TSKgel® AMIDE-80 2 µm HILIC COLUMNS

TSKgel Amide-80 columns packed with 2 µm silica based particles are the latest addition to the well-known TSKgel Amide-80 series. The amide stationary phase provides a unique selectivity under regular normal phase conditions or in the hydrophilic interaction (HILIC) mode of chromatography. The Amide-80 phase shows higher retention of polar compounds than other Amide columns on the market.

For years, TSKgel Amide-80 columns are used successfully for HILIC separations of polar compounds such as saccharides, glycans, oligosaccharides or peptides, documented in more than 250 scientific publications. In parallel to the growth of the biotherapeutics market the use of Amide-phases for glycosylation analysis steadily increased.

Packed with spherical silica particles that are covalently bonded with non-ionic carbamoyl groups (Figure 1), TSKgel Amide-80 provides higher stability than conventional amino-phases and a unique selectivity.
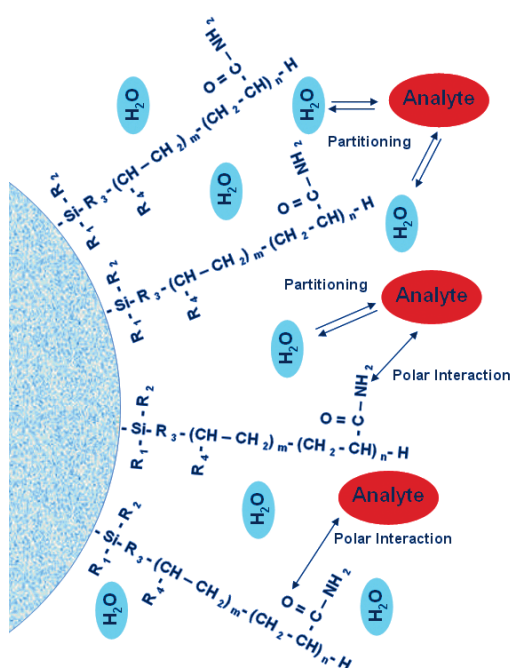
Figure 2 shows the characterization of the new 2 µm version of TSKgel Amide-80 compared to the renowned 3 µm Amide-80 based on the system proposed by Y. Kawachi et al. (J. Chromatogr. A, 1218 (2011) 5903 ff).

The new 2 µm TSKgel Amide-80 material 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.

HIGHLIGHTS

➤ Proven TSKgel Amide-80 HILIC selectivity

➤ Easy transfer of existing methods

➤ Applicable to both HPLC and UHPLC

➤ Optimized for (U)HPLC glycosylation analysis

➤ Applicable for normal phase analysis

TSKgel AMIDE-80 LIGAND CHEMISTRY AND INTERACTIONS
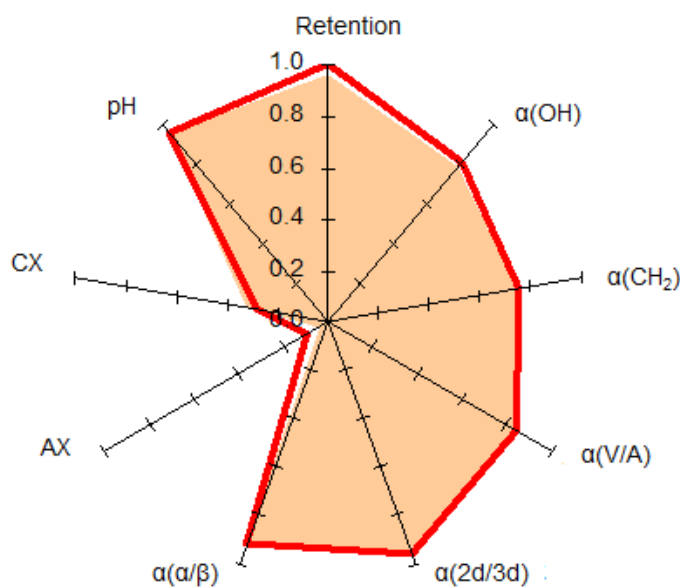

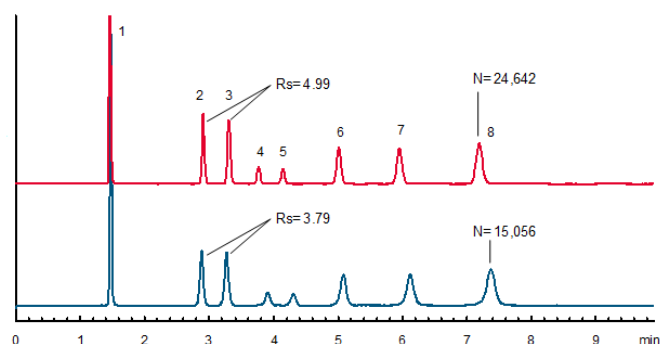➤ Figure 1

TSKgel AMIDE-80 SELECTIVITY

➤ FIGURE 2

Columns: TSKgel Amide-80 2 µm (4.6 mm ID x 15 cm, solid line)
TSKgel Amide-80 3 µm (4.6 mm ID x 15 cm, colored area)
Conditions according to Y. Kawachi et al., J. Chromatogr. A, 1218 (2011) 5903ff

HIGH RESOLUTION ANALYSIS

The reduced particle size considerably increases theoretical plates and resolution. Figure 3 shows the separation of standard samples on the new 2 µm packing compared to a conventional 3 µm TSKgel Amide-80 column. A 30% increase in resolution can be achieved when using the same method with the 2 µm material. The number of theoretical plates is increased by more than 60%.

HIGH SPEED ANALYSIS

The high resolution can be exploited to drastically reduce analysis time. Figure 4 shows an almost 10 fold reduction in total analysis time, while resolution is only reduced by about 40 percent when using a 5 cm short TSkgel Amide-80 2 µm column and increased flow rate. Despite the relatively high flow rate, the pressure drop is moderate (< 20 MPa) and allows the use of a HPLC system. Even though any system used with small particle columns should be optimized with regard to void volume and detector cell and detection parameters.

COMPARISON OF RESOLUTION

ULTRA-FAST ANAYLYSIS

➤ **FIGURE 3** ··
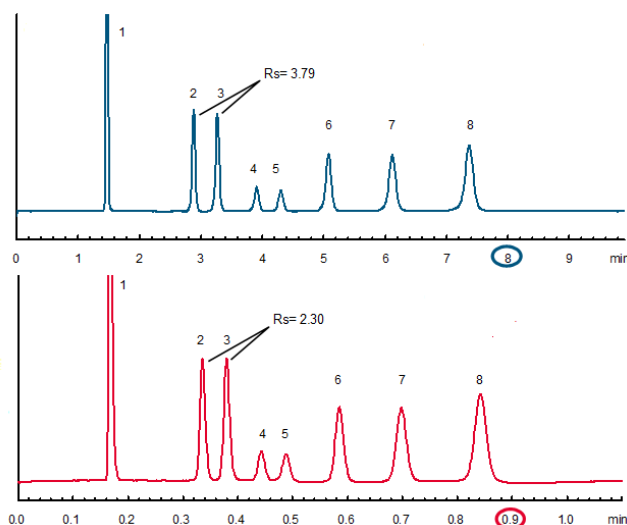
Columns: TSKgel Amide-80 2 µm (3.0 mm ID x 15 cm, red)
                 TSKgel Amide-80 3 µm (3.0 mm ID x 15 cm, blue)
Mobile phase: 20 mmol/L NH_4OAc (pH 4.7) / acetonitrile = 10 / 90
Flow rate: 0.43 mL/min;
Temperature: 40°C; Detection: UV @ 254 nm; Injection vol.: 2 µL
Samples: 1. toluene (1 g/L), 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)

➤ **FIGURE 4** ··

(A) Column: TSKgel Amide-80 2µm (3.0 mm ID x 5 cm, red)
Flow rate: 1.29 mL/min
(B) Column: TSKgel Amide-80 3µm (3.0 mm ID x 15 cm, blue)
Flow rate: 0.43 mL/min
Mobile phase: 20 mmol/L NH_4OAc (pH 4.7) / acetonitrile = 10 / 90
Temperature: 40°C; Detection: UV @ 254 nm; Injection vol.: 2 µL
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)

Ordering information

Part-No	Description	Matrix	Housing	Dimensions
0023454	TSKgel Amide-80, 2 µm, 10 nm	Silica	Stainless steel	2.0 mm ID x 5.0 cm L
0023455	TSKgel Amide-80, 2 µm, 10 nm	Silica	Stainless steel	2.0 mm ID x 10.0 cm L
0023456	TSKgel Amide-80, 2 µm, 10 nm	Silica	Stainless steel	2.0 mm ID x 15.0 cm L
0023457	TSKgel Amide-80, 2 µm, 10 nm	Silica	Stainless steel	3.0 mm ID x 5.0 cm L
0023458	TSKgel Amide-80, 2 µm, 10 nm	Silica	Stainless steel	3.0 mm ID x 10.0 cm L
0023459	TSKgel Amide-80, 2 µm, 10 nm	Silica	Stainless steel	3.0 mm ID x 15.0 cm L
0023460	TSKgel DC-Guardcolumn Amide-80, 2 µm	Silica	Stainless steel	2.0 mm ID x 1.0 cm L

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