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Rapid Analysis of Added Sugars in Meat Samples by UHPLC-ELSD using a Traditional HPLC Amino Column Chemistry

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Background

The simple sugars fructose and glucose along with the disaccharides sucrose, maltose, and lactose are commonly added to flavour or preserve (cure) meats and are required to accurately quantify for formulation and USDA nutritional label claims. Hydrophilic interaction chromatography (HILIC) separations over polar amine stationary phases have been popular methodologies implemented by HPLC. The desire to decrease run-time, increase throughput, and decrease solvent consumption through UHPLC steps have been a challenge with this application due to limited UHPLC stationary phases available for scale down.

Introduction

HILIC separations of simple sugars leverage extensive hydrogen bonding interactions of saccharide hydroxyl groups with column stationary phase amine groups. The orientation of the hydroxyl groups around the mono and disaccharide rings are what dictate their chromatographic selectivity. Some modern amine stationary phases can fail in their selectivity of some critical saccharide pairs. For example, Luna NH_2 is an optimized amine phase with ligand cross-linking for added reproducibility and durability, and maintains the ability to resolve all simple sugars and most disaccharides. However, it fails to separate lactose and maltose. For saccharide separations that include the lactose and maltose pair, a traditional propyl amine phase is necessary to achieve their selectivity. Here we leverage the selectivity of a traditional column chemistry, SphereClone™ NH_2, in a scaled down HPLC format to achieve the desired reduced run-time, increased throughput, and decreased solvent consumption.

Experimental Conditions

Original 20-Minute HPLC-RI Conditions

Conditions for both separations:

Column: SphereClone 5 µm NH_2
Dimensions: 250 x 4.6 mm
Part No.: 00G-4147-E0
Mobile Phase: Acetonitrile/Water (80:20)
Injection Volume: 15 µL
Flow Rate: 1.25 mL/min
Temperature: 50 °C
Detector: Refractive Index (RI)
Analytes: 1. Fructose
2. Glucose
3. Sucrose
4. Maltose
5. Lactose
Note- First Peak = Solvent front/Matrix

Figure 1.
Typical Standard Chromatogram in Original HPLC-RI method

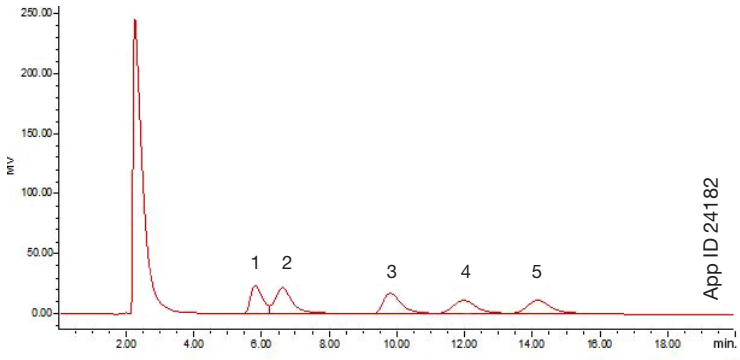

Figure 2.
Example Sample Chromatogram using Original HPLC-RI method

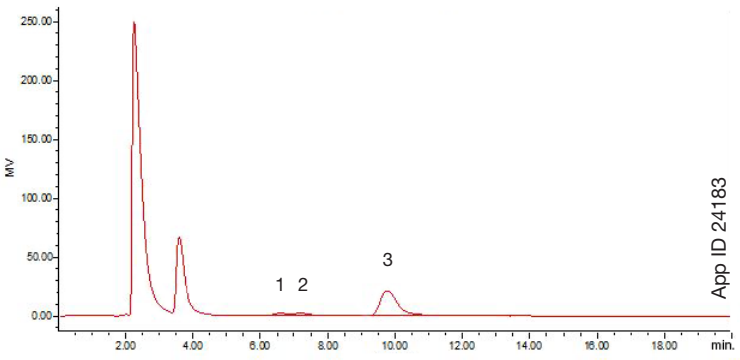

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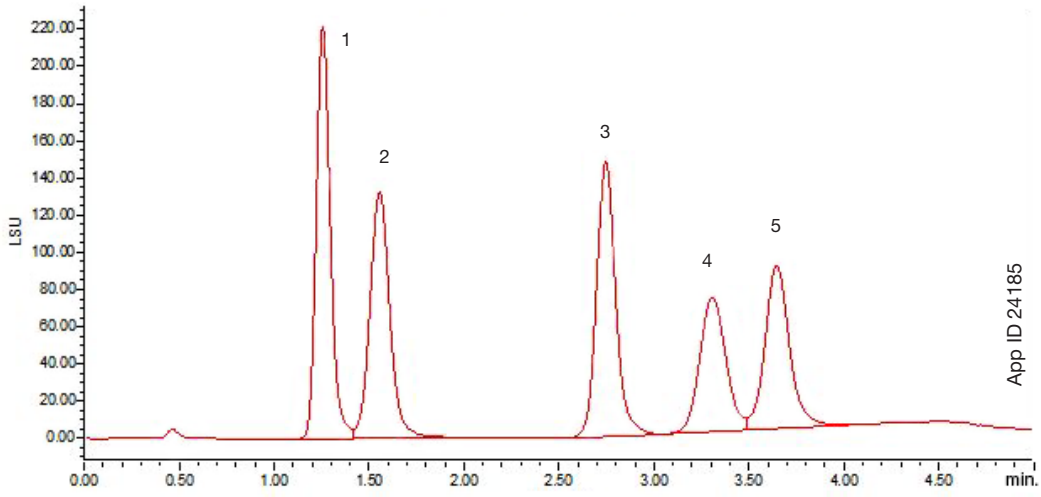
Figure 3.
Typical Standard Chromatogram using UHPLC-ELSD method



5-Minute UHPLC-ELSD Conditions

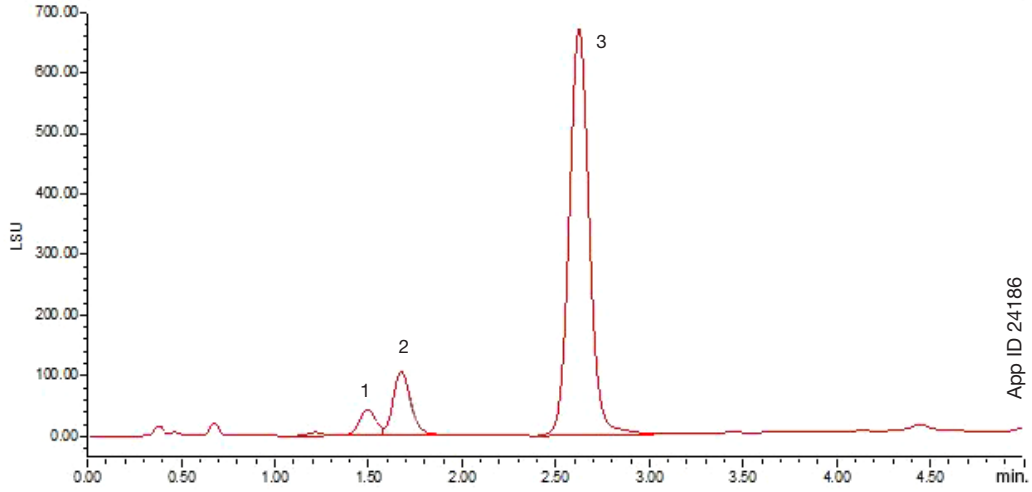
Conditions for both separations:

Column: SphereClone™ 3 µm NH_2
Dimension: 150 x 2.1 mm
Part No.: 00F-4137-AN
Mobile Phase: A: MilliQ® water
B: Acetonitrile

Gradient:	**Time (min)**	**% B**
	0	83
	2	77
	4	83
	5	83

Injection Volume: 7.5 µL
Flow Rate: 1.0 mL/min
Temperature: 50˚C
Backpressure: 5000 psi
Detector: ELSD Detector Settings
Gain: 20
Gas Pressure: 30 psi
Nebulizer Mode: Cooling
Drift Tube Temp: 60 °C
Analytes: 1. Fructose
2. Glucose
3. Sucrose
4. Maltose
5. Lactose

Figure 4.
Example Sample Chromatogram using UHPLC-ELSD method



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Results and Discussion

The sugar method utilizing UHPLC-ELSD performs equivalent to the current HPLC-RI method with the modification of using a narrow bore 3µm column with a HILIC gradient. Standards and spiked recoveries are comparable to the current methodology. Therefore, both pieces of equipment can be used to accomplish the same results only with a modification in mobile phases due to detector requirements.

- Instrument precision for all five analytes from 5 to 0.1 mg/mL (ppm) below 10%. Everything except for Lactose was below 6%.
- Two routine matrices spiked with all five analytes
  - o **Table 1**- Pet Treats
  - o **Table 2**- Mechanically Separated Chicken (MSC)

Table 1.
Pet Treats

	MDL (ppm)	MQL (ppm)	IDL (ppm)	ILQ (ppm)	Average % Recovery (40 ppm)	Average % Recovery (20 ppm)	Average % Recovery (10 ppm)
Fructose	0.53	2.13	0.014	0.056	113	105	90
Glucose	0.78	3.11	0.014	0.055	105	94	82
Sucrose	3.97	15.88	0.025	0.1	95	100	104
Maltose	0.87	3.49	0.013	0.053	107	103	97
Lactose	0.93	3.72	0.028	0.11	105	99	95

Table 2.
Mechanically Separated Chicken (MSC)

	MDL (ppm)	MQL (ppm)	IDL (ppm)	ILQ (ppm)	Average % Recovery (40 ppm)	Average % Recovery (20 ppm)	Average % Recovery (10 ppm)
Fructose	0.32	1.28	0.014	0.056	96	88	79
Glucose	0.52	2.05	0.014	0.055	101	93	85
Sucrose	0.54	2.16	0.025	0.1	95	86	77
Maltose	0.45	1.79	0.013	0.053	91	81	69
Lactose	0.79	3.18	0.028	0.11	95	88	79

Conclusion

HILIC separations using amine phases for saccharides employ conditions with low viscosity (acetonitrile-rich mobile phases at elevated temperatures). As such, the run-time can be decreased in many cases by simply increasing the flow rate. Utilizing an ELSD instead of an RI detector increased sensitivity and allowed for a gradient to further reduce the run-time, and then a narrow-bore internal diameter allowed for a decrease in solvent consumption with a faster linear velocity rather than an increase in volumetric mobile phase flow.

While UHPLC technology has expanded the range of chromatographic power, often by exploiting higher efficiencies generated from smaller particle sizes or core-shell particle morphologies, here we utilized the selectivity of an older column chemistry to achieve the same desired benefits, reducing a 20 minute run to less than 5 minutes.

Acknowledgements

We would like to provide special thanks to Tyson Foods, Inc. and WBA Analytical Laboratories for their contributions.

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SphereClone™ Ordering Information

3 µm Columns (mm)			SecurityGuard™ Cartridges (mm)	
Phase	150 x 2.1	150 x 4.6	4 x 2.0	4 x 3.0
			/10pk	/10pk
NH_2	00F-4137-AN	00F-4137-E0	AJ0-4301	AJ0-4302
			for ID: 2.0-3.0 mm	3.2-8.0 mm

5 µm Columns (mm)				SecurityGuard™ Cartridges (mm)
Phase	50 x 4.6	150 x 4.6	250 x 4.6	4 x 3.0
				/10pk
NH_2	00B-4147-E0	00F-4147-E0	00G-4147-E0	AJ0-4302
				for ID: 3.2-8.0 mm

guarantee

If SphereClone analytical columns do not provide at least an equivalent separation as compared to Spherisorb columns of the same phase, particle size and dimensions, return the column with comparative data within 45 days for a FULL REFUND.

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SecurityGuard is patented by Phenomenex. U.S. Patent No. 6,162,362
CAUTION: this patent only applies to the analytical-sized guard cartridge holder, and does not apply to SemiPrep, PREP or Ultra holders, or to any cartridges.

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