Analysis of saccharides by hydrophilic interaction liquid chromatography (HILIC) using TSK-GEL NH_2-100 columns

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Introduction

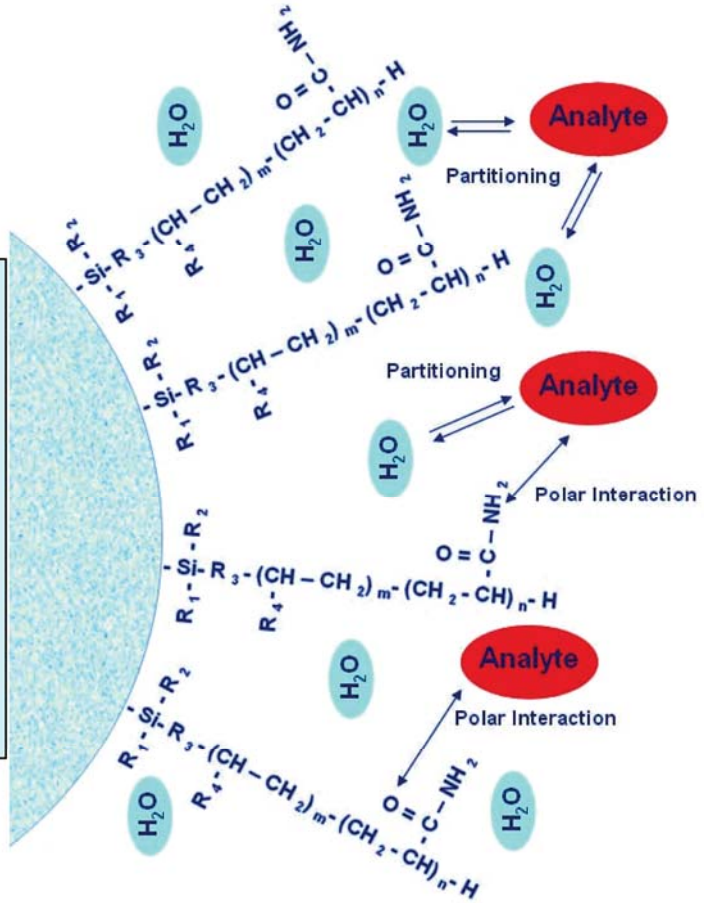
- Saccharides are fundamental substances that express various bioactivities and may exist independently or form complexes with proteins or lipids.
- Saccharides can be classified into monosaccharides, disaccharides, oligosaccharides, polysaccharides etc., based upon the degrees of polymerization and condensation.
- A polyol is an alcohol containing multiple hydroxyl groups. Sugar alcohols are a class of polyols. Sugar alcohols are commonly added to foods since they are of lower calorie content than the corresponding sugars.
- The analysis of saccharides provides valuable information for the medical, research and food industries.

Introduction

- In the past various analytical techniques have been used to analyze saccharides, including all modes of high performance liquid chromatography (HPLC).
- 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.
- Hydrophilic interaction liquid chromatography (HILIC) selectively retains saccharides and polyhydric alcohols, such as sugar alcohols, while most of the substances with low polarity, as well as monohydric alcohols, elute in the void or very close to the void volume of the column.
- Separation is valuable in method development and in quality control for the identification and quantification of these compounds.

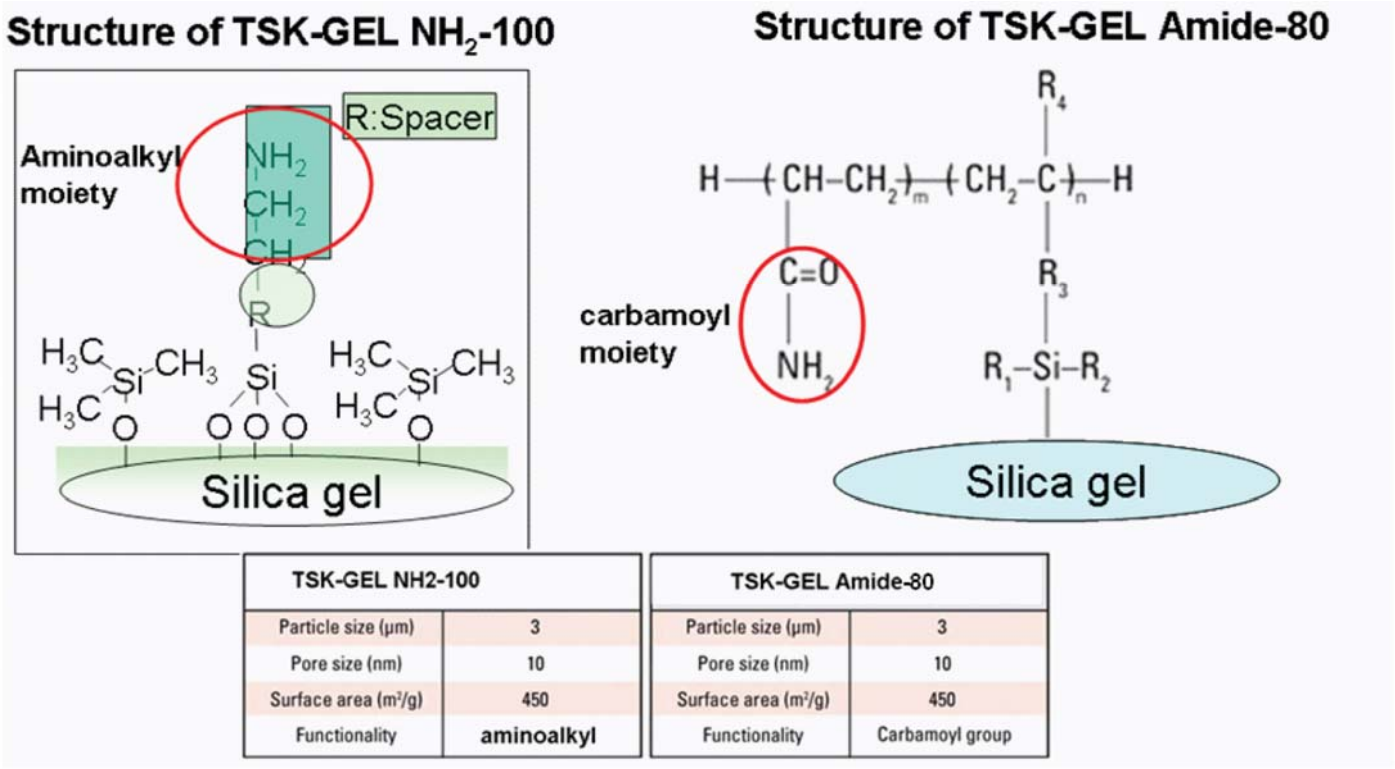
Introduction

- Polar stationary phase as in normal phase LC
- Mobile phase similar to reversed phase (high organic)
- Elution in order of increasing hydrophylicity



Mechanism of Hydrophilic Interaction Liquid Chromatography (HILIC)

Introduction



TSK-GEL NH2-100	
Particle size (µm)	3
Pore size (nm)	10
Surface area (m^2/g)	450
Functionality	aminoalkyl

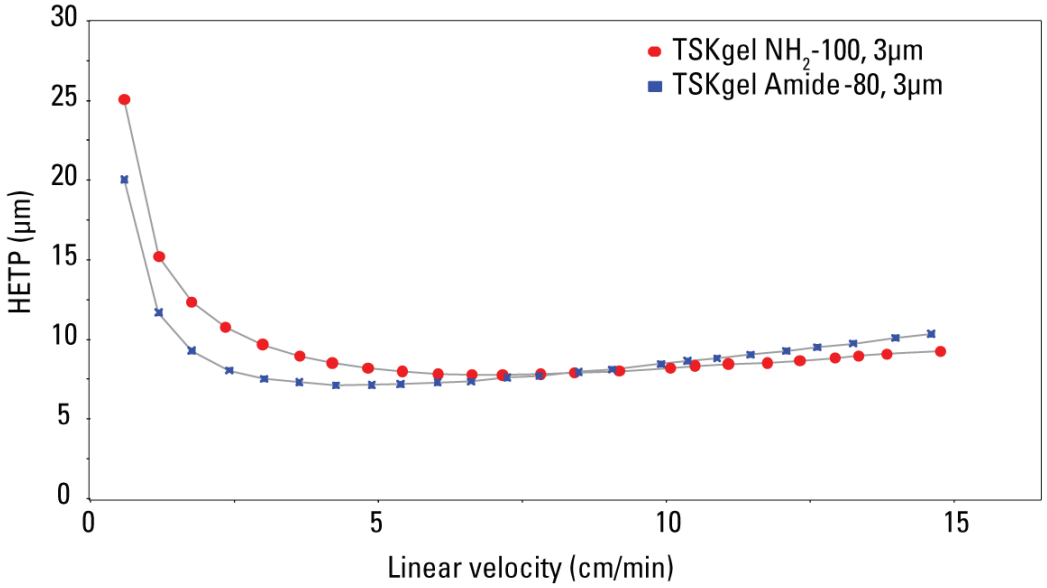
TSK-GEL Amide-80	
Particle size (µm)	3
Pore size (nm)	10
Surface area (m^2/g)	450
Functionality	Carbamoyl group

HILIC Column Products from Tosoh Bioscience: TSK-GEL Amide-80 and NH_2-100 Columns were designed for HILIC

Both can be used with evaporative light scattering (ELS) and mass spec (MS) detectors.

The 3 µm material is ideal for use in LC/MS applications for the analysis of active pharmaceutical ingredients and their metabolites.

Introduction

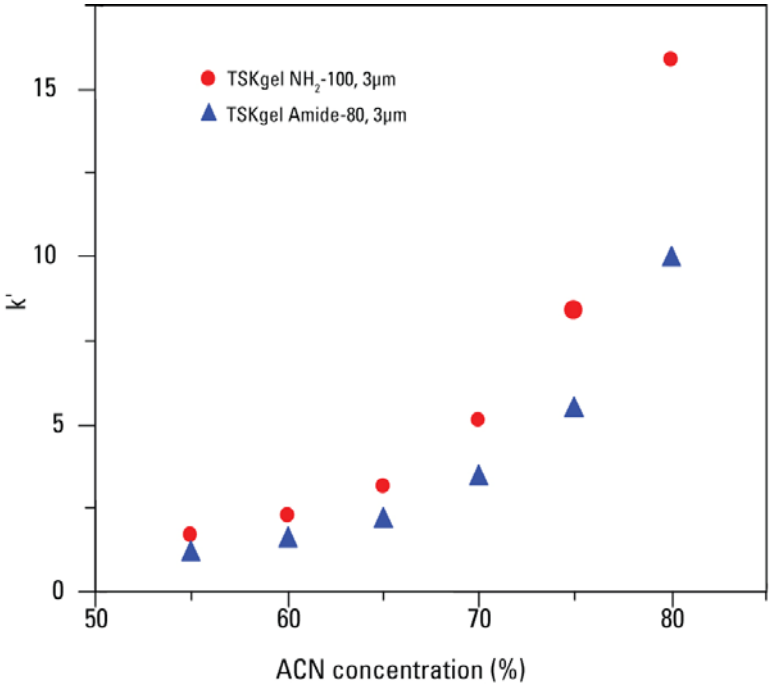


TSKgel NH_2-100, 3µm, 4.6mm ID x 15cm
TSKgel Amide-80, 3µm, 4.6mm ID x 15cm

Mobile phase:	H_2O/ACN = 10/90
Flow Rate:	0.1 ~ 2.4mL/min
Detection:	UV@254nm
Temperature:	40°C
Injection vol.:	10µL
Sample:	uracil

As expected, HETP vs. Linear Velocity is similar for both columns, since the TSK-GEL NH_2-100 and Amide-80 columns are prepared from the same spherical 3µm silica particles.

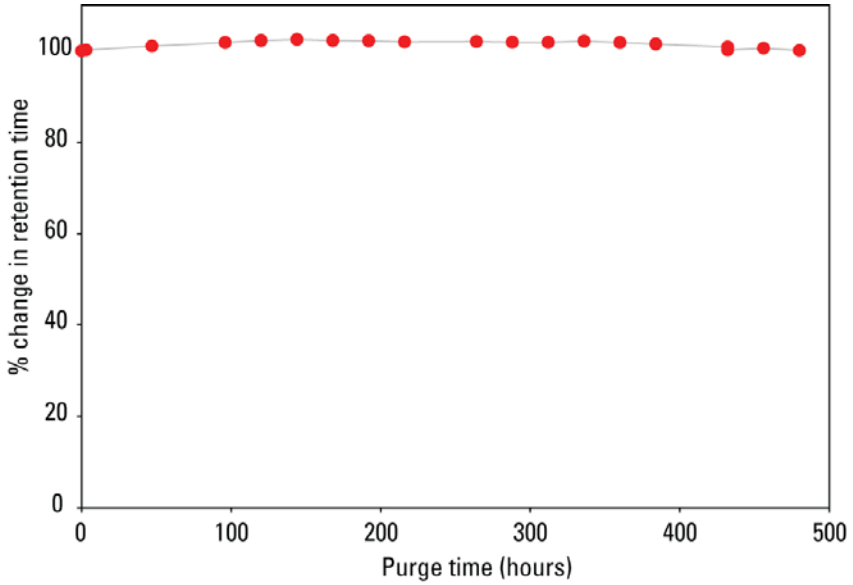
Introduction



Columns:	TSKgel NH_2-100, 3µm, 4.6mm ID x 15cm
	TSKgel Amide-80, 3µm, 4.6mm ID x 15cm
Mobile phase:	H_2O/ACN = 10/90
Flow rate:	1.0mL/min
Detection:	RI
Temperature:	40°C
Injection Vol.:	10µL
Sample:	inositol

The amino-based TSK-GEL NH_2-100 columns expand the selectivity range of HILIC solutions while offering high chemical stability, a pre-requisite for reproducible results.

Introduction: stability of a TSKgel NH_2-100 column



Column:	TSKgel NH_2-100, 3µm, 4.6mm ID x 5cm
Mobile phase:	H_2O/ACN = 25/75
Flow rate:	1.0mL/min
Detection:	RI
Temperature:	40°C
Injection vol.:	10µL
Sample:	inositol

After purging the TSKgel NH_2-100 column for 300 hours, the retention time of inositol barely changed.

Objective

To show the usefulness of the silica based TSKgel NH_2-100, 3µm, 2.0mm ID x 5cm HILIC column for analysis of different types of saccharides using a conventional HPLC system.

Material and methods

All analyses were carried out using an Agilent 1200 HPLC system run by Chemstation (ver B.04.01).

Optimal chromatographic conditions:

- Column: TSKgel NH_2-100, 3µm, 2.0mm ID x 5cm
- Detection: RI
- Column temp: 50˚C
- Flow rate: 0.2 mL/min
- Injection vol.: 2µL
- Mobile phase (Isocratic): 80% ACN in H_20

Material and methods (contd.)

The following saccharides were used to prepare the standards:

- Glucose (Supelco R422080 LB69702)
- Sucrose (Fisher S2-500)
- Trehalose dihydrate (Fisher BP2687-10)
- Maltose (ACROS Organics 329911000, Lot A0280581)
- Maltitol (ACROS Organics, Belgium, 295800250, Lot A0243754)
- Mannitol (Sigma M-4125, Lot 22K0111)

All the standards and samples were filtered through a 0.45µm filter before injecting onto the column.

High purity chemicals and HPLC grade solvents were used for the preparation of stock standards, samples and mobile phases.

Material and methods (contd.)

Preparation of standards

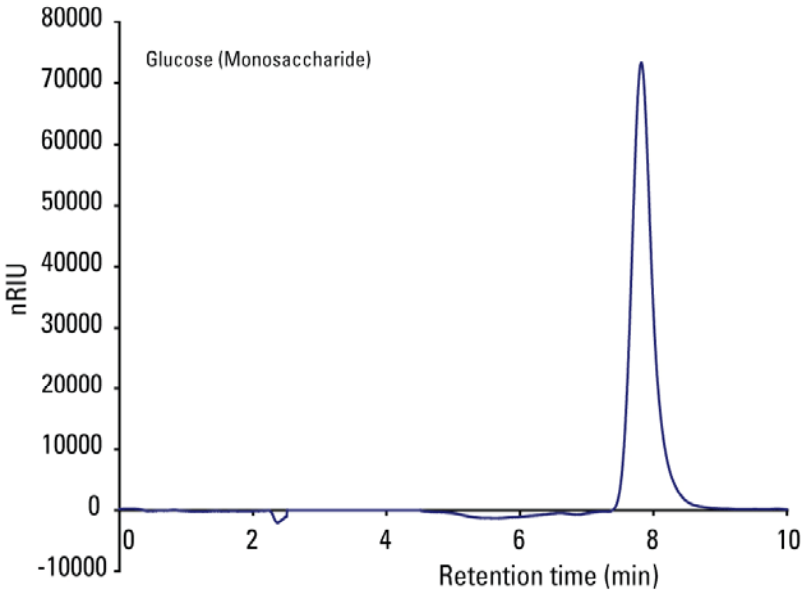
Saccharides*	Weight	Stock Standard (mg/mL)
Glucose	0.0506g in 200µL H_2O	25.3
Sucrose	0.1023g in 10.0mL of 50% ACN in H_2O	10.23
Trehalose	0.1179g in 10.0mL of 50% ACN in H_2O	11.79
Maltose	0.1171g in 10.0mL of 50% ACN in H_2O	11.71

*20µL of glucose stock standard was diluted to 140µL water and used as working standard (36.5mg/mL).
Sucrose, trehalose and maltose stock standards were used as such without further dilution.

Polyols*	Weight	Stock Standard (mg/mL)	Working Standard (mg/mL)
Maltitol	0.1071g in 10.0mL 50% ACN in H_2O	10.71	Same as stock
Mannitol	0.1011g in 10.0mL 50% ACN in H_2O	10.11	Same as stock

* Mannitol was rapidly soluble in 50% ACN in water. Maltitol was dissolved in 10.0 mL of 50% ACN in water with 2 minutes of vortex.
Maltitol and mannitol stock standards were not further diluted and used as such.

Analysis of glucose (monosaccharide) using a TSKgel NH_2-100, 3µm, 2.0mm ID x 5cm column

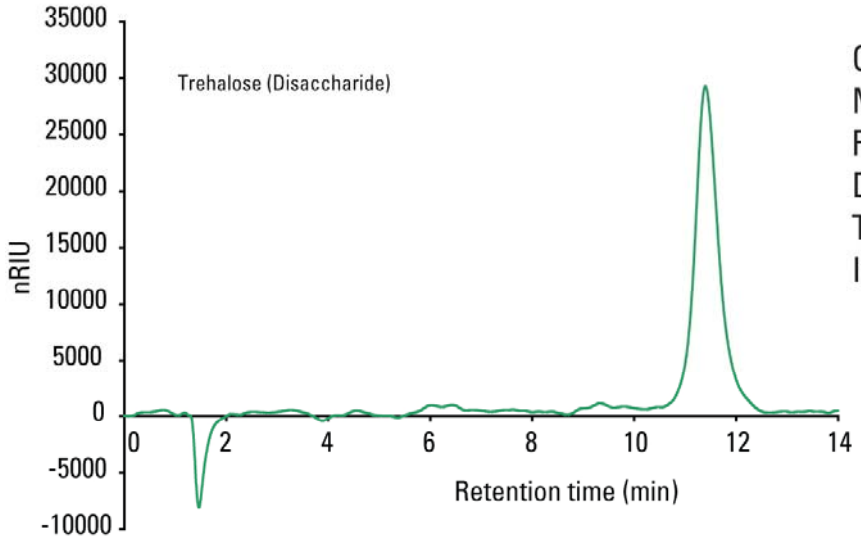


Columns:	TSKgel NH_2-100, 3µm, 2.0mm ID x 5cm
Mobile phase:	80% ACN in H_2O
Flow rate:	0.2mL/min
Detection:	RI
Temperature:	50°C
Injection Vol.:	2µL

RT (min)	k	Area (mAU*S)	A_s	Plates (N)
7.822	11.4	1.59×10^6	1.25	3377

Limit of detection (LOD) of glucose – 100 ppb

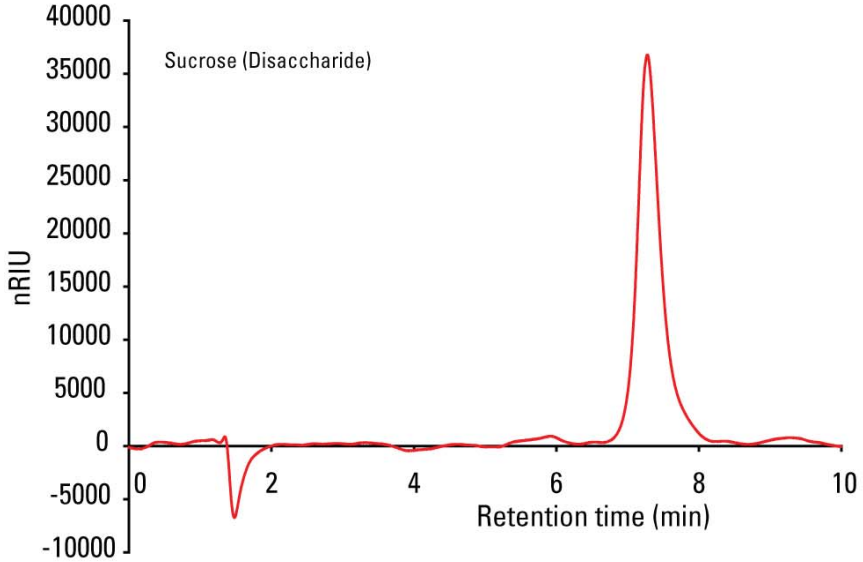
Analysis of trehalose (disaccharide) using a TSKgel NH_2-100, 3µm, 2.0mm ID x 5cm column



Columns:	TSKgel NH_2-100, 3µm, 2.0mm ID x 5cm
Mobile phase:	80% ACN in H_2O
Flow rate:	0.2mL/min
Detection:	RI
Temperature:	50°C
Injection Vol.:	2µL

RT (min)	k	Area (mAU*S)	A_s	Plates (N)
11.4	17.15	0.09×10^6	1.25	3339

Analysis of sucrose (disaccharide) using a TSKgel NH_2-100, 3µm, 2.0mm ID x 5cm column



Columns:	TSKgel NH_2-100, 3µm, 2.0mm ID x 5cm
Mobile phase:	80% ACN in H_2O
Flow rate:	0.2mL/min
Detection:	RI
Temperature:	50°C
Injection Vol.:	2µL

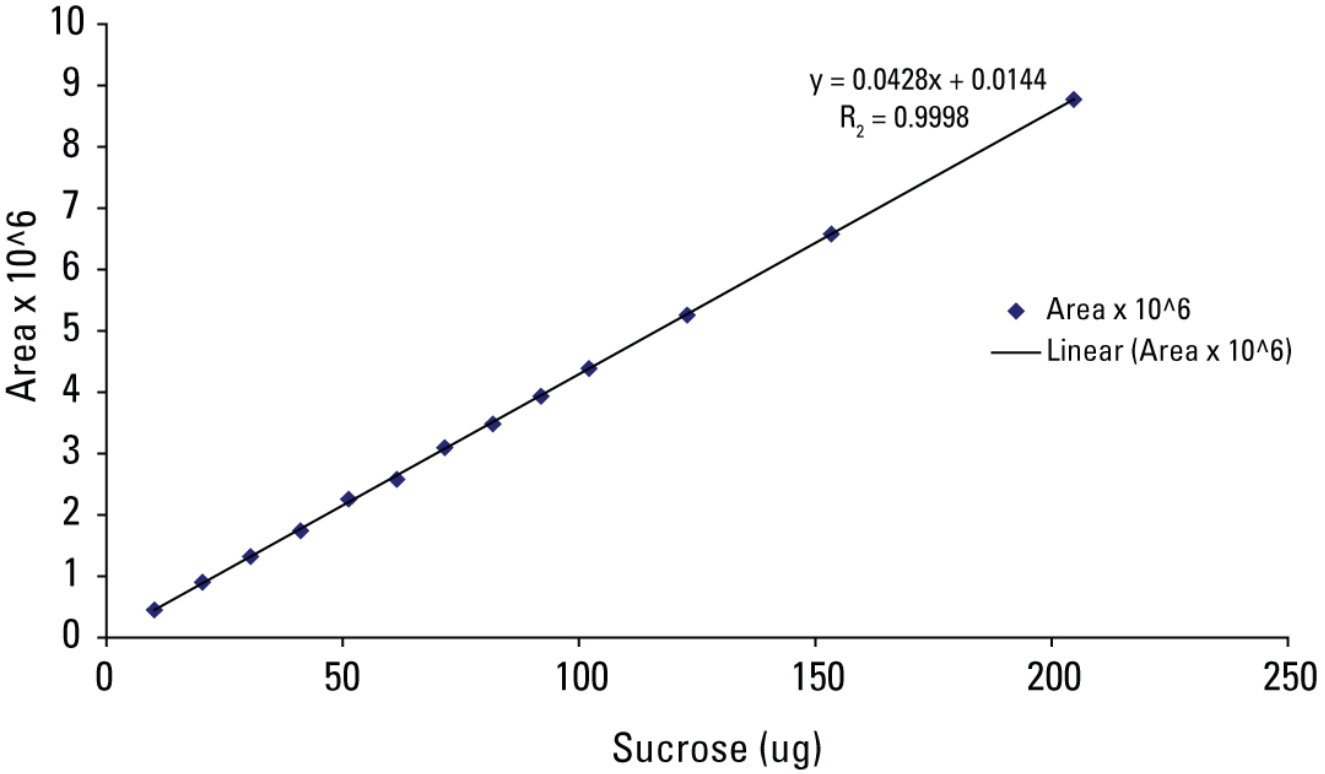
System suitability study

Sucrose

Run	RT (min)	k	Area (mAU*S)	A_s	Plates (N)
1	7.275	10.58	0.863×10^6	1.4	2732
2	7.28	10.59	1.07×10^6	1.4	2408
3	7.277	10.59	0.842×10^6	1.4	2734
Average	7.277	10.59	0.925×10^6	1.4	2624.6
Stdev	0.003	0.006	0.126×10^6	0.006	187.6
%RSD	0.000	0.000	0.136×10^6	0.008	0.071

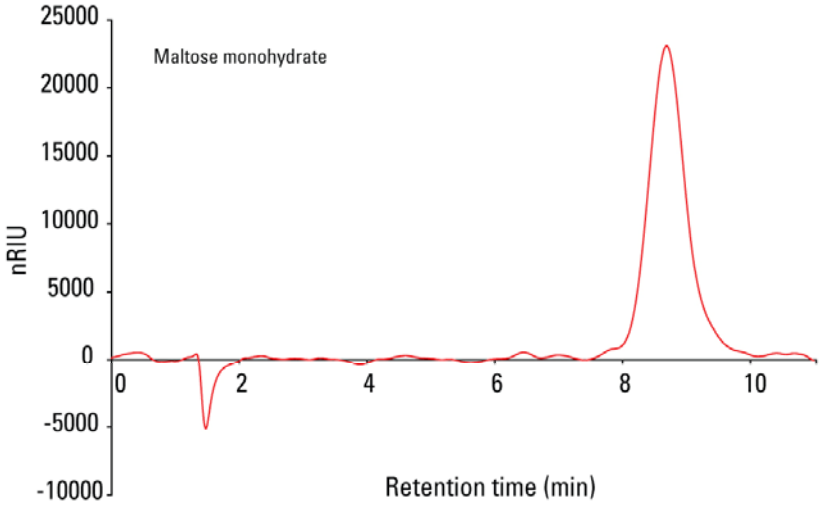
Three consecutive injections of sucrose yielded a very consistent result in case of all the peak parameters to determine the suitability of the system and method.

Loading capacity



Sucrose can be analyzed with a high degree of linearity over the experimental concentration range shown in this figure.

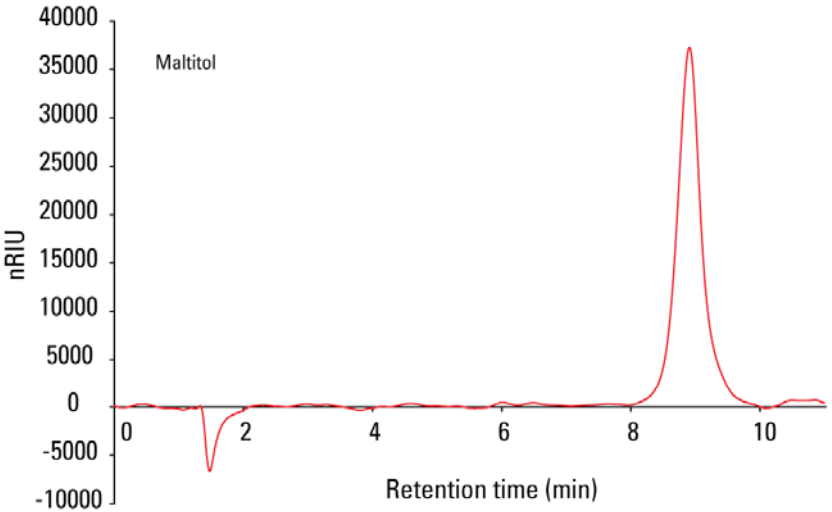
Analysis of maltose using a TSKgel NH_2-100, 3µm, 2.0mm ID x 5cm column



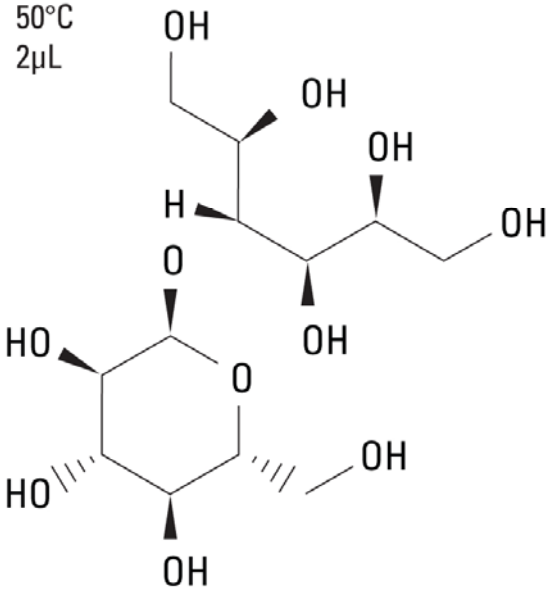
Column:	TSKgel NH_2-100, 3µm, 2.0mm ID x 5cm
Mobile phase:	80% ACN in H_20
Flow rate:	0.2mL/min
Detection:	RI
Temperature:	50°C
Injection vol.:	2µL

RT	k	Area x 10^6	A_s	Plates
8.688	12.83	0.911	1.19	1143

Analysis of maltitol (polyol or sugar alcohol) using a TSKgel NH_2-100, 3µm 2.0mm ID x 5cm column

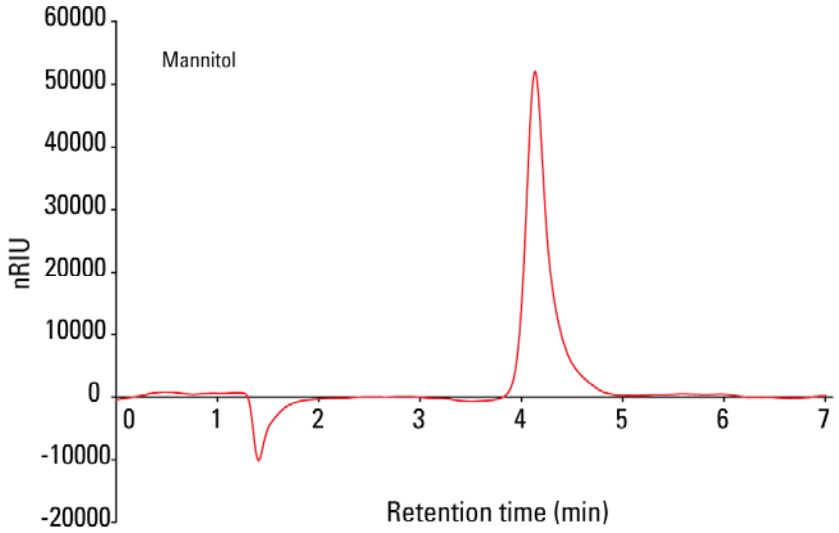


Column:	TSKgel NH_2-100, 3um, 2.0mm ID x 5cm
Mobile phase:	80% ACN in H_20
Flow rate:	0.2mL/min
Detection:	RI
Temperature:	50°C
Injection vol.:	2µL

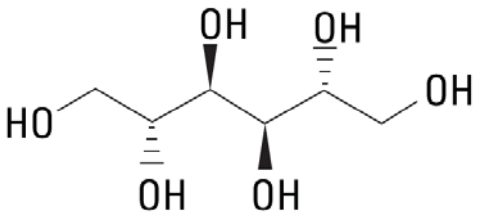


RT	k	Area x 10^6	A_s	Plates
8.908	13.18	104	1.05	3019

Analysis of mannitol (polyol or sugar alcohol) using TSKgel NH_2-100, 3µm, 2.0mm ID x 5cm column



Column:	TSKgel NH_2-100, 3um, 2.0mm ID x 5cm
Mobile phase:	80% ACN in H_20
Flow rate:	0.2mL/min
Detection:	RI
Temperature:	50°C
Injection vol.:	2µL



System suitability

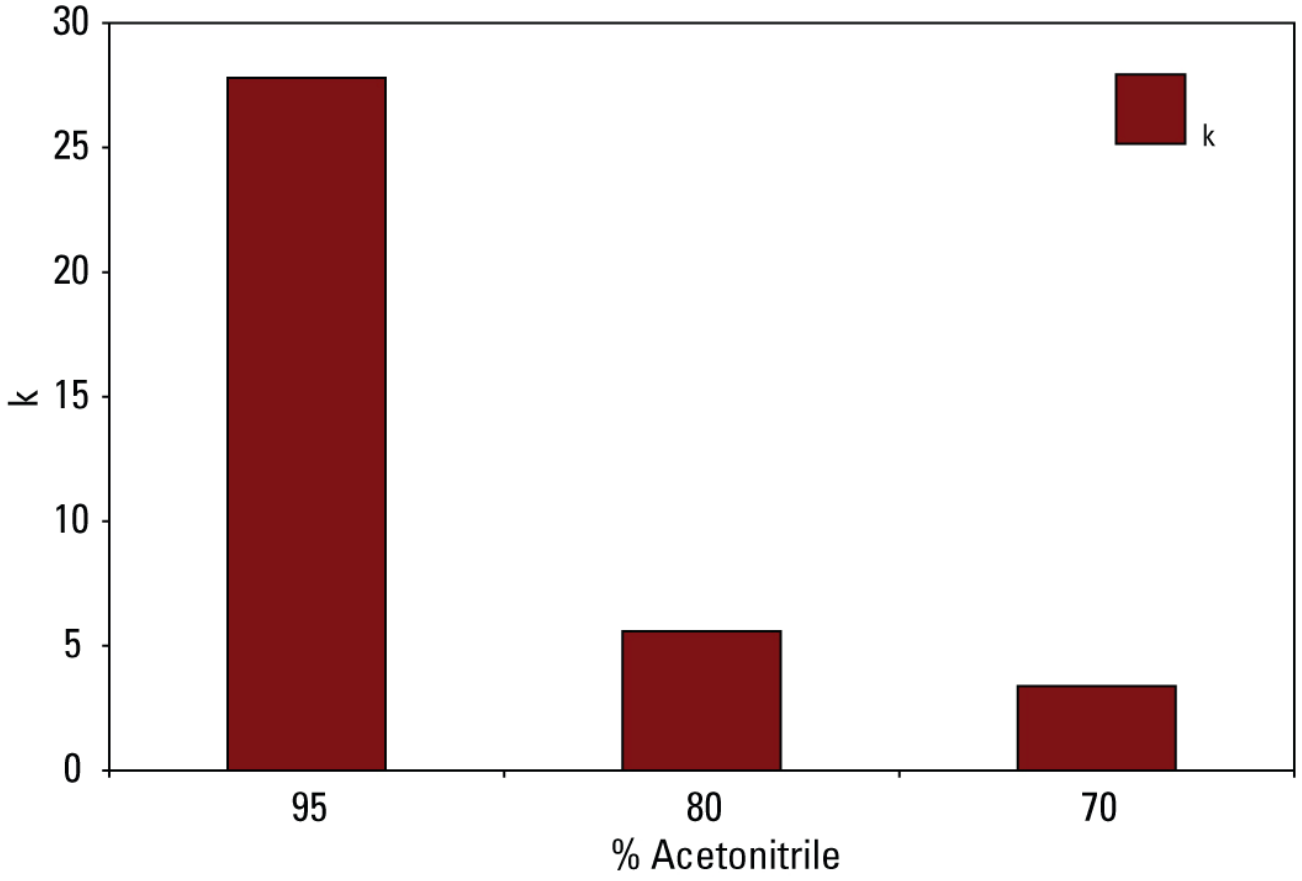
Mannitol

Run	RT (min)	k	Area x 10^6	A_s	Plates (N)
1	4.135	5.58	0.829	1.58	2124
2	4.135	5.58	0.923	1.63	2030
3	4.131	5.58	0.95	1.58	2027
4	4.134	5.58	0.915	1.69	2029
5	4.133	5.58	0.886	1.66	2028
Average	4.134	5.58	0.901	1.63	2047.6
Stdev	0.002	0.000	0.046	0.018	42.7
%RSD	0.000	0.000	0.051	0.029	0.021

Mannitol showed more tailing compared to maltitol possibly due to the difference in their interaction with the stationary phase inherent to the difference in their structure.

The analysis of mannitol, a sugar alcohol, could be reproduced with a high degree of consistency as in the case of sucrose, a disaccharide.

Effect of acetonitrile concentration on the retention of mannitol using TSKgel NH_2-100, 3µm, 2.0mm ID x 5cm column



Conclusions

- Different kinds of saccharides and sugar alcohols could be separated on a TSKgel NH_2-100 with good symmetry and efficiency.
- Calibration curve of sucrose show high loading capacity with high degree of linearity within the experimental range.
- System suitability studies (sucrose and mannitol) show that the analyses could be reproduced with very low %RSD in peak parameters using the TSKgel NH_2-100 column.
- The concentration of acetonitrile has considerable effect on the peak parameters such as retention time, capacity factor, symmetry and efficiency as seen in the analysis of mannitol using a TSKgel NH_2-100, 3µm, 2.0mm ID x 5cm column.
- This study shows that TSK-GEL NH_2-100 is stable.
- Limit of detection of glucose in the ppb level show high sensitivity of this column.
- Overall, this study shows that a TSKgel NH_2-100 column is suitable for the analysis of different kind of saccharides.