Analysis of Saccharides by Hydrophilic Interaction Chromatography (HILIC) using TSKgel® NH_2-100 Columns

TSKgel
APPLICATION NOTE

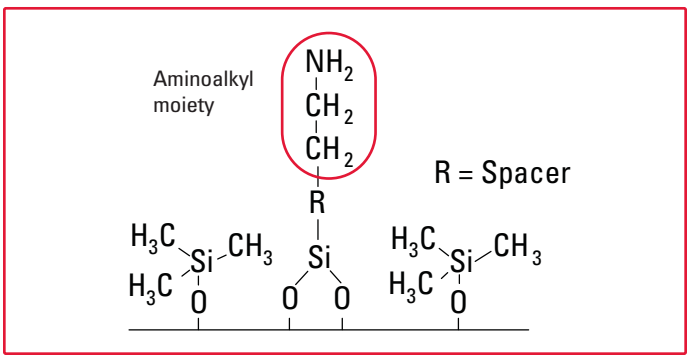
Introduction

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

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 or very close to the void volume of the column.

We report the successful analysis of different kinds of saccharides and sugar alcohols using a TSKgel NH_2-100 HILIC HPLC column. TSKgel NH_2-100 columns are packed with 3µm silica particles containing 100Å pores. A novel bonding strategy was adopted to improve chemical stability of the bonded phase. First the silica is reacted with a trimethylsilane endcapping reagent at a low stoichiometric ratio, before reacting residual and accessible silanol groups with trifunctional alkylaminosilane reagent. The resulting bonded phase provides a better safeguard against hydrolysis of the underlying silica. TSKgel NH_2-100 columns are unique in that the bonded phase ligand not only, as expected, has a terminal primary amino group, but that the spacer also incorporates secondary as well as tertiary amino groups *(see Figure 1)*. TSKgel NH_2-100 columns are well suited for the analysis of all types of hydrophilic compounds, including saccharides and sugar alcohols.

Figure 1. Structure of TSKgel NH_2-100



Experimental conditions

LC System:	HP-1100 HPLC with Chemstation (ver B.03.01)
Column:	TSKgel NH_2-100, 3µm, 2.0mm ID x 5cm
Mobile phase (Isocratic):	80% ACN in H_20
Flow rate:	0.2mL/min
Detection:	RI
Temperature:	50°C
Injection vol.:	2µL

The following commercially available compounds were used to prepare standard solutions:
Sucrose (Fisher S2-500)
Mannitol (Sigma M-4125, Lot 22K0111)

All the standards and samples were filtered through a 0.45µm PVDF filter (Gelman) before injecting onto the column. High purity chemicals and HPLC grade solvents were used for the preparation of stock standards, samples and mobile phases.

Table 1. Preparation of Standards

Saccharides	Weight	Stock Standard (mg/mL)
Sucrose	0.1023g in 10mL of 50% ACN in H_2O	10.23

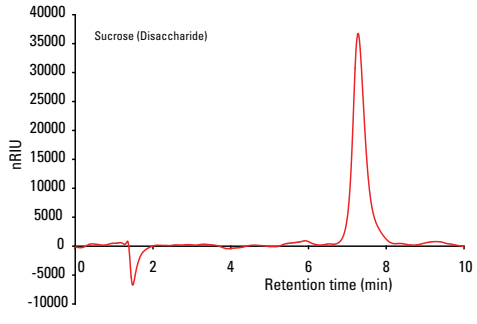
Polyols*	Weight	Stock Standard (mg/mL)	Working Standard (mg/mL)
Mannitol	0.1011g in 10mL of 50% ACN in H_2O	10.11	Same as stock

* Mannitol was readily soluble in 50% ACN in water.

Results and Discussion

The symmetry and efficiency of the eluted sucrose standard, a disaccharide, from the TSKgel NH_2-100 column is shown in *Figure 2*.

Figure 2. Analysis of Sucrose Standard



Column:	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

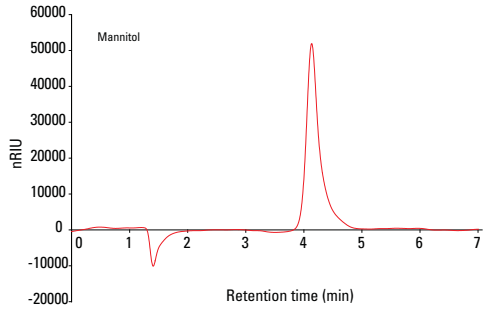
To determine the suitability of the system and method, three consecutive injections of sucrose were loaded onto the TSKgel NH_2-100 column. The study yielded a very consistent result across all peak parameters *(see Table 2)*. This column shows very high reproducibility for the analysis of sucrose.

Table 2. System Suitability Study Results - Sucrose Analysis

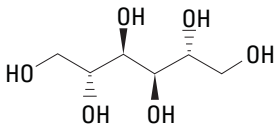
Sucrose	Run	RT	k	Area	AF	N
	1	7.275	10.58	0.863	1.40	2732
	2	7.280	10.59	1.070	1.40	2408
	3	7.277	10.59	0.842	1.40	2734
	Average	7.277	10.59	0.925	1.40	2625
	Stdev	0.003	0.01	0.126	0.01	187.6
	%RSD	0.03%	0.05%	13.6%	0.71%	7.1%

Mannitol, a sugar alcohol, was successfully analyzed on a TSKgel NH_2-100 column within five minutes *(Figure 3)*.

Figure 3. Analysis of Mannitol Standard



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



As in the case of sucrose, the analysis of mannitol could be reproduced with a high degree of consistency *(Table 3)*.

Table 3. System Suitability Study Results - Mannitol Analysis

Mannitol	Run	RT	k	Area	AF	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	0.950	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	5.580	0.901	1.63	2048
	Stdev	0.002	0.01	0.046	0.049	42.72
	%RSD	0.04%	0.18%	5.12%	2.99%	2.09%

Conclusions

This study shows that a TSKgel NH_2-100 column is suitable for the analysis of sucrose, a disaccharide, and mannitol, a sugar alcohol. Both standards separated on this column with good symmetry and efficiency. System suitability studies show that the analyses of sucrose and mannitol can be reproduced with very low %RSD in peak parameters using the TSKgel NH_2-100 column.

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