TSKgel Amide-80 Normal Phase Chromatography Columns for the Separation of Saccharides - Now Available in 3µm!

TSKgel
APPLICATION NOTE

Introduction

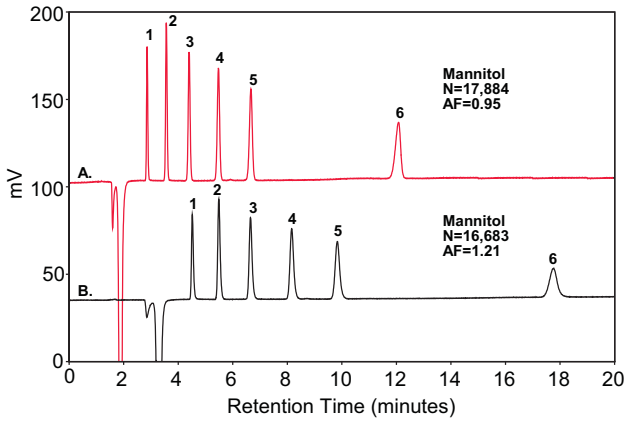
Saccharides are fundamental substances that express various bioactivities and may exist independently or form complexes with proteins or lipids. The analysis of saccharides and sugar chains in complex carbohydrates provides valuable information for the medical, research and food industries. Normal phase chromatography, in tandem with a differential refractometer as a detector, has long been used for the analysis of saccharides, as it provides good selectivity with relatively short analysis times.

TSKgel Amide-80 normal phase columns from Tosoh Bioscience contain a packing material in which the stationary phase consists of nonionic carbamoyl groups that are chemically bonded to the silica gel particles. Having a nonionic stationary phase, compared to so-called amino-bonded phases, affords TSKgel Amide-80 excellent chemical stability. The H-atom in the -NH group in the stationary phase can form a hydrogen bond with oxygen atoms in hydroxyl groups or with a carbonyl group. As a result, a water-rich layer is created in the bonded phase that allows for partitioning of solutes with the more organic-rich mobile phase, enabling separation in normal phase partition mode. TSKgel Amide-80 retains the saccharides and other polyols favorably and can be used under more practical elution conditions compared to nonionic diol-bonded silica gel. Now available in a 3µm particle size, these TSKgel Amide-80 columns provide higher column efficiency at a reduced analysis time for the separation of saccharides.

Results

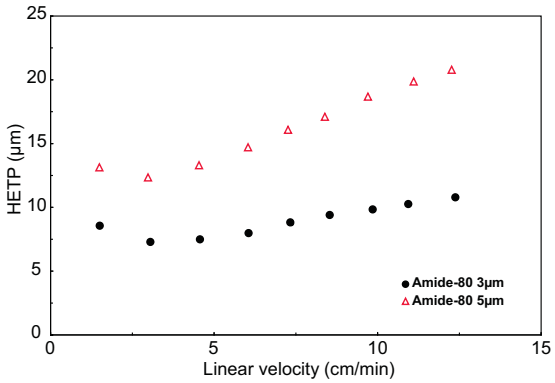
TSKgel Amide-80 retains polar compounds such as saccharides and polyols in organic/water solvent systems such as acetonitrile/water. To illustrate this point, the chromatograms in *Figure 1* show the separation of various sugar alcohols using 3µm and 5µm TSKgel Amide-80 columns. Basically, the more hydroxyl groups in a compound, the more polar it will be and the longer it will be retained on the columns. Comparison of retention between mannitol and inositol, each with 6 hydroxyl groups, shows that inositol, which has a cyclic structure and lower solubility in the acetonitrile/water mobile phase, is retained longer. Despite the fact that a shorter column is used, mannitol elutes with a higher number of theoretical plates from the 3µm TSKgel Amide-80 column. Overall the 3µm column provides better resolution at a greatly reduced analysis time when compared to the 5µm TSKgel Amide-80 column. *Figure 2* illustrates that the minimum of the HETP-linear velocity curve is lower and is obtained at higher linear velocity for the 3µm column. Also, as expected, the slope of the curve is less steep for the smaller particle size. The benefits for the user are two-fold. The higher column efficiency allows for improved results when analyzing complex samples, at the cost of increased column back pressure. Alternatively, the higher efficiency can be traded in for higher throughput by using a shorter column length.

Figure 1. Chromatogram comparison for sugar alcohols on TSKgel Amide-80 3µm and 5µm columns.



Columns:	A. TSKgel Amide-80, 3µm (4.6mm ID x 15cm)
	B. TSKgel Amide-80, 5µm (4.6mm ID x 25cm)
Eluent:	H_2O/ACN=25/75
Flow rate:	1.0mL/min
Detection:	RI
Temp.:	25ºC
Inj. volume:	10µL
Sample:	1. ethyleneglycol 2. glycerin 3. erythritol
	4. xylitol 5. mannitol 6. inositol

Figure 2. H/u curves for TSKgel Amide-80 3µm and 5µm columns.



Columns:	TSKgel Amide-80, 3µm (4.6mm ID x 15cm)
	TSKgel Amide-80, 5µm (4.6mm ID x 25cm)
Eluent:	H_2O/ACN=25/75
Flow rate:	1.0mL/min
Detection:	RI
Temp.:	40ºC
Inj. volume:	10µL
Sample:	mannitol

Conclusion

TSKgel Amide-80 high-performance normal phase chromatography columns have been developed to simplify and speed up the analysis of polyols, such as saccharides.

They have overcome the weaknesses of conventional normal phase chromatography columns and achieve high precision as well as favorable reproducibility. In addition, 3µm TSKgel Amide-80 columns are now available, which provide better resolution at reduced analysis time and offer higher column efficiency.

TOSOH

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