



TSKgel HILIC COLUMNS

HILIC
HYDROPHILIC
INTERACTIONS
LIQUID
CHROMATOGRAPHY

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TOSOH HISTORY

- 1935 FOUNDED 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 TOSOH US OPERATIONS FORMED IN MONTGOMERYVILLE
- 1989 TOSOH 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 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.

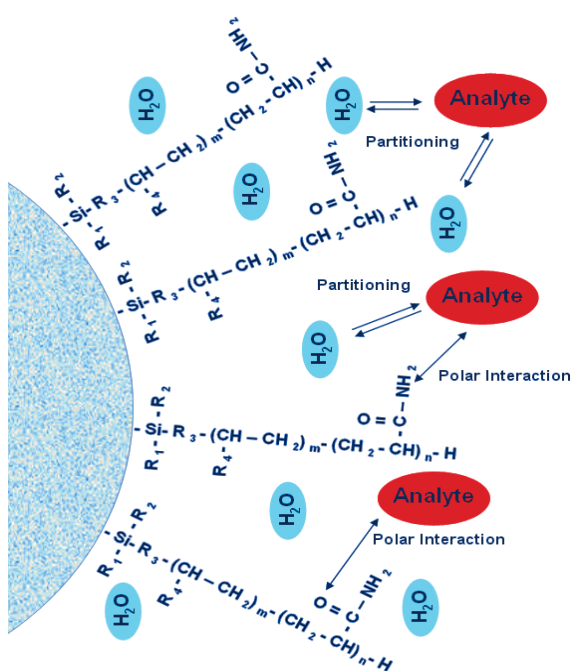
At low acetonitrile concentrations HILIC columns show a reversed phase mode of retention. The HILIC mode can only be executed when starting at high acetonitrile concentrations.

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.

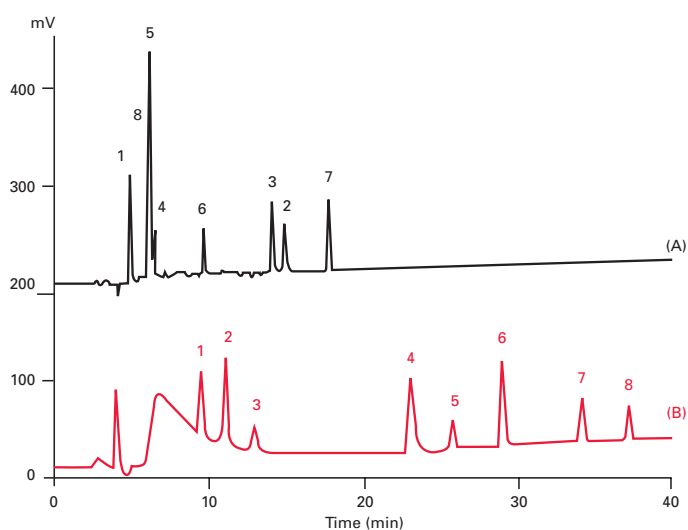
In method development HILIC is an option as soon as polar compounds have to be analyzed and retention on reversed phase columns is too low. Since common RPC solvents can be used, TSK-GEL HILIC columns can be implemented in method development systems using automated column selection. A range of reversed 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 particle 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



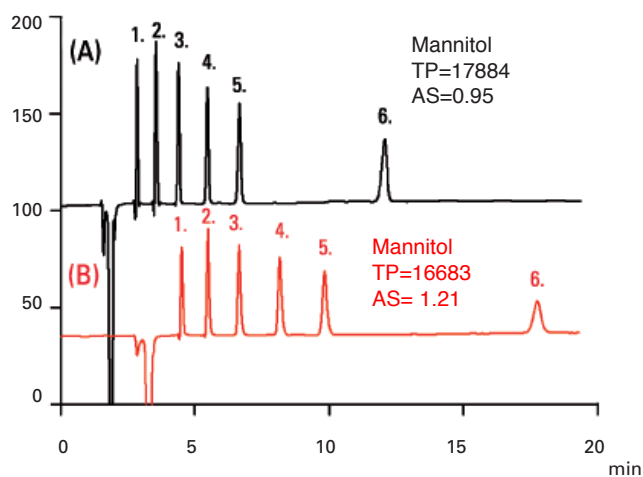
TSKgel Amide-80 columns with small particle size (3 μm) and a new high resolution type of TSKgel Amide-80 5 μm columns are the latest additions to the well-known TSKgel Amide-80 series. For years TSKgel Amide-80 columns are used successfully for HILIC separations of polar compounds, documented in more 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

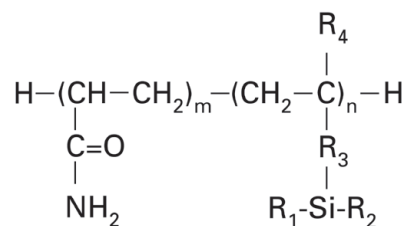
Figure 4 shows the separation of sugar alcohols on a TSKgel Amide-80 3 μm column compared to a TSKgel Amide-80 5 μm column. Basically, the more hydroxyl groups in a compound the more polar it will be and the longer it will be retained on the column.

FIGURE 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: $\text{H}_2\text{O}/\text{CH}_3\text{CN} = 25/75$
Flow rate: 1.0 mL/min
Detection: Refractive index
Temp.: 25 $^\circ\text{C}$
Inj. volume: 10 μL
Sample: 1. Ethyleneglycol 2. Glycerin
3. Erythritol 4. Xylitol
5. Mannitol 6. Inositol

FIGURE 3
Structure of TSKgel Amide-80

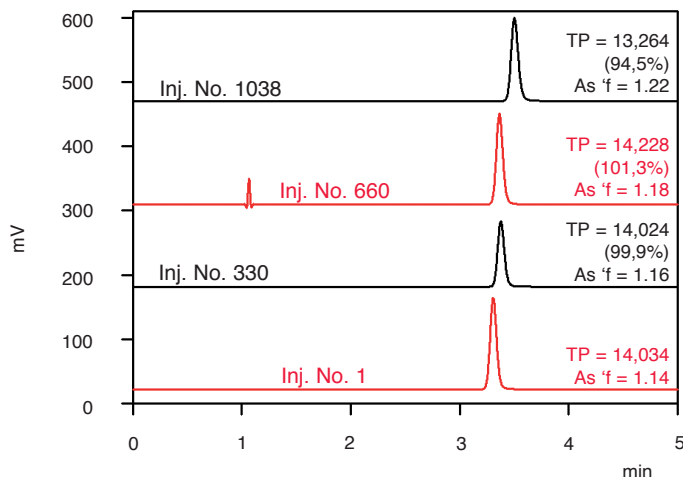


Comparison of the retention between mannitol and inositol, each with 6 hydroxyl groups, shows that inositol, which has a cyclic structure and lower solubility in the mobile phase is retained longer. Overall the 3 μm column provides better resolution at reduced analysis time when compared to the 5 μm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



Column: TSKgel Amide-80 3 μm (2.0 mm ID x 15 cm L)
Eluent: $\text{H}_2\text{O}/\text{CH}_3\text{CN} = 15/85$
Flow rate: 0.2 mL/min
Detection: UV@254 nm
Temp.: 25 $^\circ\text{C}$
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.

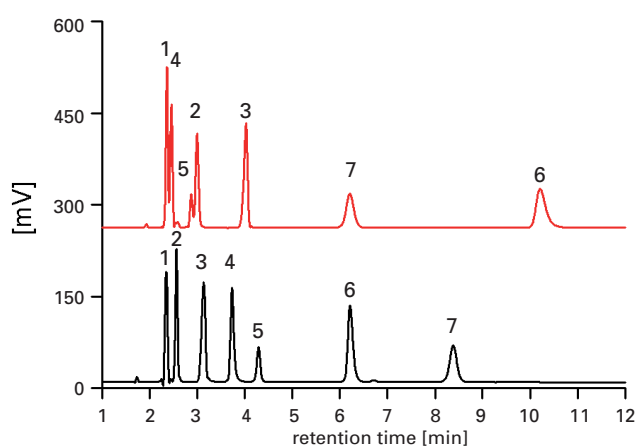
The NH2-100 HILIC phase is based on a silica particle with 3 μm particle and 100 \AA pore size, treated with a special endcapping procedure. Amino groups are introduced step wise after endcapping (Figure 6). The amino groups act as HILIC functional groups without any peak splits. Due to their high ligand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

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

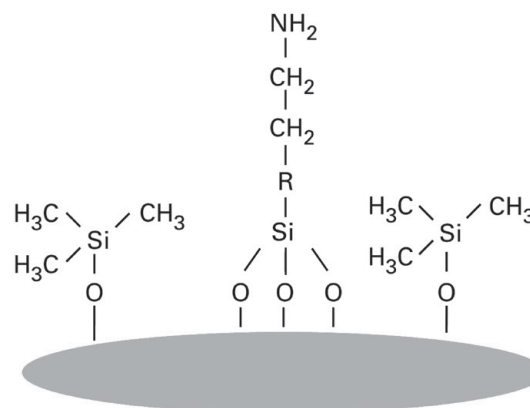
Temp.: 40°C

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 6 Structure of TSKgel NH2-100

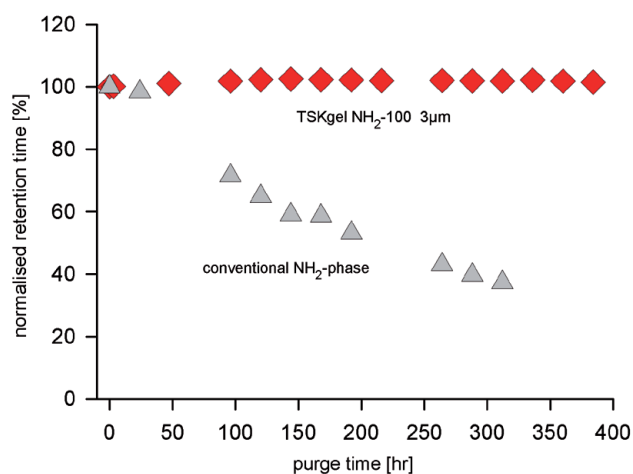


column shows stronger retention for nicotinic acid, vitamin C, and vitamin B12, while retention of vitamin B1, B2, and pyridoxine is reduced.

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 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: H₂O/ACN (25/75)

Flow rate: 1.0 mL/min

Detect: RI

Temp.: 40 °C

Injection.: 10 μL

Sample: Inositol

HILIC APPLICATIONS

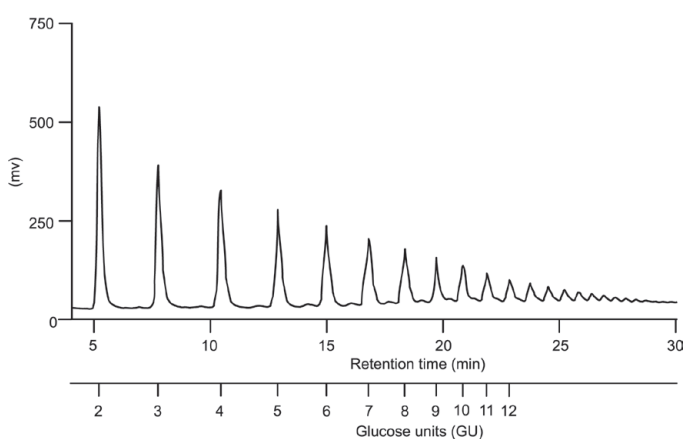
GLYCAN ANALYSIS



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 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.

FIGURE 9
Separation of a 2AB-labeled Dextran Ladder on TSKgel Amide-80



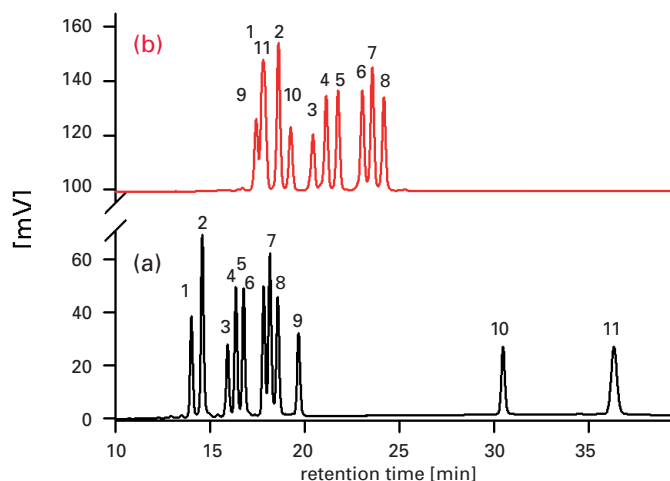
Column: TSKgel Amide-80 (3 μm , 2.0 mm ID x 15 cm L)
 Eluent: A) 50 mM Ammonium formate (pH 4.3)
 B) Acetonitrile
 Gradient: 0-35 min - 75-35 % B
 Flow rate: 0.22 mL/min
 Detection: Fluorescence Ex@360 nm, Em@425 nm
 Temperature: 50 °C
 Injection vol.: 3 μL
 Sample: CAB-GHP dextran ladder
 (Ludger; ~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

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

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 10
Separation of PA-Glycans on TSKgel NH2-100



Column: (a): TSKgel NH2-100 3 μm , 4.6 mm ID x 15 cm L
 (b): TSKgel Amide-80 3 μm , 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

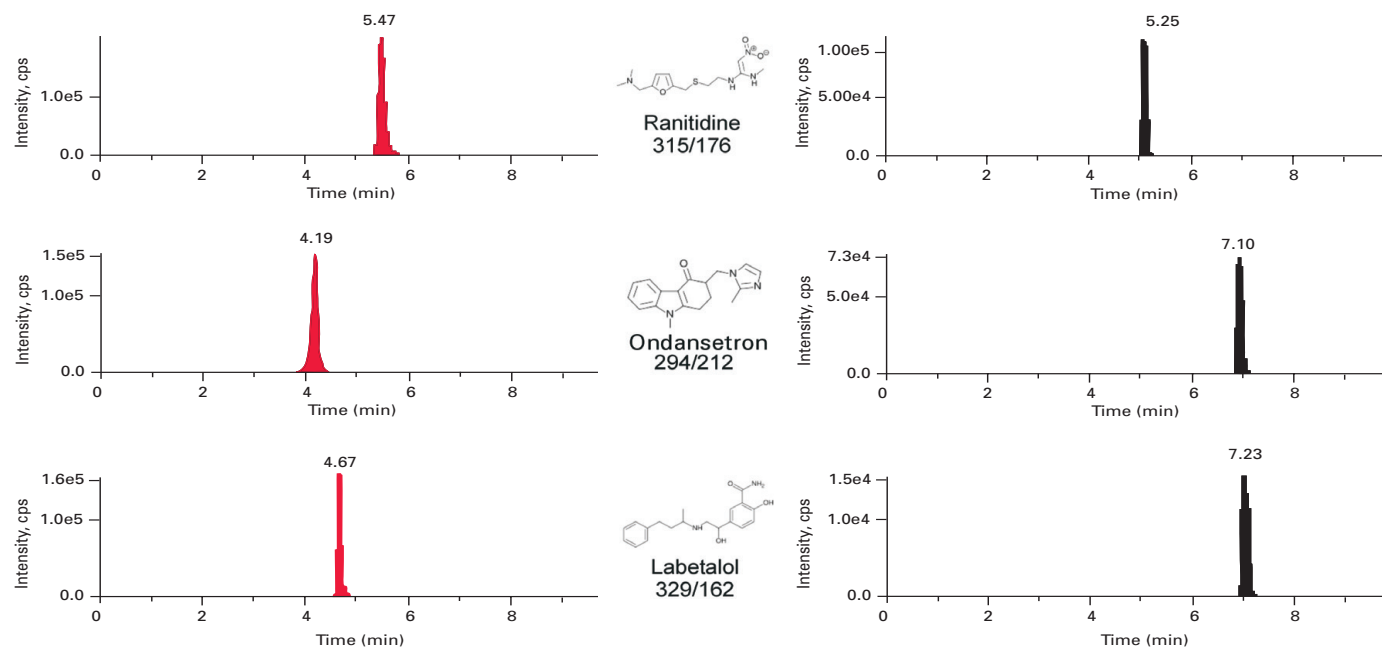
High-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) has become a powerful tool when detection sensitivity is an issue. HILIC offers unique advantages for MS detection of very polar compounds when compared to reversed phase mode. The higher organic content of the eluent in HILIC 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 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 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+

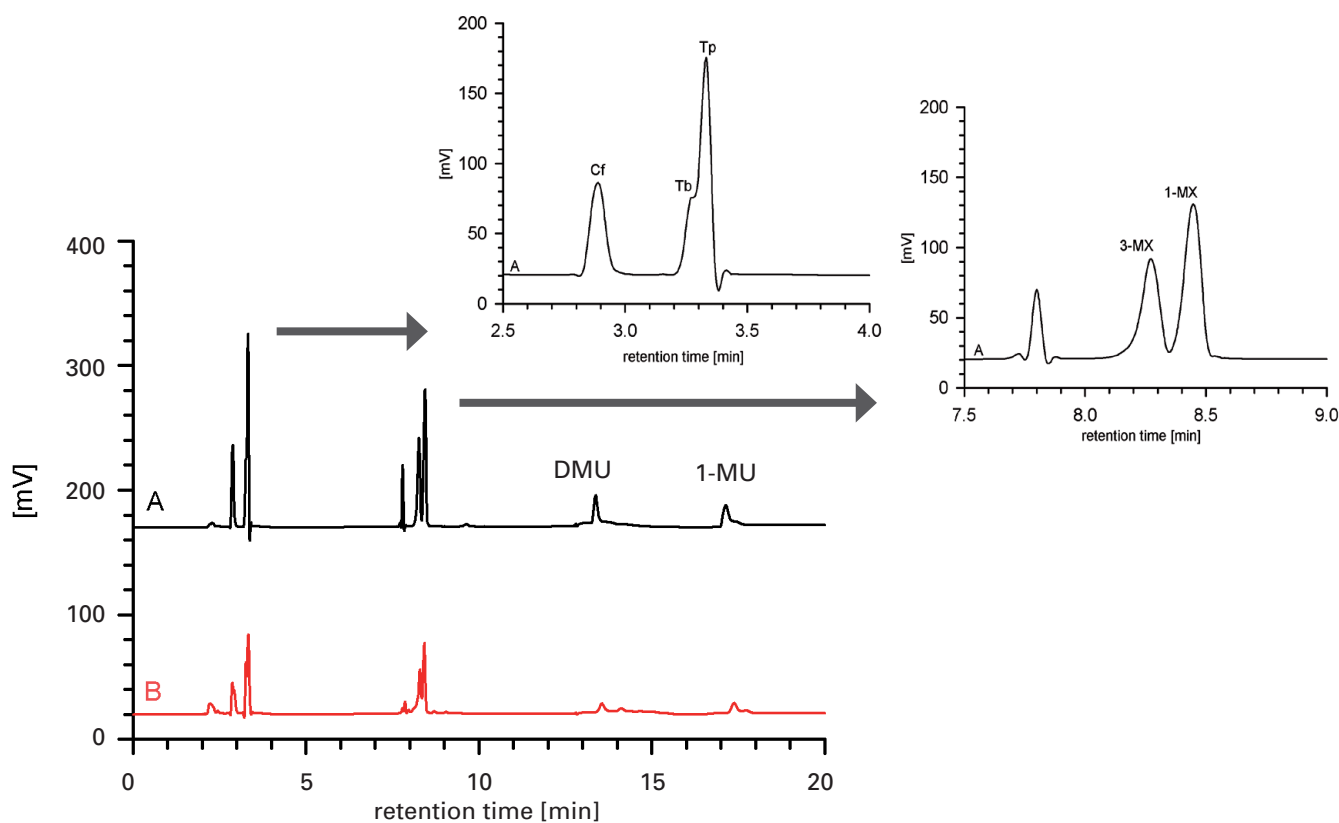
HILIC APPLICATION DRUG METABOLITES



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 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 12
Separation of Theophylline and its Metabolites in Serum after online Deproteination



Column: Analysis: TSKgel NH2-100 3 μ m, 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: $\text{H}_2\text{O}/\text{ACN}=15/85$
 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 $^\circ\text{C}$
 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	100 Å	100 Å
Particle size	3 µm, 5 µm & 10 µm	3 µm
Functional group	Carbamoyl	Aminoethyl

➤ **ORDERING INFORMATION**

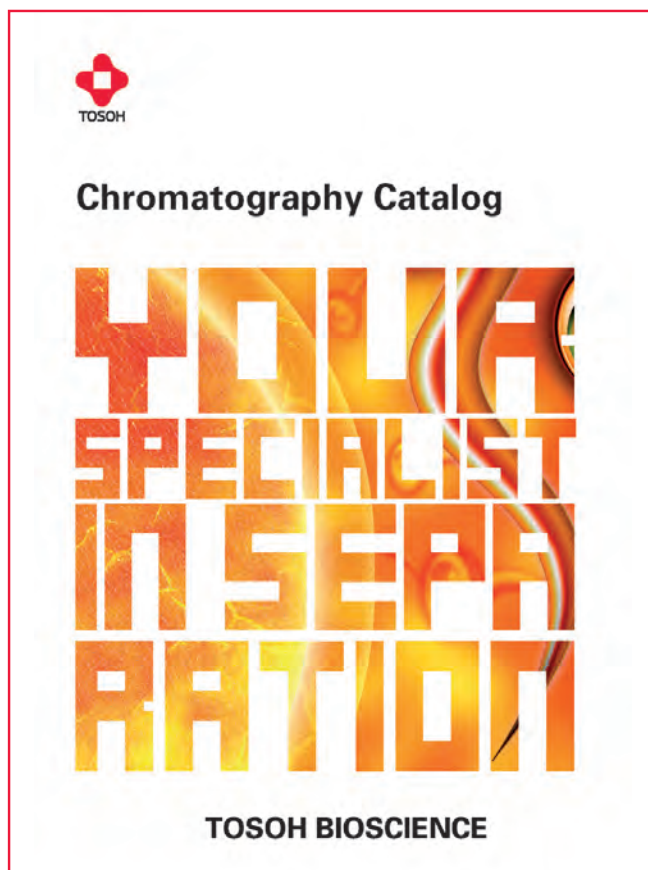
Part #	Description	ID (mm)	Length (cm)	Particle Size (µm)	Number Theoretical Plates	Flow Rate (mL/min)		Maximum Pressure Drop (kg/cm ²)
						Range	Max.	
Stainless steel columns								
21864	Amide-80	2.0	5.0	3	≥ 3,500			200
21865	Amide-80	2.0	15.0	3	≥ 13,000			200
21866	Amide-80	4.6	5.0	3	≥ 6,000			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.tb@tosoh.com



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