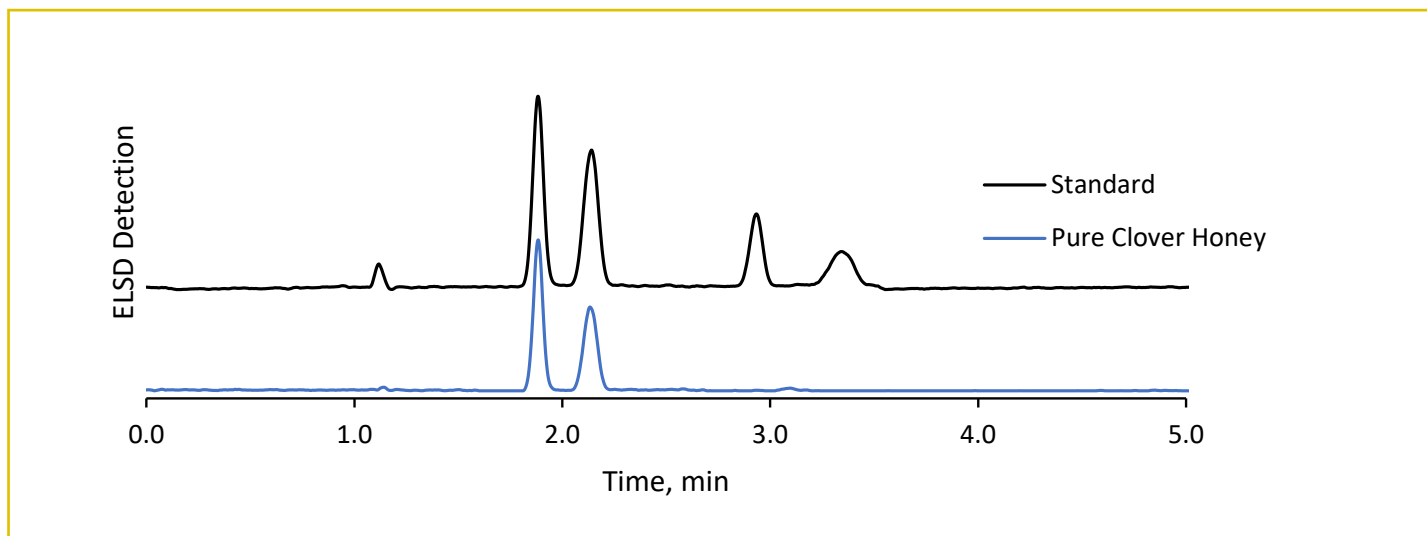




## Analysis of Sugars in Pure Honey using HALO® Penta-HILIC

215-SU



### PEAK IDENTITIES:

1. D-(-) Fructose
2. D-(+) Glucose
3. Sucrose
4. D-(+) Maltose

### TEST CONDITIONS:

**Column:** HALO 90 Å Penta-HILIC, 2.7  $\mu\text{m}$ , 4.6 x 150 mm

**Part Number:** 92814-705

**Mobile Phase A:** Water

**Mobile Phase B:** Acetonitrile

Isocratic: 80% B

**Flow Rate:** 1.4 mL/min

**Initial Pressure:** 213 bar

**Temperature:** 65 °C

**Detection:** ELSD, 40 °C, 3.3 bar

**Injection Volume:** 15  $\mu\text{L}$

**Sample Solvent:** 80/20 ACN/ Water

**Data Rate:** 10 Hz

**Response Time:** 0.10 sec

**LC System:** Shimadzu Nexera X2

Honey can significantly range in quality depending on its purity and levels of sucrose and maltose. Natural honey primarily consists of fructose and glucose, while adulterated honey can contain high levels of sucrose and maltose.

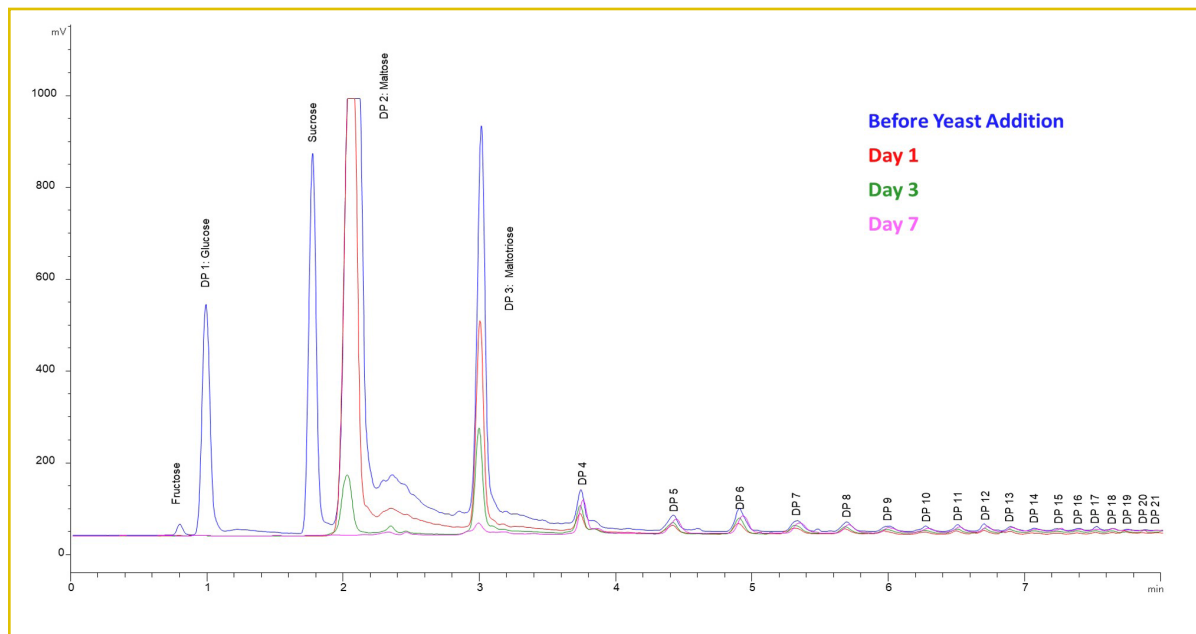
A HALO® Penta-HILIC column separates the primary monosaccharides in pure honey clover showing no signs of adulteration.





### Beer Fermentation Analysis using HALO® Penta-HILIC

269-SU



#### TEST CONDITIONS:

**Column:** HALO 90 Å Penta-HILIC, 2.7 µm, 3.0 x 50 mm

**Part Number:** 92813-405

**Mobile Phase A:** Water

**Mobile Phase B:** Acetonitrile

Gradient:	Time	%B
	0.0	92
	8.0	52

**Flow Rate:** 0.75 mL/min

**Temperature:** 65 °C

**Detection:** ELSD, 40°C, 45 psi

**Injection Volume:** 2 µL

**Data Rate:** 10 Hz, 2 sec filter

*Data Courtesy of Merlin K. L. Bicking, Ph. D. (ACCTA, Inc.)*

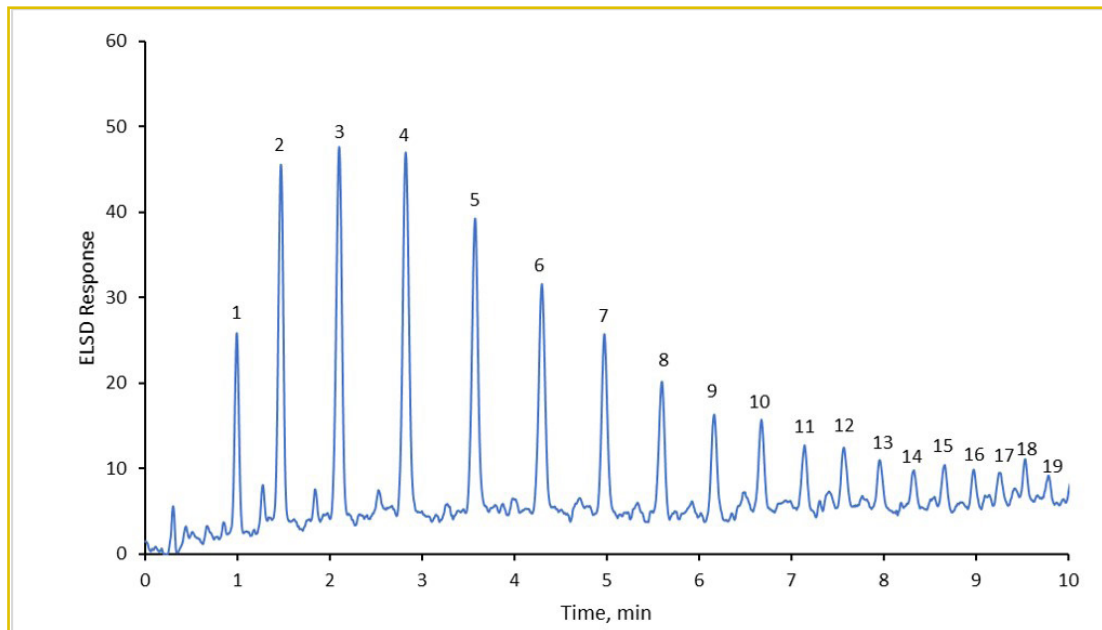
A Belgian ale is analyzed with a HALO® Penta-HILIC column using an evaporative light scattering detector (ELSD). Sugars, oligosaccharides, and polysaccharide levels are monitored throughout the fermentation process in order to track yeast behavior. These levels will decrease over time as the yeast converts the sugars to ethanol. The Penta-HILIC/ ELSD combination is a great way to perform rapid sugar analysis providing high resolution and good peak shape at elevated temperatures.





## Fast Separation of Oligosaccharides using HALO 90 Å Penta-HILIC

273-SU



### PEAK IDENTITIES:

1. DP 3
2. DP 4
3. DP 5
4. DP 6
5. DP 7
6. DP 8
7. DP 9
8. DP 10
9. DP 11
10. DP 12
11. DP 13
12. DP 14
13. DP 15
14. DP 16
15. DP 17
16. DP 18
17. DP 19
18. DP 20
19. DP 21

### TEST CONDITIONS:

**Column:** HALO 90 Å Penta-HILIC, 2.7 µm, 4.6 x 50 mm

**Part Number:** 92814-405

**Mobile Phase A:** Water

**Mobile Phase B:** ACN

**Gradient:** 75-55% B in 10 min

**Flow Rate:** 2.0 mL/min

**Pressure:** 105 bar

**Temperature:** 65 °C

**Detection:** ELSD, 40 °C, 3.3 bar

**Injection Volume:** 20 µL

**Sample Solvent:** 70/30 ACN/Water

**Data Rate:** 10 Hz

**Response Time:** 0.10 sec

**LC System:** Shimadzu Nexera X2

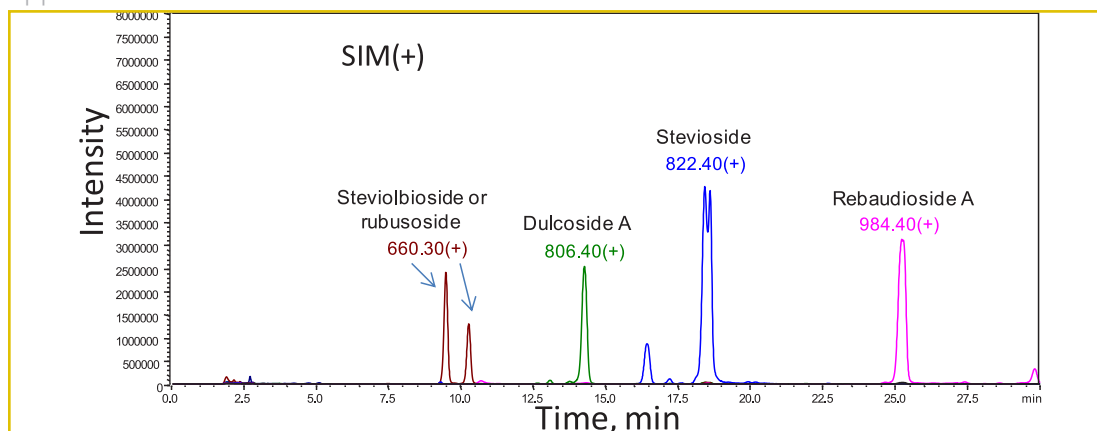
The combination of evaporative light scattering detection (ELSD) and a short 50 mm HALO® Penta-HILIC column enables a fast analysis of oligosaccharides in under 10 minutes whereas traditional columns could have analysis times as long as 30 minutes to more than an hour. Using ELSD eliminates the need to label the sugar with either a UV or fluorescent tag, which simplifies the analysis. Peak identities are labeled by degree of polymerization (DP).





## LC-MS Analysis of Stevia Extract on HALO® Penta-HILIC, 5 µm

Application Note 124-F



Stevia is a natural sweetener and is used as a substitute for sugar. LC/MS analysis of Stevia glycosides from a Stevia extract is easily accomplished using a HALO® Penta-HILIC, 5 µm column due to its unique bonded phase containing five OH groups and the high efficiency of the 5-micron Fused-Core® particles.

### TEST CONDITIONS:

**Column:** HALO 90 Å Penta-HILIC, 5 µm,  
3.0 x 250 mm  
**Part Number:** 95813-905  
**Mobile Phase:**  
A: 50/50 water/acetonitrile with 5 mM ammonium formate, pH 3.0  
B: 5/95 water/acetonitrile with 5 mM ammonium formate, pH 3.0  
**Gradient:** 90% B to 67% B in 30 min  
**Flow Rate:** 0.5 mL/min  
**Pressure:** 60 bar  
**Temperature:** Ambient  
**Injection Volume:** 5.0 µL  
**Sample Solvent:** 80/20 acetonitrile/water  
**LC System:** Shimadzu Nexera  
**MS:** Shimadzu LCMS 2020 (single quadrupole)  
**ESI:** +4.5 kV  
**Scan Range:** 200-1200 m/z  
**Scan Rate:** 2 pps  
**Capillary:** 250 °C  
**Heat Block:** 350 °C  
**Nebulizing Gas Flow:** 1.5 L/min  
**Drying Gas Flow:** 15 L/min

### EXTRACTION PROCEDURE:

1. Weigh 400 mg of Stevia rebaudiana leaves (Sigma S5381)
2. Crush leaves with mortar and pestle and transfer to vial
3. Add 8.0 mL of 50/50 (v/v) acetonitrile/water
4. Sonicate vial contents for 15 minutes
5. Filter sample using 25 mm syringe filter having 0.2 µm PTFE membrane (VWR 28145-495)
6. Centrifuge @ 10K rpm (5 min) and collect supernate
7. Dilute 400 µL of extract in 600 µL of acetonitrile for overall concentration of 80/20 acetonitrile/water
8. Centrifuge diluted sample @ 10K (5 min.) rpm and inject the supernate

