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Rapid Analysis of Oligosaccharides on the Hitachi LaChromUltra® Liquid Chromatography System Using Refractive Index Detection

Hitachi High Technologies America, Inc.

Carbohydrate analysis of foods is necessary for federally mandated nutritional labeling. Here we present an oligosaccharide analysis using the LaChromUltra liquid chromatography system and a short, small particle sized column for fast analysis. Sucrose and three of its oligomers (stachyose, raffinose, and verbascose) are studied using refractive index (RI) detection. Traditional separations that employ specialized carbohydrate columns can take as long as 15 minutes[1] to analyze these oligosaccharides. These separations typically use gradient elution, which is not appropriate for RI detection. This application note shows separation of these sugars in under 2 minutes, while keeping actual injection to injection time under 3 minutes. This rapid analysis allows for higher sample throughput, as well as an overall reduction in solvent usage.

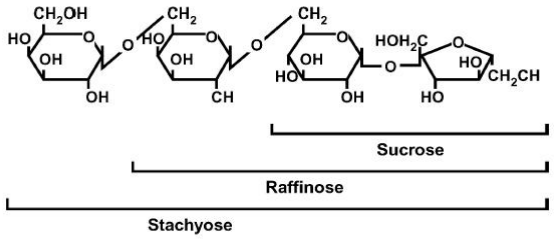

Figure 1: The structures for the sugars. Verbascose is stachyose plus another β-galactoside linked glucose molecule. Graphic courtesy Vinjamoori et al[2]:

Experimental Conditions:

Module	Conditions and Other
Pump (L-2160U)	Mobile Phase: Isocratic ACN/water 80/20
Autosampler (L-2200U)	Injection Volume: 3, 5, and 7 µL of a 0.1% solution of each component
Oven (L-2300)	Column Temperature: 40°C
Refractive Index (L-2490)	Flow Cell Temperature: 40°C
Column	Thermo Hypersil APS-2 3 µm 3.0 x 50 mm
Standards	0.1% of each Oligosaccharide in mobile phase: Sucrose: Sigma 077K0085 D-(+)-Raffinose: Sigma 039K0016 Stachyose Hydrate: Sigma 078K380 Verbascose: Fluka 1332709

Results — Reproducibility of Standards (5 µL Inj. Vol.):

Oligosaccharide	Sucrose	Raffinose	Stachyose	Verbascose
Peak Area Rep 1	147997	131200	108780	116696
Peak Area Rep 2	146676	129955	107842	113277
Peak Area Rep 3	146951	129479	109085	115102
Peak Area Rep 4	149271	131356	111194	116954
Peak Area Rep 5	151272	132741	109317	113145
Mean	148433.4	130946.2	109243.6	115034.8
Std Dev	1887	1283	1226	1810
%RSD	1.3	1.0	1.1	1.6

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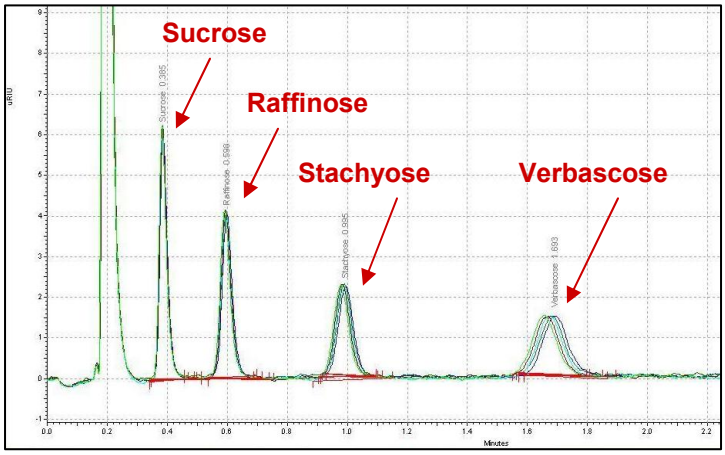
Results – Example Chromatogram of Oligosaccharides:
Five Replicate Injections with a 5µL Injection Volume



RI Result Peak #	Name	Retention Time	Area	Theo. Plates	Asymmetry
1	Sucrose	0.39	147997	1074	1.41
2	Raffinose	0.6	131200	1462	1.18
3	Stachyose	1.04	108780	1731	1.02
4	Verbascose	1.69	116696	1907	1.03

Results – Linearity of Standards (3, 5 and 7 µL):

Oligosaccharide	Sucrose	Raffinose	Stachyose	Verbascose
r-squared value	1.0000	0.9999	0.9998	0.9997

Discussion:

Hitachi's LaChromUltra liquid chromatography system, equipped with a 3 µm particle size aminopropylsilane column, resolves the oligosaccharides sucrose, raffinose, stachyose, and verbascose in far less time than traditional separations. The system is suitable and reproducible. Reproducibility (<2 %RSD) and system suitability (theoretical plates: N > 1000 and asymmetry (tailing factor): T < 2.0) are shown for the 5 µL oligosaccharide injection volume.

References:

1 – Alltech Prevail ES Carbohydrate Column Literature. Alltech Associates, Deerfield, IL. 2002.

2 – Vinjamoori, D. et al. "Challenges and opportunities in the analysis of raffinose oligosaccharides, pentosans, phytate, and glucosinolates." *J Anim Sci*, 82: 319-328 2004.

Hitachi High Technologies America, Inc.
Life Sciences Division
5100 Franklin Drive
Pleasanton, CA 94588
Toll Free: (800) 548-9001
E-Mail: Sales-LS@hitachi-hta.com
Web site: www.hitachi-hta.com/ultra