APPLICATIONS

Separation of Common Sugar Alcohols Used as Excipients with the Luna® Omega SUGAR LC Column - A Novel HILIC Selectivity

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Introduction

Traditionally, excipients are considered inert ingredients such as diluents, fillers, binders, lubricants, coatings, solvents, and dyes used in pharmaceutical formulation for drug products.[1] However, interest has increased for some excipients due to an observed influence on the overall absorption and/or bioavailability regarding the active pharmaceutical ingredient. For example, mannitol has been shown to decrease the bioavailability of cimetidine in oral dosage forms.[2] Because of the potential impact on the pharmacological effect of the active ingredient, analytical analysis of excipients has received broad industry focus.

Sugar alcohols are common excipients that are used as sweeteners and diluting agents for orally administered formulations. Erythritol, xylitol, sorbitol, mannitol, and maltitol are common sugar alcohols that are typically poorly absorbed and therefore osmotically active.[3,4] Because of the potential impact on absorption of co-administered drugs, accurate and robust analysis of sugar alcohols are important to pharmaceutical quality monitoring.

For the purpose of this technical note, we demonstrated the separation of sugar alcohols commonly utilized as excipients in pharmaceutical formulations. We prioritized the achievement of chromatographic resolution between sugar alcohols because of the strong chemical similarities, especially in the case of sorbitol and mannitol. Sorbitol and mannitol are positional isomers that differ by the orientation of a hydroxyl group on the 2-carbon. We first achieved chromatographic resolution of a 5-sugar alcohol standard that contained erythritol, xylitol, sorbitol, mannitol, and maltitol. After, we applied the same chromatographic mode of separation to a melatonin base sleeping aid that contained the excipients xylitol and mannitol. This was done to demonstrate the applicability of the Luna Omega 3 µm SUGAR LC column for the analysis of sugar alcohols within an orally administered tablet.

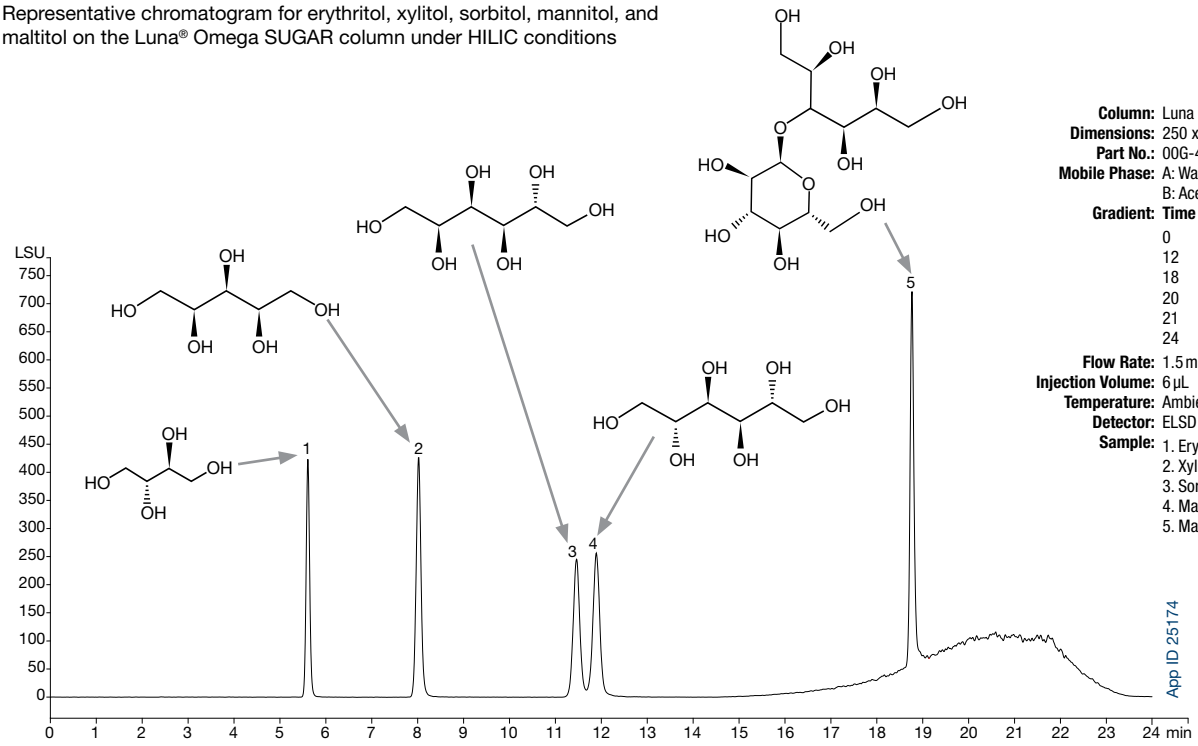
Methods and Materials

The elution implications of a 5-sugar alcohol analytical reference standard obtained from Sigma-Aldrich (St. Louis, MO) that con-tained erythritol, xylitol, sorbitol, mannitol, and maltitol was eval-uated. For this analysis, we used the Luna Omega SUGAR col-umn which is a fully porous, thermally modified silica-based LC column. The chemistry consists of a functionalized amide polyol/amine-based phase with aqueous stable end-capping. The col-umn dimension used for this demonstration and for the analysis of a sleeping aid pill was a 3 µm 250 x 4.6 mm column. The system used for the evaluation was a Waters ACQUITY I class (Milford, MA) equipped with an ELSD detector. The ELSD was set to a gas pressure of 45 psi with a drift tube temperature of 50 °C. A mobile phase that promoted HILIC based chromatography was selected. Part A consisted of a 10 mM ammonium acetate buffer and part B was acetonitrile, pH was left unadjusted. A flow rate of 1.5 mL/min was utilized with an injection volume of 6 µL.
A mixture of neat sugar alcohols standards was used to confirm the applicability of the Luna Omega SUGAR column to the separation of closely related sugar alcohols, under HILIC conditions. The concentration of the standard used was 2 mg/mL.

After the Luna Omega SUGAR demonstrated the ability to resolve a 5-sugar alcohol standard, we applied similar conditions to a sleeping aid pill that contained melatonin as the active ingredient. The same mobile phase convention was utilized with a pH unadjusted 10 mM ammonium acetate buffer and acetonitrile as the strong organic solvent. The sleeping aid pill was dissolved in 10 mL of water/acetonitrile (50:50), vortexed, and filtered using a Phenex™ 0.45 µm Nylon syringe filter. Peak identification was confirmed for xylitol and mannitol by running a neat standard mixture of these two sugar alcohols, dissolved in the water/acetonitrile mixture. The standard concentration of xylitol and mannitol was 2 mg/mL with a 6 µL injection volume.

Special attention was given to the initial conditioning and equilibration of the column prior to analysis. It has been shown that the retention mechanism of hydrophilic interaction chromatography (HILIC) is dependent on the establishment of an aqueous-rich layer on the surface of the column's stationary phase.[5] In order to propagate a reproducible hydrophilic layer, a 50:50 mixture of water with 10 mM ammonium acetate and acetonitrile was used to isocratically flush the Luna Omega 3 µm SUGAR column prior to use. The column (250 x 4.6 mm) was flushed for 20 minutes at a flow rate of 1 mL/min for a final flush volume of 20 mL. After the isocratic flush, the column was equilibrated with the initial gradient conditions for 15 minutes at 1.5 mL/min to establish the aqueous-rich layer that is necessary for HILIC separations.

Figure 1.
Representative chromatogram for erythritol, xylitol, sorbitol, mannitol, and maltitol on the Luna® Omega SUGAR column under HILIC conditions

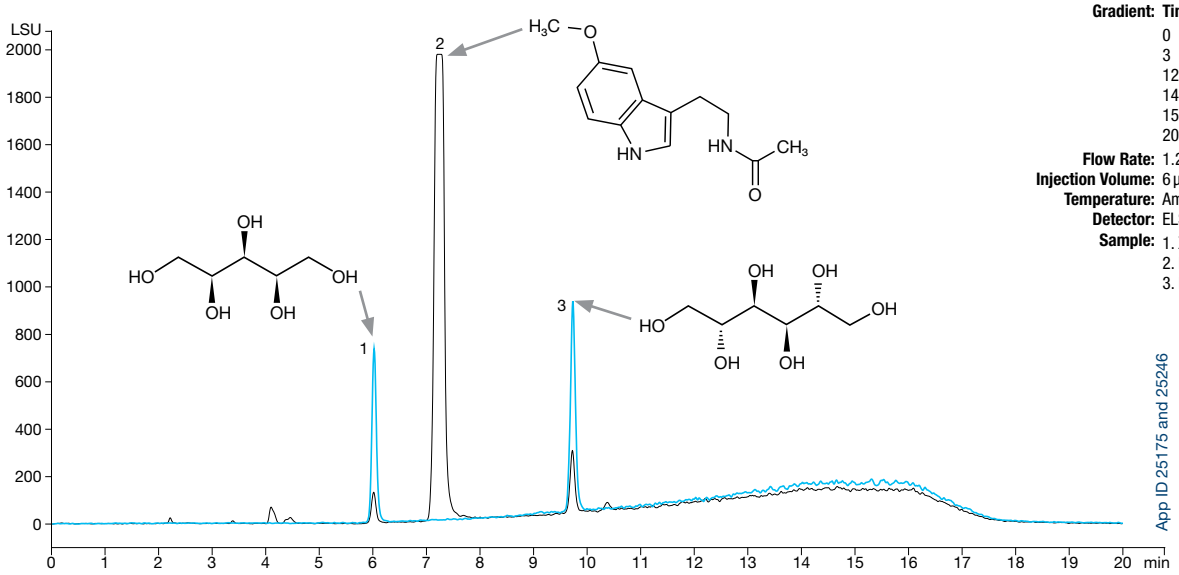


Column: Luna Omega 3 µm SUGAR
Dimensions: 250 x 4.6 mm
Part No.: 00G-4775-E0
Mobile Phase: A: Water with 10 mM Ammonium acetate
B: Acetonitrile

Gradient:	**Time (min)**	**% B**
	0	84
	12	84
	18	65
	20	65
	21	84
	24	84

Flow Rate: 1.5 mL/min
Injection Volume: 6 µL
Temperature: Ambient
Detector: ELSD (50 °C)
Sample: 1. Erythritol
2. Xylitol
3. Sorbitol
4. Mannitol
5. Maltitol

Figure 2.
Representative chromatogram overlay of sleeping aid pill (melatonin) and reference standard for xylitol and mannitol on the Luna Omega SUGAR column under HILIC conditions



Column: Luna Omega 3 µm SUGAR
Dimensions: 250 x 4.6 mm
Part No.: 00G-4775-E0
Mobile Phase: A: Water with 10 mM Ammonium acetate
B: Acetonitrile

Gradient:	**Time (min)**	**% B**
	0	75
	3	75
	12	60
	14	60
	15	75
	20	75

Flow Rate: 1.2 mL/min
Injection Volume: 6 µL
Temperature: Ambient
Detector: ELSD (50 °C)
Sample: 1. Xylitol
2. Melatonin
3. Mannitol

Results and Discussion

The Luna® Omega 3 µm SUGAR LC column demonstrated the ability to separate a 5-sugar alcohol standard (erythritol, xylitol, sorbitol, mannitol, and maltitol) in **Figure 1** under HILIC conditions. Specifically, the unique amide polyol/amine stationary phase of the Luna Omega SUGAR column was able to achieve baseline resolution between the positional isomers mannitol and sorbitol. Additionally, the method was able to provide good peak shape and retention for all 5 sugar alcohols, referenced above. In **Table 1** are the reported values of tailing, as prescribed by the USP definition for peak symmetry.[6]

Table 1.
Summary of 5-sugar alcohol standard mixture on Luna Omega SUGAR

Peak No.	Analyte	Retention Time (min)	Area	Height	USP Resolution	USP Tailing
1	Erythritol	5.6	2267846	423016		1.07
2	Xylitol	8.0	3133420	426963	14.24	1.05
3	Sorbitol	11.5	2470421	245102	14.67	1.09
4	Mannitol	11.9	2691776	255362	1.57	0.97
5	Maltitol	18.8	4008678	665672	32.05	1.10

In **Figure 2**, we applied similar running conditions to the qualitative representation of a sleeping aid pill that contained melatonin as the active ingredient and xylitol/mannitol as excipients. For peak identification, we utilized a 2 mg/mL standard injection for xylitol and mannitol, overlaid in **Figure 2**. Therefore, it was confirmed that under the presented conditions xylitol had an observed retention time of 6.0 and mannitol was 9.7 minutes (**Table 2**). All peaks featured good peak shape and were fully resolved from one another.

Table 2.
Qualitative results of sleeping aid pill on Luna Omega SUGAR

Peak No.	Analyte	Retention Time (min)	Area	Height	USP Resolution	USP Tailing
1	Xylitol	6.0	797927	130274		1.12
2	Melatonin	7.2	25794227	1964535	5.48	1.30
3	Mannitol	9.7	1619553	260544	11.35	1.00

Conclusion

In this technote, we first demonstrated the separation of 5-sugar alcohols commonly utilized as excipients in pharmaceutical formulations and then applied similar running conditions to a formulated melatonin sleeping aid pill that contained xylitol/mannitol as excipients. The Luna Omega 3 µm SUGAR column was able to provide adequate resolution and good peak shape for both standards and pill samples.

References

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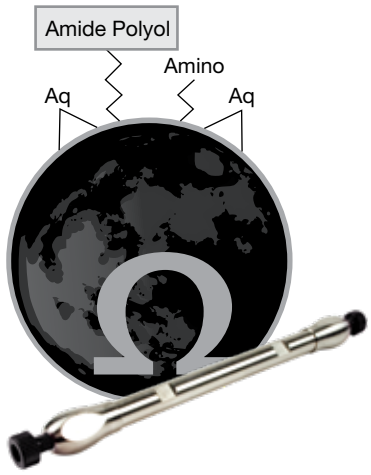
APPLICATIONS

Novel nitrogen containing stationary phase that greatly increases the retention of sugars and sugar alcohols under HILIC conditions



Luna® Omega SUGAR

Ordering Information

3 µm Minibore Columns (mm)				SecurityGuard™ Cartridges (mm)
Phases	50 x 2.1	100 x 2.1	150 x 2.1	4 x 2.0*
				/10pk
SUGAR	00B-4775-AN	00D-4775-AN	00F-4775-AN	AJ0-4496
				for ID: 2.0-3.0 mm

3 µm MidBore™ Columns (mm)		SecurityGuard Cartridges (mm)
Phases	150 x 3.0	4 x 2.0*
		/10pk
SUGAR	00F-4775-Y0	AJ0-4496
		for ID: 2.0-3.0 mm

3 µm Analytical Columns (mm)				SecurityGuard Cartridges (mm)
Phases	100 x 4.6	150 x 4.6	250 x 4.6	4 x 3.0*
				/10pk
SUGAR	00D-4775-E0	00F-4775-E0	00G-4775-E0	AJ0-4495
				for ID: 3.2-8.0 mm

* SecurityGuard Standard Analytical Cartridges require holder, Part No.: KJ0-4282

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