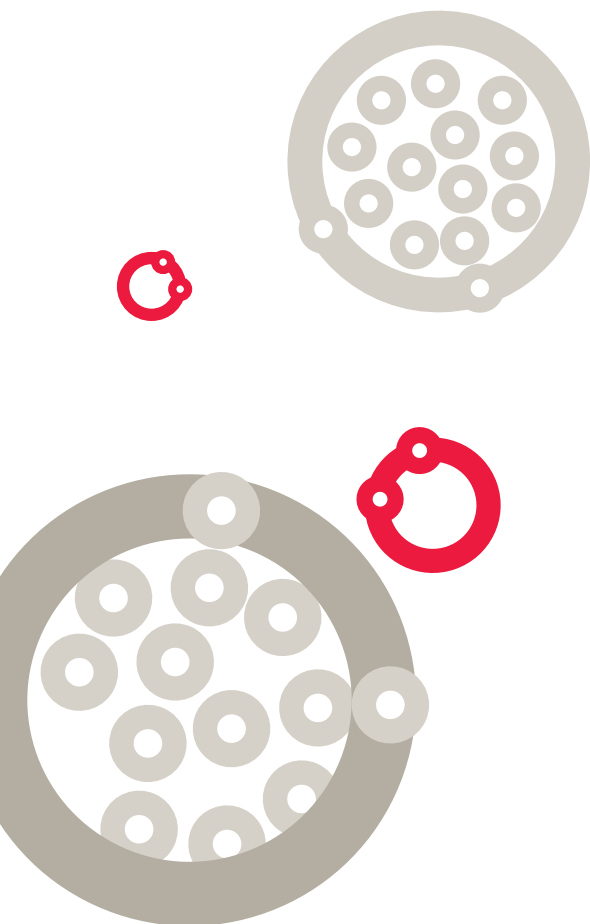
The versatility and robustness of TSKgel Amide-80 enables fast and efficient separation with extended column lifetime, needed for many applications in the food and pharmaceutical industry.

FIGURE 4

DURABILITY OF TSKGEL AMIDE-80 3 μm



Column: TSKgel Amide-80 3 μm (2.0 mm ID x 15 cm L)  
 Mobile phase : H<sub>2</sub>O/CH<sub>3</sub>CN = 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 Amide-80

## INFLUENCE OF PARTICLE SIZE

Figure 5 shows the separation of sugar alcohols on a TSKgel Amide-80 3  $\mu\text{m}$  column compared to a TSKgel Amide-80 5  $\mu\text{m}$  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  $\mu\text{m}$  column provides better resolution at reduced analysis time when compared to the 5  $\mu\text{m}$  TSKgel Amide-80 column.

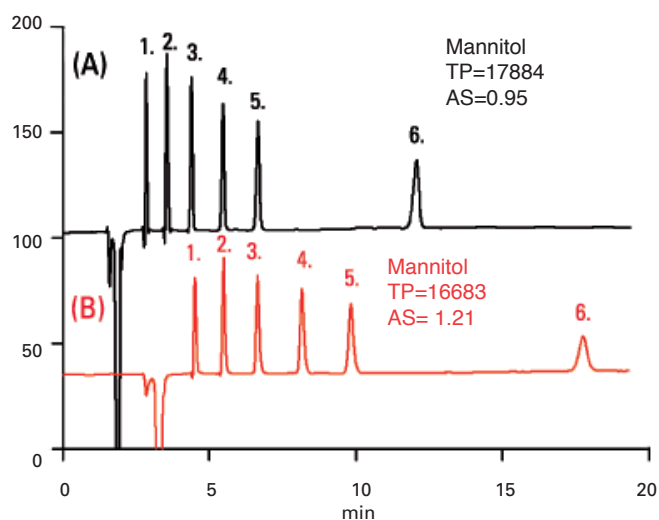
The new 2  $\mu\text{m}$  TSKgel Amide-80 phase further improves peak capacity and sensitivity for both, (U)HPLC and LC-MS analysis. When using short columns this can be exploited to considerably shorten analysis time. The columns are especially suited for use in UHPLC systems, as their reduced system volume and optimized detector specifications help to maintain the high resolution that can be achieved with 2 micron columns.

Figure 6 shows the separation of standard samples on the new 2  $\mu\text{m}$  packing compared to a conventional 3  $\mu\text{m}$  TSKgel Amide-80 column. A 30% increase in resolution can be achieved when using the same method with the 2  $\mu\text{m}$  material. The number of theoretical plates is increased by more than 60%.

➤ MORE INFORMATION ON [bit.ly/TSKgelAmide80](http://bit.ly/TSKgelAmide80)

➤ **FIGURE 5**

### SEPARATION OF POLYALCOHOLS



Columns: A) TSKgel Amide-80 3  $\mu\text{m}$  (4.6 mm ID x 15 cm L)  
B) TSKgel Amide-80 5  $\mu\text{m}$  (4.6 mm ID x 25 cm L)

Mobile phase:  $\text{H}_2\text{O}/\text{CH}_3\text{CN} = 25/75$

Flow rate: 1.0 mL/min

Detection: Refractive index

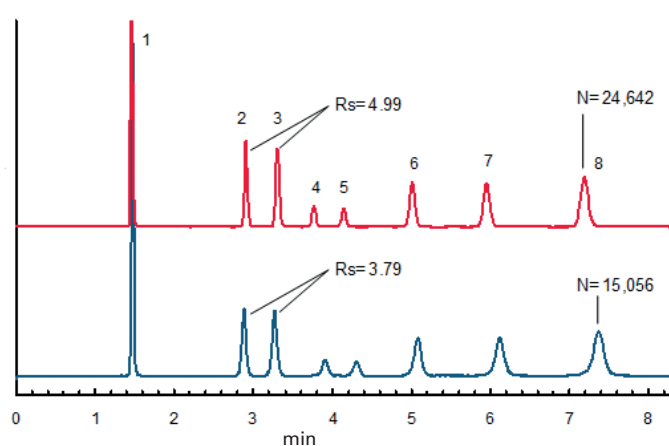
Temp.: 25  $^\circ\text{C}$

Inj. volume : 10  $\mu\text{L}$

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

➤ **FIGURE 6**

### COMPARISON OF RESOLUTION OF 2 $\mu\text{m}$ AND 3 $\mu\text{m}$ PARTICLES



Columns: **TSKgel Amide-80 2  $\mu\text{m}$**  (3.0 mm ID x 15 cm)  
TSKgel Amide-80 3  $\mu\text{m}$  (3.0 mm ID x 15 cm)

Mobile phase: 20 mmol/L  $\text{NH}_4\text{OAc}$  (pH 4.7) / acetonitrile = 10/90

Flow rate: 0.43 mL/min;

Temperature: 40  $^\circ\text{C}$ ;

Detection: UV @ 254 nm;

Injection vol.: 2  $\mu\text{L}$

Samples: 1. toluene (1 g/L), 2. theophylline (0.1 g/L),  
3. theobromine (0.1 g/L), 4. NP Glu (0.1 g/L),  
5. NP Glu (0.1 g/L), 6. 2'-deoxyuridine (0.1 g/L),  
7. 5-methyluridine (0.1 g/L), 8. uridine (0.1 g/L)

# HILIC

## TSKgel NH2-100



TSKgel NH2-100 3  $\mu\text{m}$  columns expand the selectivity range of TSKgel HILIC solutions by a new, robust amino-phase. In contrast to conventional silica-based amino phases it 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.

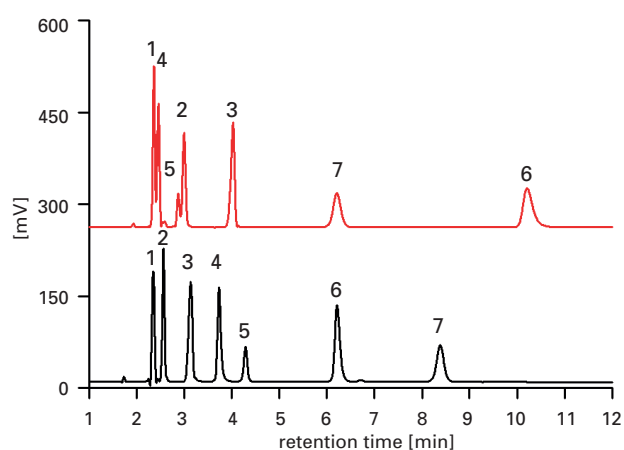
The NH2-100 phase is based on a silica particle with 3  $\mu\text{m}$  particle and 100  $\text{\AA}$  pore size, treated with a special endcapping procedure. Amino groups are introduced step wisely after endcapping (Figure 8). They act as HILIC functional groups without any peak splits. Due to their high ligand density and large surface area TSKgel NH2-100 3  $\mu\text{m}$  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  $\mu\text{m}$ ), 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 7

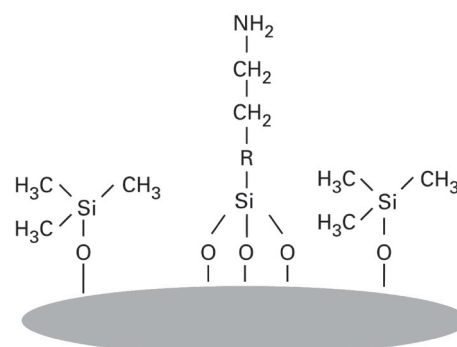
#### SEPARATION OF WATER SOLUBLE VITAMINS



Columns: TSKgel Amide-80 3  $\mu\text{m}$ , 4.6 mm ID x 15 cm L  
 TSKgel NH2-100 3  $\mu\text{m}$ , 4.6 mm ID x 15 cm L  
 Mobile phase: 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  $\mu\text{L}$

### FIGURE 8

#### STRUCTURE OF TSKgel NH2-100



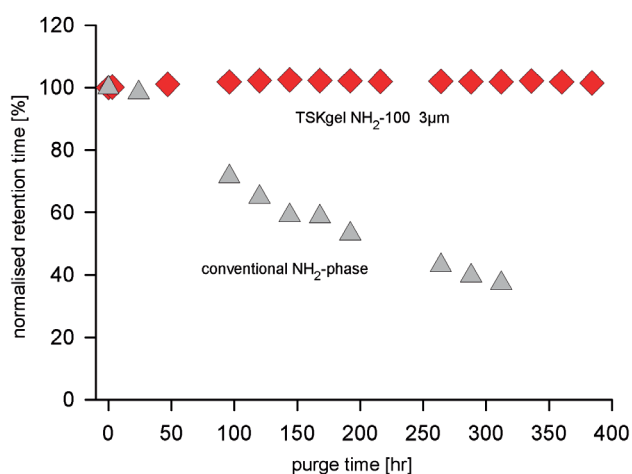
### TSKgel NH2-100 LONG TERM STABILITY

The high stability of TSKgel NH2-100 columns is demonstrated in Figure 9 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.

### MORE INFORMATION ON [bit.ly/TSKgelNH2](http://bit.ly/TSKgelNH2)

### FIGURE 9

#### LONG TERM STABILITY OF TSKgel NH2-100 COLUMNS



Column: TSKgel NH2-100 3  $\mu\text{m}$ , 4.6 mm ID x 15 cm L  
 Conventional Amino column, 4.6 mm ID x 25 cm L  
 Mobile phase: H<sub>2</sub>O/ACN (25/75)  
 Flow rate: 1.0 mL/min  
 Detect: RI  
 Temp.: 40 °C  
 Injection.: 10  $\mu\text{L}$   
 Sample: Inositol



# HILIC APPLICATIONS GLYCAN ANALYSIS

Glycosylation 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  $\mu\text{m}$  columns have been used successfully in glycan analysis. Amide-80 chemistry is ideally suited for the separation of carbohydrate structures.

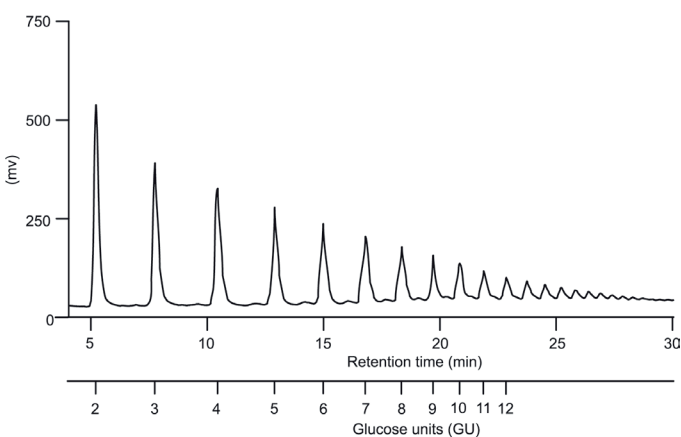
With the 3  $\mu\text{m}$  particles resolution and sensitivity can be further enhanced. Figure 10 shows the high-resolution separation of a 2-aminobenzamide (2AB) labeled dextran ladder within 30 minutes on a TSKgel Amide-80 3  $\mu\text{m}$  column.

The selectivity of the TSKgel NH<sub>2</sub>-100 series differs from TSKgel Amide-80 selectivity as shown in Figure 11. 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 10**

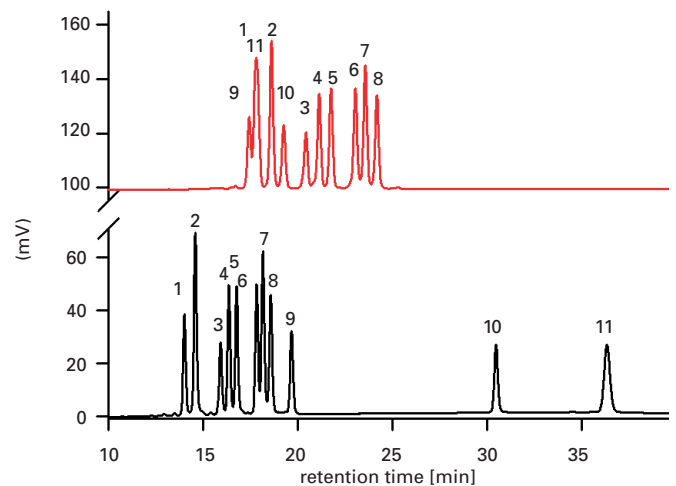
SEPARATION OF A 2AB-LABELED DEXTRAN LADDER ON TSKgel AMIDE-80



Column: TSKgel Amide-80 (3  $\mu\text{m}$ , 2.0 mm ID  $\times$  15 cm L)  
 Mobile phase: A) 50 mM Ammonium formate (pH 4.3)  
 B) Acetonitrile  
 Gradient: 0-35 min - 75-35 % B  
 Flow rate: 0.22 mL/min  
 Detection: Fluorescence Ex@360 nm, Em@425 nm  
 Temperature: 50  $^{\circ}\text{C}$   
 Injection vol.: 3  $\mu\text{l}$   
 Sample: CAB-GHP dextran ladder  
 (Ludger; ~ 300 fmol for GU<sub>2</sub>)

➤ **FIGURE 11**

SEPARATION OF PA-GLYCANS ON TSKgel NH<sub>2</sub>-100



Columns: (a): TSKgel NH<sub>2</sub>-100 3  $\mu\text{m}$ , 4.6 mm ID  $\times$  15 cm L  
 (b): TSKgel Amide-80 3  $\mu\text{m}$ , 4.6 mm ID  $\times$  15 cm L  
 Mobile phase: (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  $^{\circ}\text{C}$   
 Inj. vol.: 10  $\mu\text{L}$





# HILIC APPLICATIONS UHPLC GLYCAN ANALYSIS

The new TSKgel Amide-80 2µm column provides the same unique selectivity as TSKgel Amide-80 3 µm or 5 µm that are applied for glycan analysis in many QC labs for years.

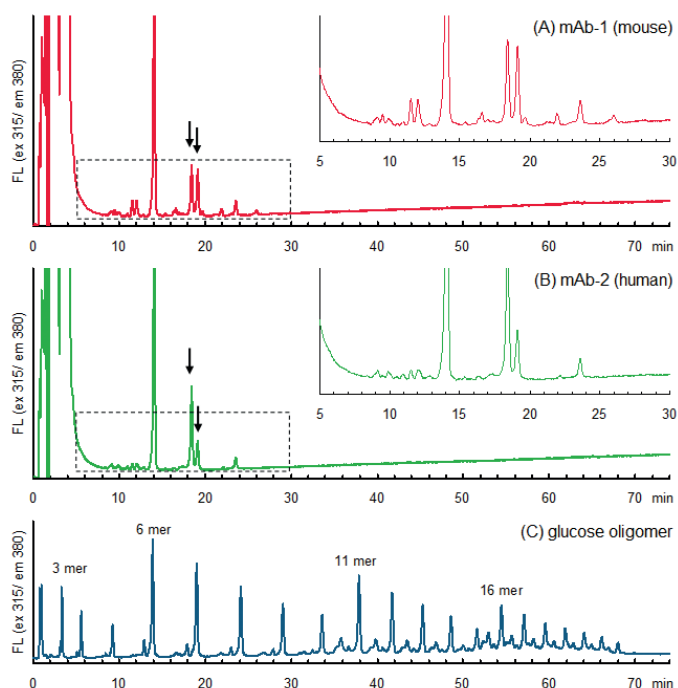
The new 2 µm material improves peak capacity and sensitivity for both, (U)HPLC and LC-MS analysis and allows a smooth transfer of established methods from HPLC to UHPLC.

The columns are especially suited for use in UHPLC systems, as their reduced system volume and optimized detector specifications help to maintain the high resolution that can be achieved with 2 micron stationary phase.

The suitability of the new 2 micron material for glycosylation analysis of labelled glycans by both fluorescence detection (Figure 12) and mass spectrometric detection (Figure 13) is demonstrated for various antibody samples.

**FIGURE 12**

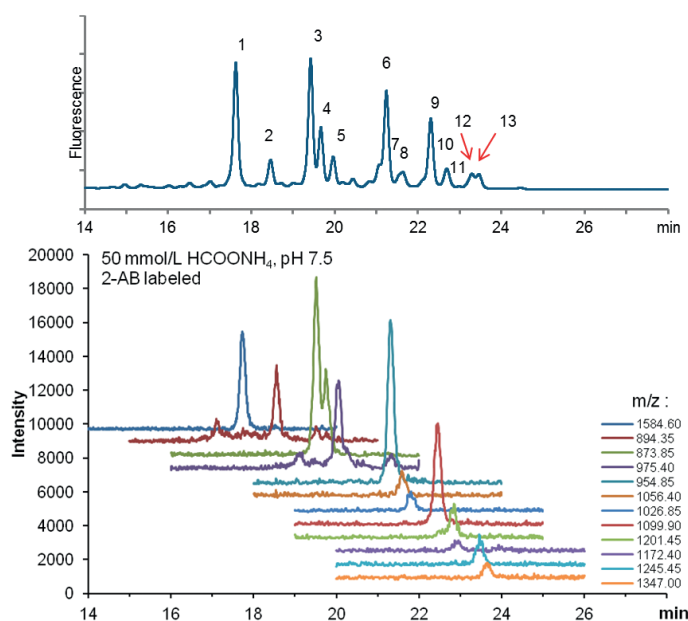
GLYCOSYLATION ANALYSIS OF ANTIBODIES



Column: TSKgel Amide-80 2 µm (2.0 mm ID x 15 cm)  
 Mobile phase: A: 200 mmol/L acetic acid + triethylamine (pH 7.3)  
 B: acetonitrile  
 Gradient: 75% B (0-5min), 75-50% B (5-80 min, linear)  
 Flow rate: 0.5 mL/min  
 Temperature: 40 °C  
 Detection: fluorescence (EX @ 315 nm, EM @ 380 nm)  
 Injection vol.: 50 µL  
 Sample: (A) pyridylaminated oligosaccharides released from mAb-1 (mouse)  
 (B) pyridylaminated oligosaccharides released from mAb-2 (human)  
 (C) PA-glucose ladder (3-22 mer) (TaKaRa Bio)

**FIGURE 13**

UHPLC-MS ANALYSIS OF 2-AB GLYCANS ON TSKgel AMIDE-80 2 µm



Column: TSKgel Amide-80 2 µm (2.0 mm ID x 15 cm)  
 Mobile phase: A: 50 mmol/L HCOONH<sub>4</sub>, pH 7.5  
 B: acetonitrile  
 Gradient: 75 %B (0-5 min), 75-50 %B (5-30 min, linear)  
 Flow rate: 0.3 mL/min  
 Temperature: 40 °C  
 Detection: (a) fluorescence (EX @ 315 nm, EM @ 380 nm)  
 (b) LC/MS, ESI positive, SIM (Shimadzu LCMS-8030)  
 Injection vol.: 50 µL  
 Sample: 2-AB labelled N-glycans released from human IgG (Ludger, cat.# CLIBN-IGG-01)



# 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.

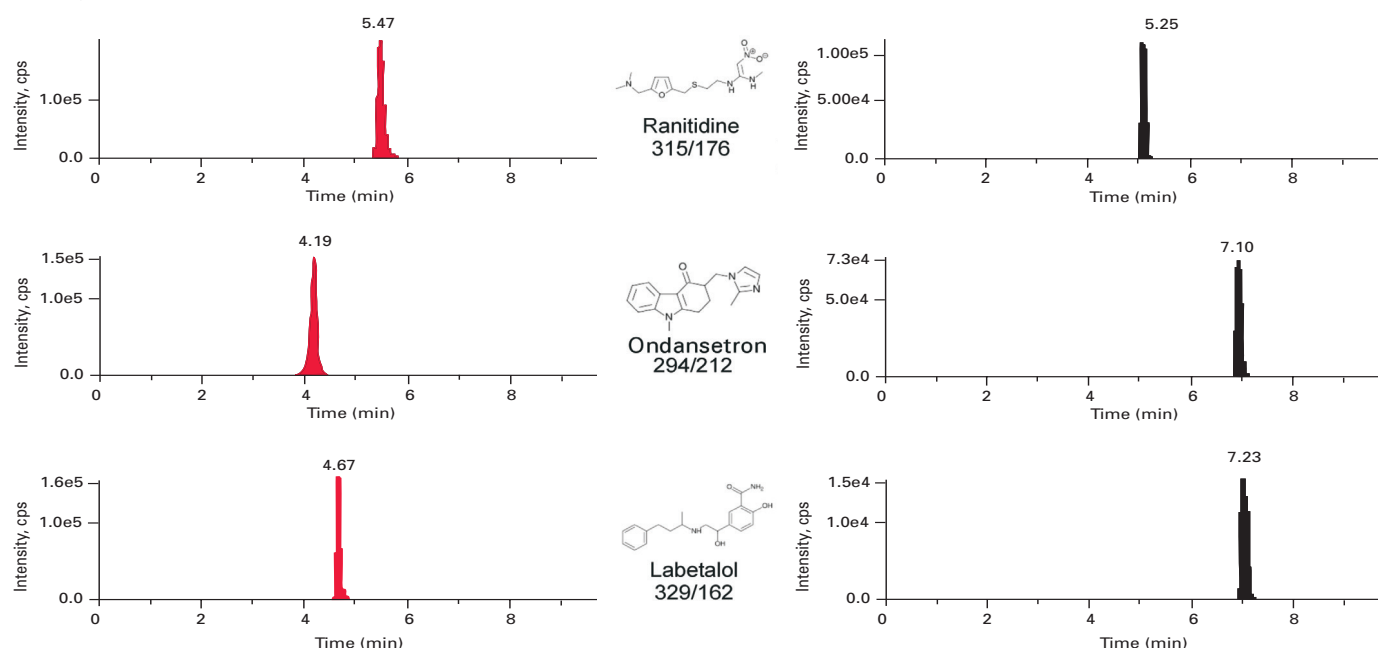
HILIC separations are performed with gradients starting with high percentage of organic solvent and ending with a high portion of aqueous solvent - opposite to typical reversed phase gradients. The elution order of compounds is usually inverted as well. As a result polar compounds are very well separated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

Figure 14 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 14**

### 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)  
 Mobile phase: 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)  
 Mobile phase: 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

## HILIC-MS FOR BIOGENIC AMINES



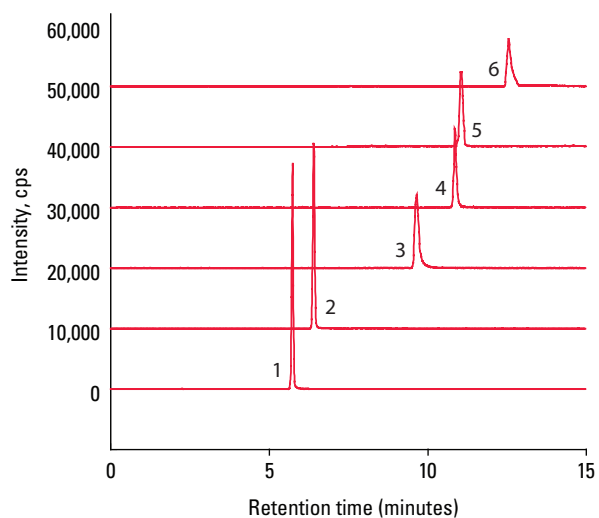
Biogenic amines, such as histamine or tyramine are low molecular weight organic bases generated through decarboxylation of free amino acids by microorganisms. They are present in a wide range of foods products, including fish, meat, cheese, wine, beer, vegetables, fruits, and nuts. In fermented foods their presence is a result of the fermentation process.

Analysis methods for biogenic amines include reversed phase HPLC with fluorescence detection using derivatization. The drawbacks of these methods are a 40 minute analysis time, derivatization, and liquid phase extraction.

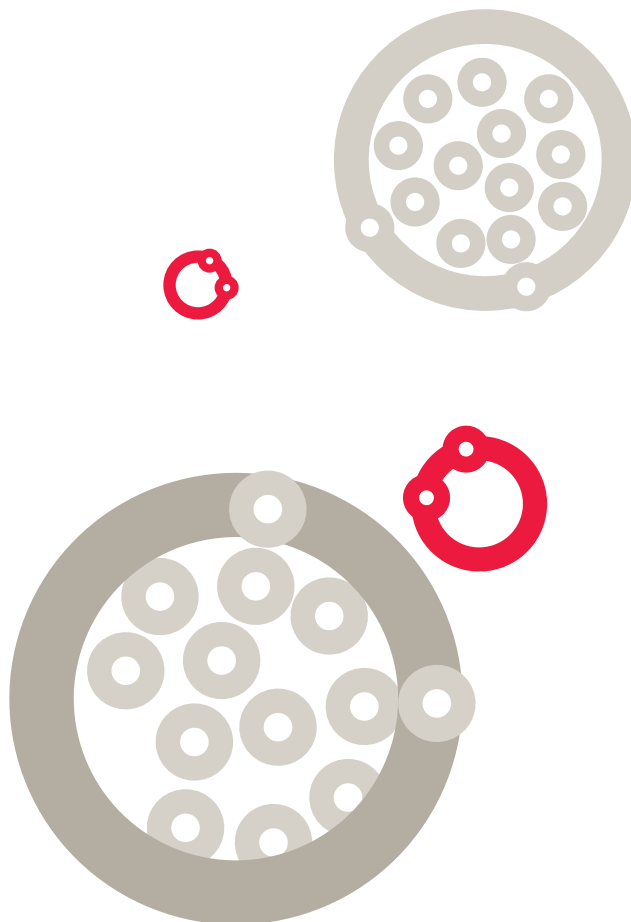
TSKgel Amide-80 can be used to develop a simple, highly sensitive and direct analytical method with electrospray ionization MS/MS detection (Figure 15). Six biogenic amines were analyzed in 15 minutes without the need for a complex and time-consuming derivatization procedure.

FIGURE 15

### HILIC MS/MS ANALYSIS OF BIOGENIC AMINES



Column: TSKgel Amide-80 3  $\mu$ m (2mm ID x 15 cm L)  
 Mobile phase: A: 30 mmol/L ammonium formate, pH 4.0  
 B: Acetonitrile; Flow rate: 0.2 mL/min;  
 Temperature: 50°C;  
 Injection Vol.: 2  $\mu$ L;  
 Gradient: 90% B to 40% B in 12 min;  
 Sample: (1) tryptamine (50  $\mu$ g/L; 161.0/115.0 amu),  
 (2) tyramine (20  $\mu$ g/L; 138.0/121.0 amu);  
 (3) histamine (50  $\mu$ g/L; 112.0/95.0 amu);  
 (4) cadaverine (250  $\mu$ g/L; 103.1/86.1 amu);  
 (5) putrescine (250  $\mu$ g/L; 89.1/72.1 amu);  
 (6) spermidine (50  $\mu$ g/L; 146.3/72.1 amu);  
 Detection: ESI pos. MRM (QTRAP®, AB SCIEX)





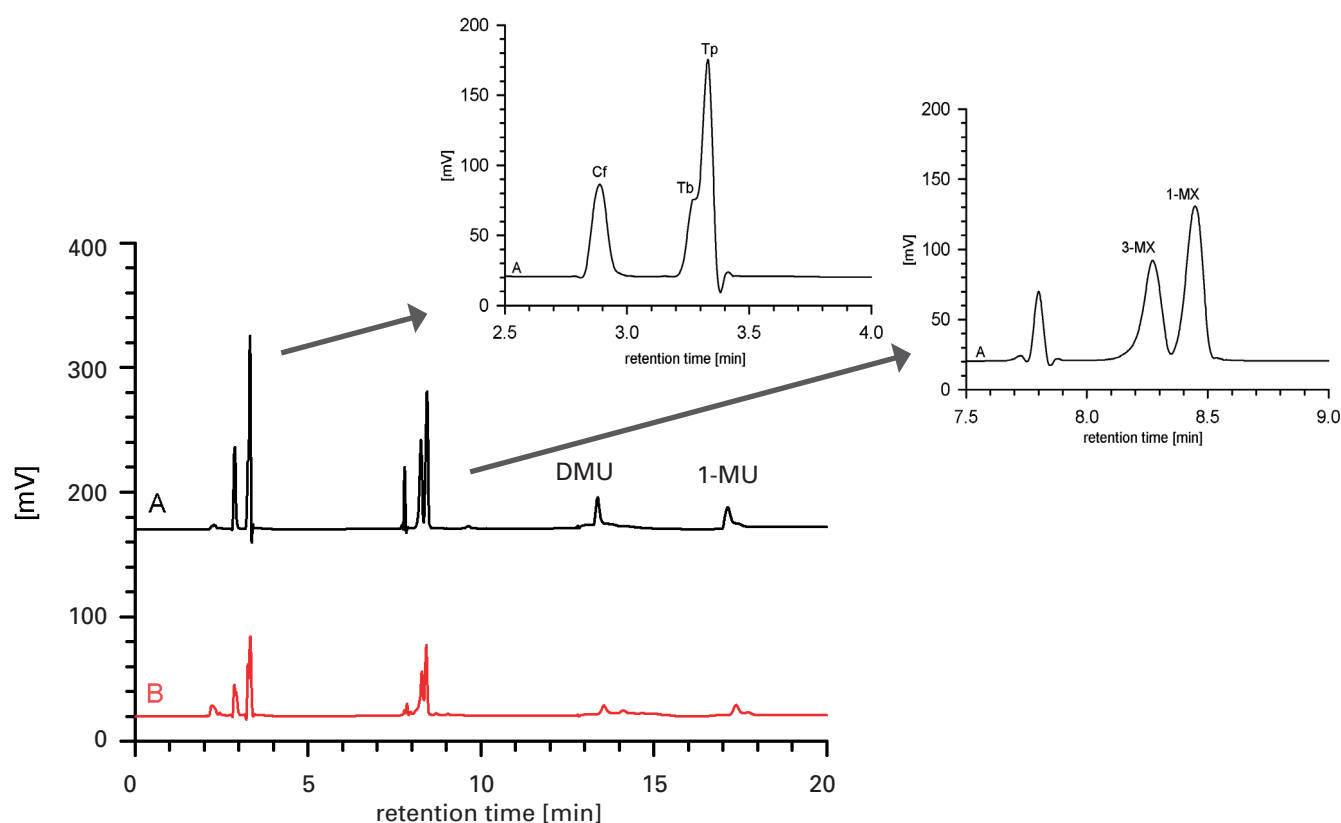
# HILIC APPLICATION DRUG METABOLITES

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 16 shows the analysis of theophylline 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 16**

SEPARATION OF THEOPHYLLINE AND ITS METABOLITES IN SERUM AFTER ONLINE DEPROTEINATION



Column: Analysis: TSKgel NH<sub>2</sub>-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

Mobile phase: Pretreatment; 0.2 M HCO<sub>2</sub>NH<sub>4</sub> (pH 3.6) 0 - 0.3 min  
 A: ACN  
 B: H<sub>2</sub>O/ACN=15/85  
 C: 0.2 M HCO<sub>2</sub>NH<sub>4</sub> (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. Theophylline (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	10 nm	10 nm
Particle size	2 / 3 / 5 / 10 μm	3 μm
Functional group	Carbamoyl	Aminoethyl

➤ **ORDERING INFORMATION**

Part #	Description	ID (mm)	Length (cm)	Particle Size (μm)	Number Theoretical Plates	Max. Pressure Drop (mPa)
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STAINLESS STEEL COLUMNS

0023454	<b>NEW!</b> Amide-80	2.0	5.0	2	≥ 5,800	40
0023455	<b>NEW!</b> Amide-80	2.0	10.0	2	≥ 14,000	60
0023456	<b>NEW!</b> Amide-80	2.0	15.0	2	≥ 21,500	80
0023457	<b>NEW!</b> Amide-80	3.0	5.0	2	≥ 8,300	40
0023458	<b>NEW!</b> Amide-80	3.0	10.0	2	≥ 16,500	60
0023459	<b>NEW!</b> Amide-80	3.0	15.0	2	≥ 24,000	80
0021864	Amide-80	2.0	5.0	3	≥ 3,500	20
0021865	Amide-80	2.0	15.0	3	≥ 13,000	20
0022850	Amide-80	3.0	5.0	3	≥ 5,500	20
0022851	Amide-80	3.0	10.0	3	≥ 11,000	20
0022852	Amide-80	3.0	15.0	3	≥ 16,000	20
0021866	Amide-80	4.6	5.0	3	≥ 6,000	20
0022849	Amide-80	4.6	10.0	3	≥ 12,000	20
0021867	Amide-80	4.6	15.0	3	≥ 18,500	20
0020009	Amide-80	1.0	5.0	5	≥ 300	3
0020010	Amide-80	1.0	10.0	5	≥ 600	6
0021486	Amide-80	1.0	15.0	5	≥ 4,000	9
0021487	Amide-80	1.0	25.0	5	≥ 6,000	12
0019694	Amide-80	2.0	5.0	5	≥ 1,000	4
0019695	Amide-80	2.0	10.0	5	≥ 2,000	8
0019696	Amide-80	2.0	15.0	5	≥ 4,000	10
0019697	Amide-80	2.0	25.0	5	≥ 6,000	15
0019532	Amide-80	4.6	5.0	5	≥ 1,500	15
0019533	Amide-80	4.6	10.0	5	≥ 3,000	15
0013071	Amide-80	4.6	25.0	5	≥ 8,000	15
0021982	Amide-80 HR	4.6	25.0	5	≥ 18,000	15
0014459	Amide-80	7.8	30.0	10	≥ 5,000	7
0014460	Amide-80	21.5	30.0	10	≥ 8,000	3
0021967	NH2-100	2.0	5.0	3	≥ 4,000	15
0021968	NH2-100	2.0	15.0	3	≥ 15,000	20
0021969	NH2-100	4.6	5.0	3	≥ 6,000	5
0021970	NH2-100	4.6	15.0	3	≥ 18,000	15
0021999	NH2-100 DC,	4.6	5.0	3	≥ 6,000	5

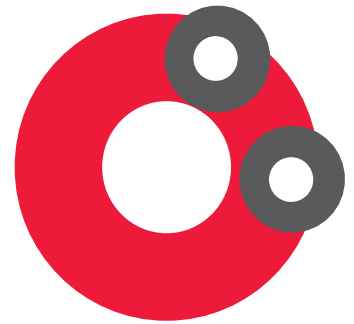
For direct coupling for 2D LC to other HPLC column





Part #	Description	ID (mm)	Length (cm)	Particle Size (µm)	
<b>Guard column products</b>					
0021862	Amide-80 Guard cartridge, pk 3	2.0	1.0	3	For 2.0 mm ID columns
0021863	Amide-80 Guard cartridge, pk 3	3.2	1.5	3	For 4.6 mm ID columns
0021941	Amide-80 Guard cartridge, pk 3	2.0	1.0	5	For all 2.0 mm ID columns
0019010	Amide-80 Guard cartridge, pk 3	3.2	1.5	5	For all 4.6 mm ID columns
0019021	Amide-80 Guard column	4.6	1.0	5	For all 4.6 mm ID columns (P/N 0013071, 0014459)
0014461	Amide-80 Guard column	21.5	7.5	10	For 21.5 mm ID column (P/N 0014460)
0023460	<b>NEW!</b> Amide-80 2 µm (DC)	2.0	1.0	2	Direct Connect Guardcolumn
0021971	NH <sub>2</sub> -100 Guard cartridge, pk 3	2.0	1.0	3	For 2.0 mm ID columns
0021972	NH <sub>2</sub> -100 Guard cartridge, pk 3	3.2	1.5	3	For 4.6 mm ID column
0019308	Guard cartridge holder				For 2.0 mm ID x 1.0 cm L guard cartridges
0019018	Guard cartridge holder				For 3.2 mm ID x 1.5 cm L guard cartridge

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## LOOKING FOR MORE?

**YOU HAVE PLENTY OF OPTIONS TO GET SUPPORT AND INSIGHTS FOR YOUR CHROMATOGRAPHY PROJECTS!**

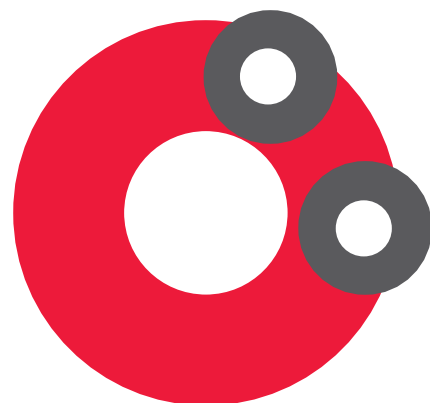


➤ ANY QUESTIONS?

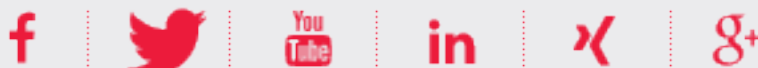
Our technical experts are happy to discuss your specific separation needs:  
+49 (0)6155-70437-36 or  
[techsupport.tbg@tosoh.com](mailto:techsupport.tbg@tosoh.com)

➤ LOOKING FOR INSTRUCTION MANUALS OR APPLICATION NOTES?

Check out the website  
[www.tosohbioscience.de](http://www.tosohbioscience.de)



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Im Leuschnerpark 4 64347 Griesheim, Germany  
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info.tbg@tosoh.com [www.tosohbioscience.de](http://www.tosohbioscience.de)