TSKgel® Amide-80, 2 µm Columns

A higher resolution, faster analysis alternative to TSKgel Amide-80, 3 µm columns with equivalent selectivity for easy method transfer

Introduction

TSKgel Amide-80 hydrophilic interaction chromatography (HILIC) columns enable the analysis of labeled glycans, peptides, oligonucleic acid, and other hydrophilic small molecules. Packed with spherical silica particles covalently bonded with carbamoyl moiety, the polar functional groups of the sample, such as hydroxy groups, form hydrogen bonds with the polar groups (amino groups) of the packing. A water-rich layer created in the bonded phase allows for partitioning of solutes with the more organic-rich mobile phase. The number of hydroxy groups, conformation and solubility in the mobile phase determines the order of elution.

TSKgel Amide-80 columns are now also available in 2 µm particle size. Offering equivalent retention and selectivity as TSKgel Amide-80, 3 µm columns, with higher resolution and a faster analysis time, TSKgel Amide-80, 2 µm columns are a suitable alternative. An additional advantage of TSKgel Amide-80, 2 µm columns is that they retain more hydrophilic compounds than existing amide columns on the market.

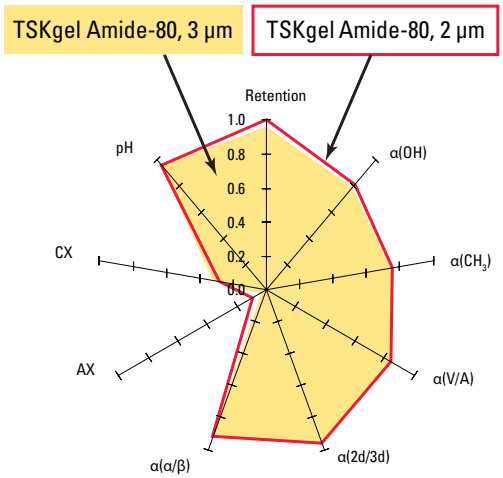
Selectivity and stability of TSKgel Amide-80, 2 µm columns:

Comparative evaluations of TSKgel Amide-80, 2 and 3 µm columns were completed based on the method used by Y. Kawachi et al.[1] Different types of selectivity arising from OH, CH_3, cation and anion exchange, and shape, as well as selectivity for regio and configurational isomers, pH of the stationary phase, and lastly, retention were used to characterize these two columns. Nucleoside derivatives, phenyl glucoside derivatives, xanthine derivatives, sodium p-toluenesulfonate, and trimethylphenylammonium chloride were used as standard samples for this study.

As a result of this evaluation, the TSKgel Amide-80, 2 µm stationary phase was found to have almost equivalent retention and selectivity as the TSKgel Amide-80, 3 µm *(Figure 1)*. This allows for smooth method transfer from a 3 µm to a 2 µm TSKgel Amide-80 column.

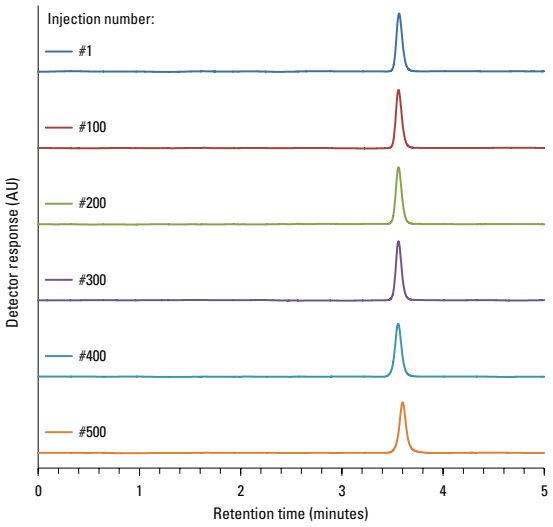
Figure 2 demonstrates the highly reproducible performance of a TSKgel Amide-80, 2 µm column after 500 injections of uracil. This figure also indicates the uncompromised packing integrity of the stationary phase over repeated consecutive injections. Throughout the study, the analytical column was protected by a 2 mm ID x 1 cm TSKgel Amide-80 guard column, which was packed with the same support as that contained in the analytical column. The usage of a guard column is recommended, as the lifetime of the column will be enhanced.

Figure 1. Selectivity of TSKgel Amide-80 Columns



Column:	TSKgel Amide-80, 2µm, 4.6 mm ID × 15 cm (prototype)
Mobile phase:	20 mmol/L NH_4OAc, pH 4.7 / acetonitrile = 10/90
Flow rate:	1.0 mL/min
Detection:	UV @ 254 nm
Temperature:	30 ˚C
Injection vol.:	5 µL

Figure 2. Stability of TSKgel Amide-80, 2 µm Column



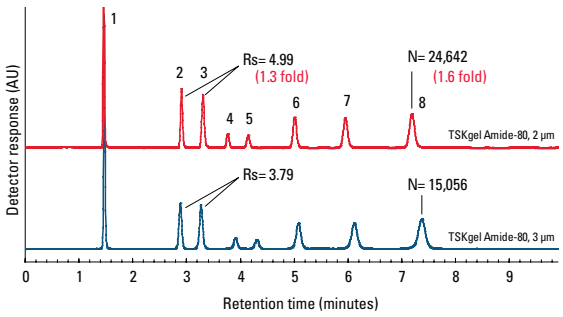
Column:	TSKgel Amide-80, 2µm, 3.0 mm ID x 15 cm + TSKgel Amide-80 guard column, 2 µm, 2.0 mm ID × 1 cm
Mobile phase:	H_2O / acetonitrile = 15/85 (v/v)
Flow rate:	0.43 mL/min
Detection:	UV @ 254 nm
Temperature:	40 ˚C
Injection vol.:	2 µL
Sample:	uracil

Applications

A set of hydrophilic molecules, such as nucleosides, sugars, hydrotropes etc., were analyzed using TSKgel Amide-80, 3.0 mm ID × 15 cm columns of 2 and 3 µm particle size. As seen in *Figure 3*, similar chromatographic profiles were obtained with similar selectivity. The smaller particle size of the TSKgel Amide-80, 2 µm column yielded a 1.6-fold increase in theoretical plates and a 1.3-fold higher resolution.

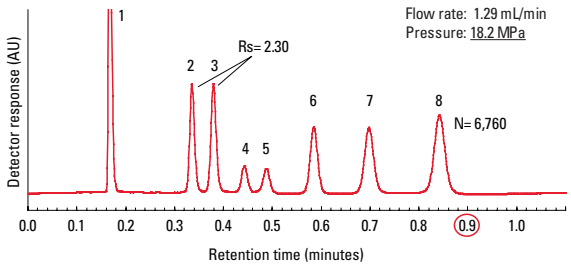
A TSKgel Amide-80, 2 µm column showed impressive results for an ultra-high speed analysis of these same samples *(Figure 4)*. A less than one minute separation was obtained using a TSKgel Amide-80, 2 µm column at a flow rate of 1.29 mL/min. In addition, the 2 µm column showed a lower pressure drop than the maximum pressure of a conventional HPLC system. Therefore, it is not necessary to use a UHPLC system for this type of ultra-high fast separation.

Figure 3. Higher Resolution with TSKgel Amide-80, 2 µm Column



Columns:	TSKgel Amide-80, 2µm, 3.0 mm ID × 15 cm TSKgel Amide-80, 3µm, 3.0 mm ID × 15 cm
Mobile phase:	20 mmol/L NH_4OAc, pH 4.7 / acetonitrile = 10/90
Flow rate:	0.43 mL/min
Detection:	UV @ 254 nm
Temperature:	40 ˚C
Injection vol.:	2 µL
Samples:	1. toluene (1 g/L) 2. theophylline (0.1 g/L) 3. theobromine (0.1 g/L) 4. NPβGlu (0.1 g/L) 5. NPαGlu (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. Ultra-high Fast Separation with TSKgel Amide-80, 2 µm Column



Column:	TSKgel Amide-80, 2 µm, 3.0 mm ID × 5 cm
Mobile phase:	20 mmol/L NH_4OAc, pH 4.7 / acetonitrile = 10/90
Flow rate:	1.29 mL/min
Detection:	UV @ 254 nm
Temperature:	40 ˚C
Injection vol.:	2 µL
Samples:	1. toluene (1 g/L) 2. theophylline (0.1 g/L) 3. theobromine (0.1 g/L) 4. NPβGlu (0.1 g/L) 5. NPαGlu (0.1 g/L) 6. 2'-deoxyuridine (0.1 g/L) 7. 5-methyluridine (0.1 g/L) 8. uridine (0.1 g/L)

Reference

[1]Y. Kawachi *et al., J. Chromatogr. A*, 1218 (2011) 5903

Ordering Information

Part#	Description	Matrix	Housing	ID (mm)	Length (cm)
23454	TSKgel Amide-80, 2 µm	Silica	Stainless Steel	2.0	5
23455	TSKgel Amide-80, 2 µm	Silica	Stainless Steel	2.0	10
23456	TSKgel Amide-80, 2 µm	Silica	Stainless Steel	2.0	15
23457	TSKgel Amide-80, 2 µm	Silica	Stainless Steel	3.0	5
23458	TSKgel Amide-80, 2 µm	Silica	Stainless Steel	3.0	10
23459	TSKgel Amide-80, 2 µm	Silica	Stainless Steel	3.0	15
23460	TSKgel guard column for TSKgel Amide-80, 2 µm	Silica	Stainless Steel	2.0	1

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