



# Fast Screening of Oligo- and Poly-saccharides in Beer

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# Overview

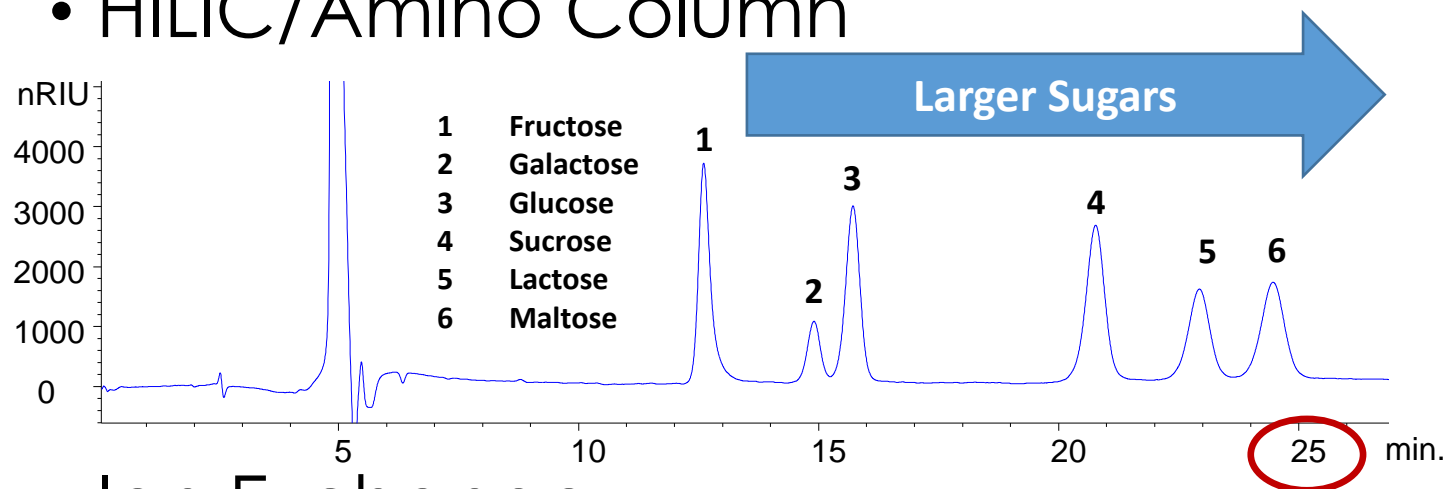
- Refine existing procedures for fast screening of sugars to identify larger sugars in beer samples
- Evaluate sample preparation procedures
  - Provide filtered aqueous sample for injection
  - Minimize interferences in chromatography
  - Minimize column contamination from highly retained components
- Evaluate
  - mash samples
  - fermentation samples
  - finished product





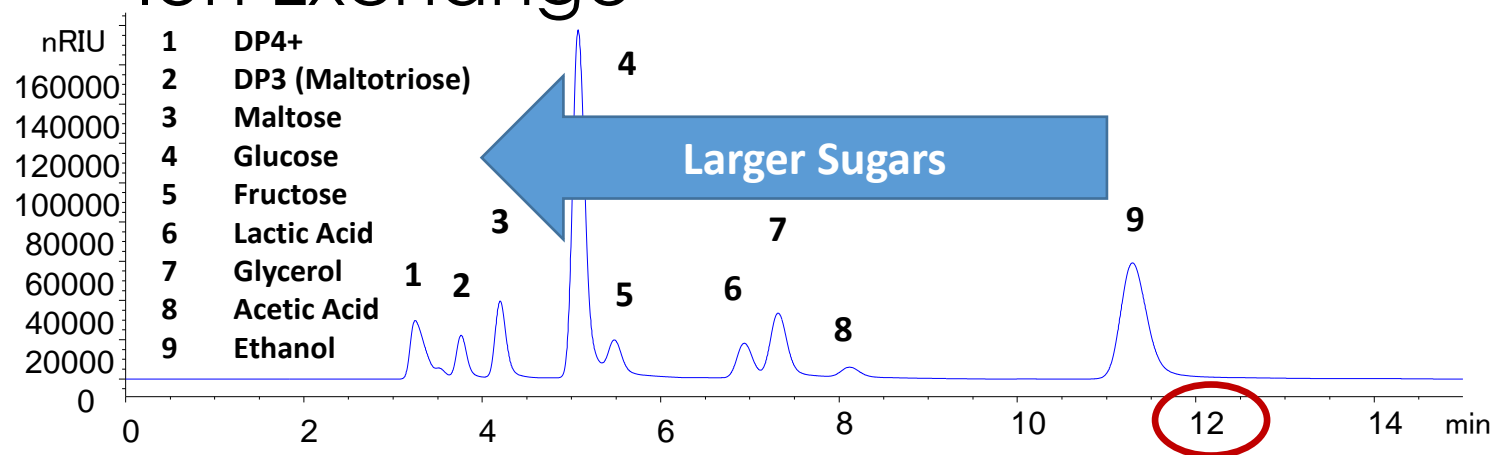
# Current Options for Separating Sugars by HPLC

- HILIC/Amino Column



2X 4.6 X 150 mm APHera NH2  
(2 columns in series)  
75/25 ACN/Water

- Ion Exchange

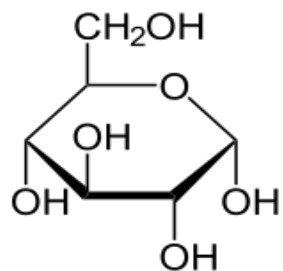
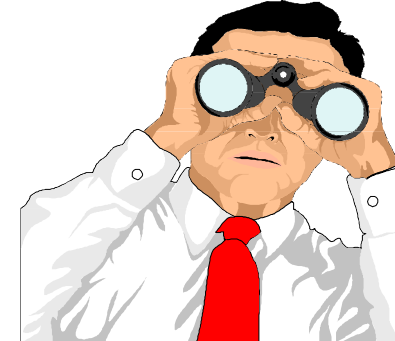


4.6 X 150 mm Ion Exchange (H+)  
0.005 N H<sub>2</sub>SO<sub>4</sub>



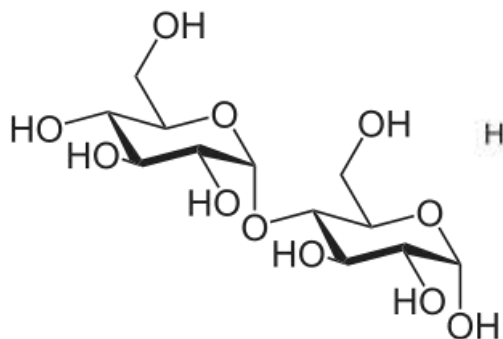


# Looking For a Better Option for Larger Sugars



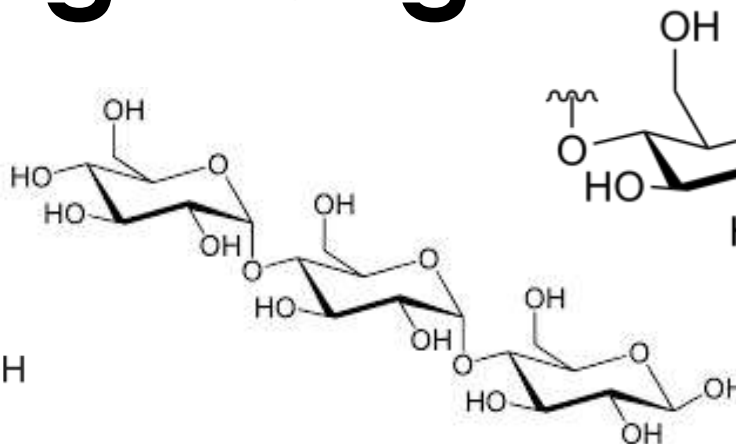
**DP 1**  
**(Glucose)**

Log P = -3.2



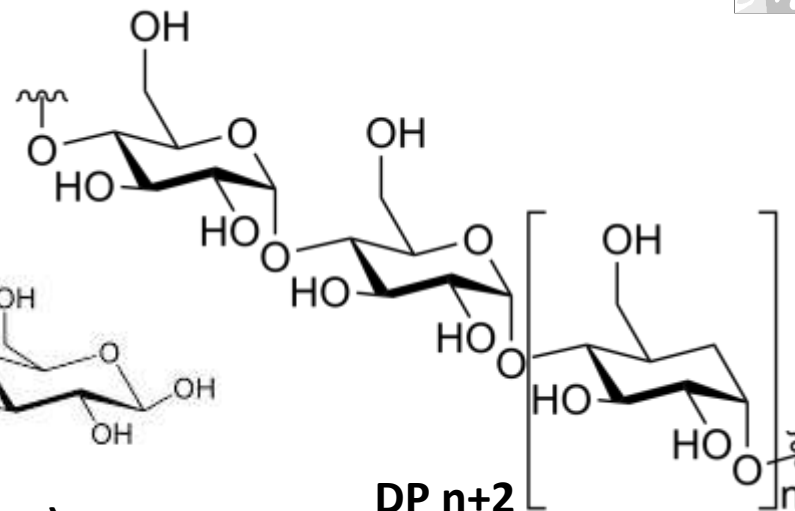
**DP 2**  
**(Maltose)**

Log P = -4.7 (calc.)

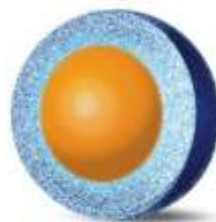


**DP 3**  
**(Maltotriose)**

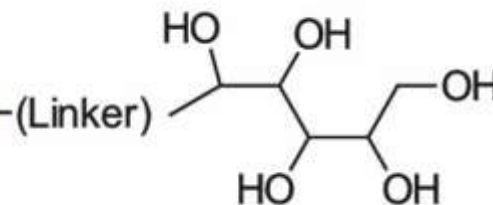
Log P = -5.6



**DP n+2**



**Halo Pentahilic**



Courtesy: AMT

**A "glycan" stationary phase!**  
**(HILIC Mode)**

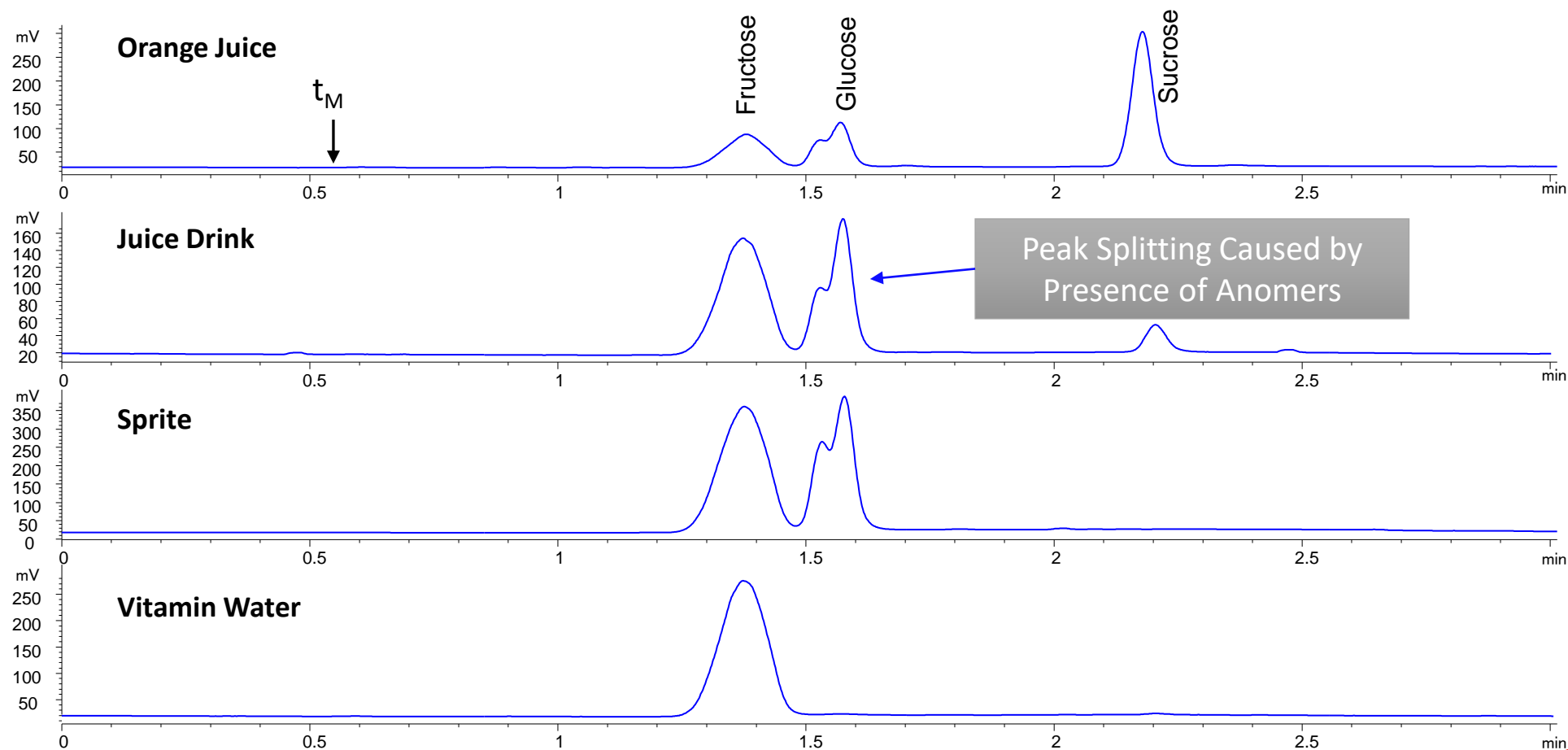




# Fruit Drinks (Fast Isocratic Method)

Column: 3.0X 100 mm, 2.7  $\mu$ m  
Mobile Phase: H<sub>2</sub>O/ACN (20/80)  
Flow: 0.75 mL/min  
Injection: 2  $\mu$ L  
Column Temperature: 35 C  
Detector: ELSD [40 °C, 45 psi]  
Sample: Diluted 1:10 with water/acn

- Analysis time less than 2.5 minutes!

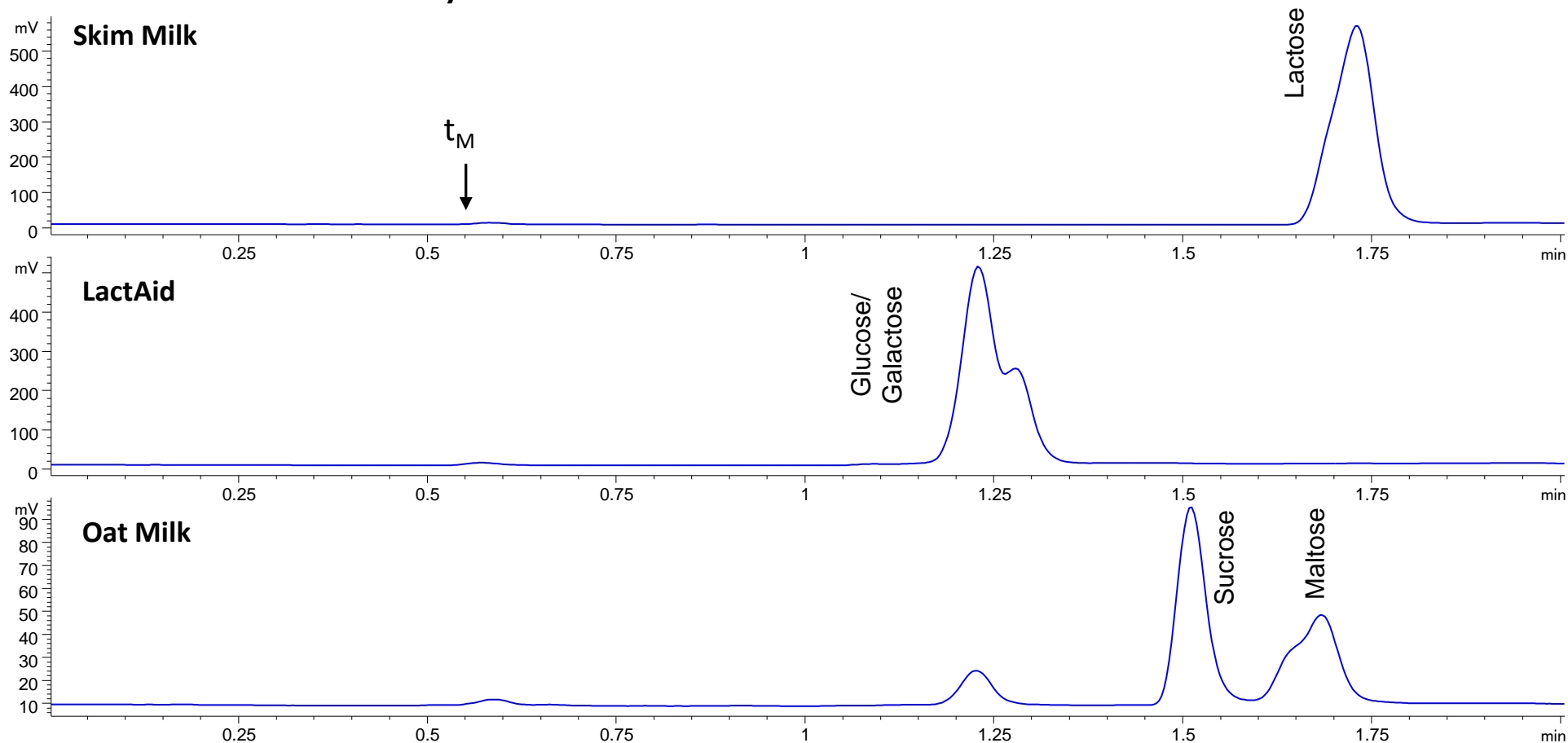




# Dairy/Plant Drinks (Fast Isocratic Method)

Column: 3.0X 100 mm, 2.7  $\mu$ m  
Mobile Phase: H<sub>2</sub>O/ACN (25/75)  
Flow: 0.75 mL/min  
Injection: 1  $\mu$ L  
Column Temperature: 35 C  
Detector: ELSD [40 °C, 45 psi]  
Sample: Diluted 1:10 with water/acn

- Analysis time less than 2 minutes!

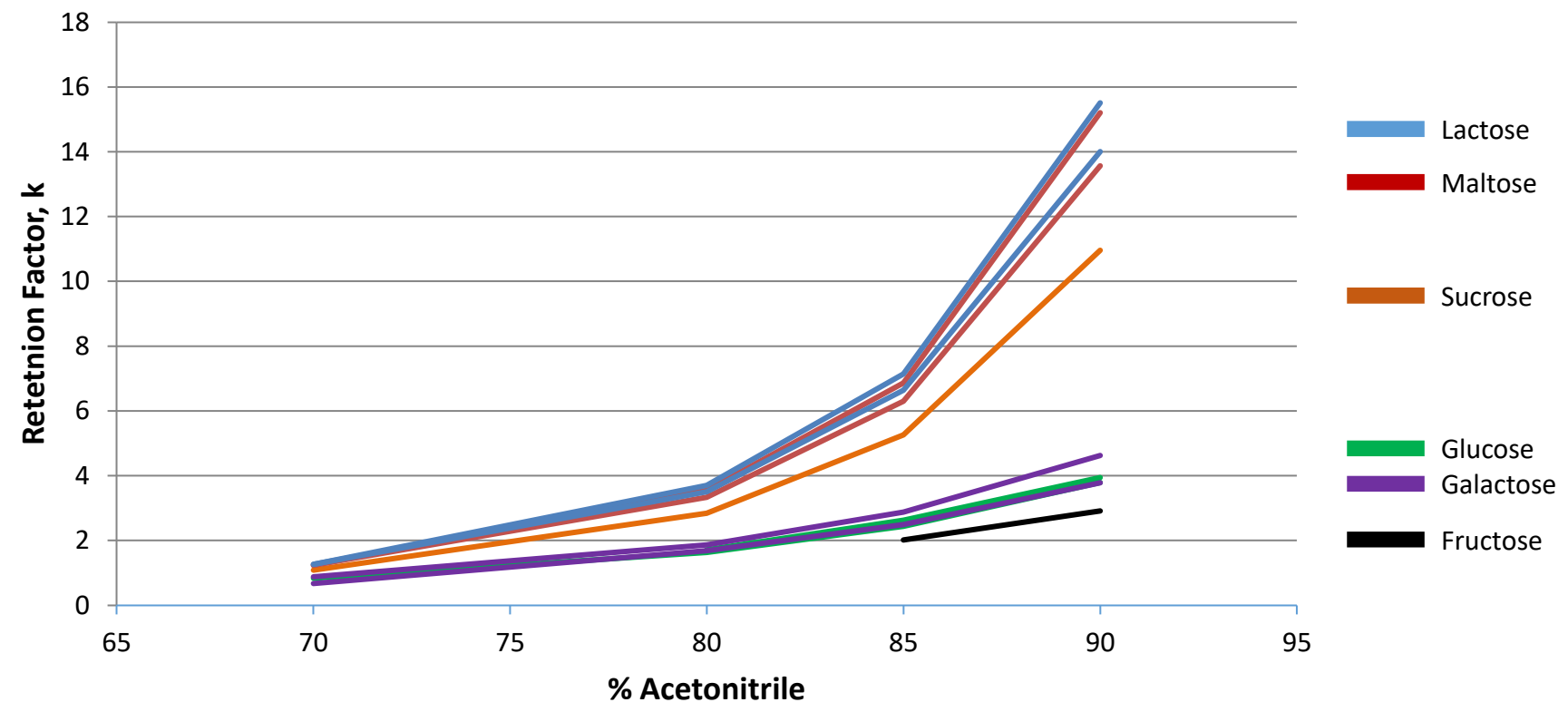




Column: 3.0X 100 mm, 2.7  $\mu$ m  
Mobile Phase: H<sub>2</sub>O/ACN  
Flow: 0.75 mL/min  
Injection: 1  $\mu$ L  
Column Temperature: 35 C  
Detector: ELSD [40 °C, 45 psi]  
Sample: 1.0 mg/mL

# Retention Pattern

- Retention behavior follows a typical HILIC trend



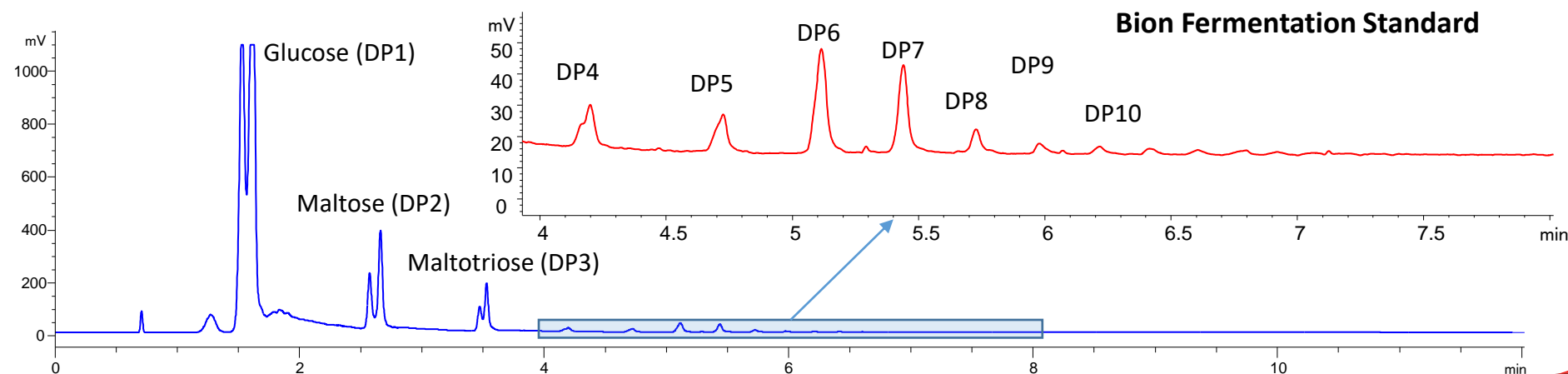
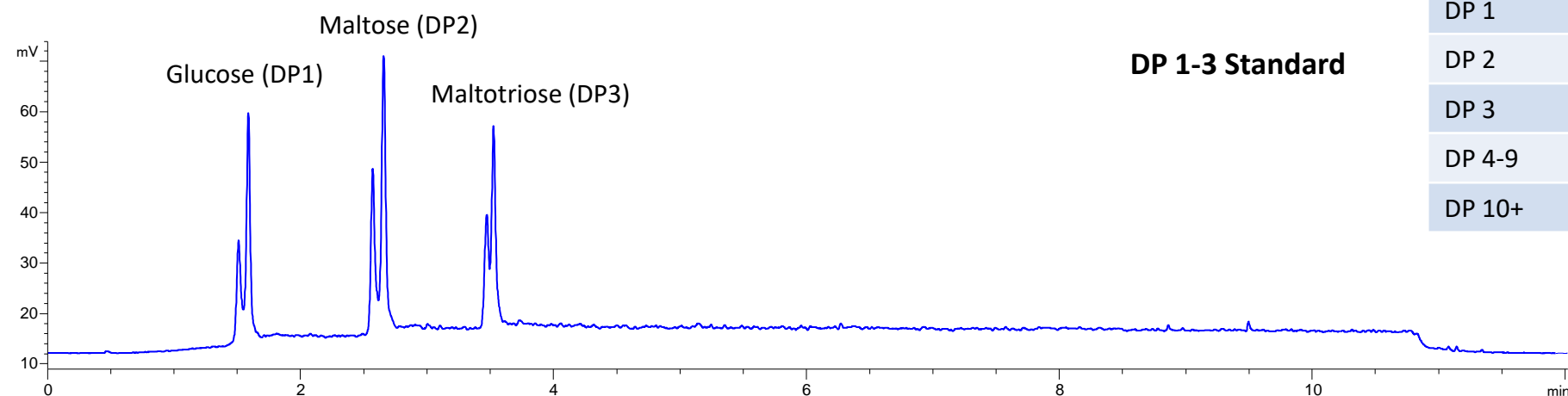


# DP Sugars

Column: 4.6X 50 mm, 2.7  $\mu$ m  
Mobile Phase: H<sub>2</sub>O/ACN (90-40% ACN/10 min.)  
Flow: 1.5 mL/min  
Injection: 1  $\mu$ L  
Column Temperature: 35 C  
Detector: ELSD [40 °C, 45 psi]  
Sample: Filtered

- Gradient elution on a fructan column allows determination of higher-level DP sugars

Abbreviation	Common Name
DP 1	Glucose
DP 2	Maltose
DP 3	Maltotriose
DP 4-9	Oligosaccharides
DP 10+	Polysaccharides

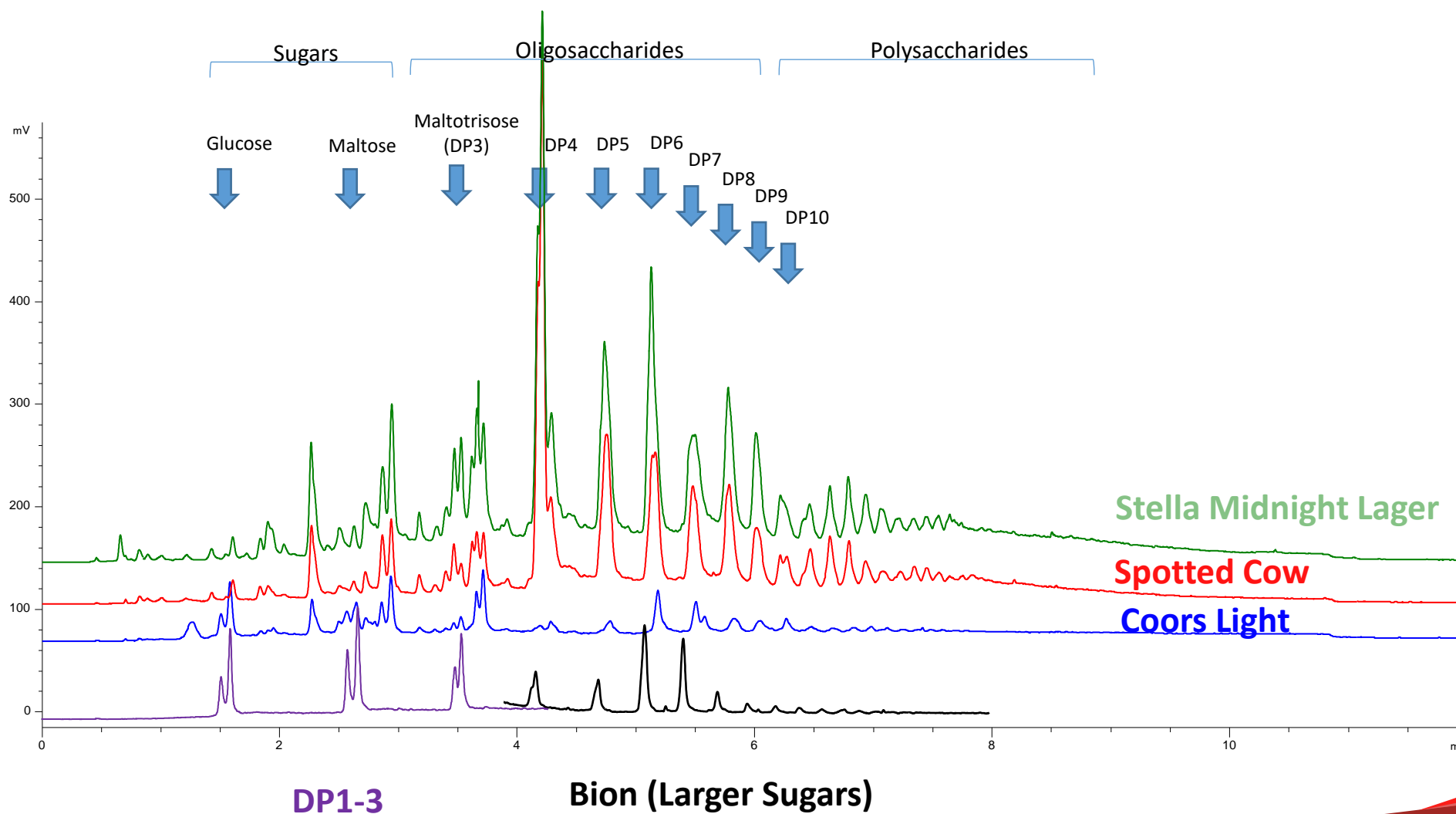






# Beer Is More Interesting

Column: 4.6X 50 mm, 2.7  $\mu$ m  
Mobile Phase: H<sub>2</sub>O/ACN (90-40% ACN/10 min.)  
Flow: 1.5 mL/min  
Injection: 1  $\mu$ L  
Column Temperature: 35 C  
Detector: ELSD [40 °C, 45 psi]  
Sample: Filtered





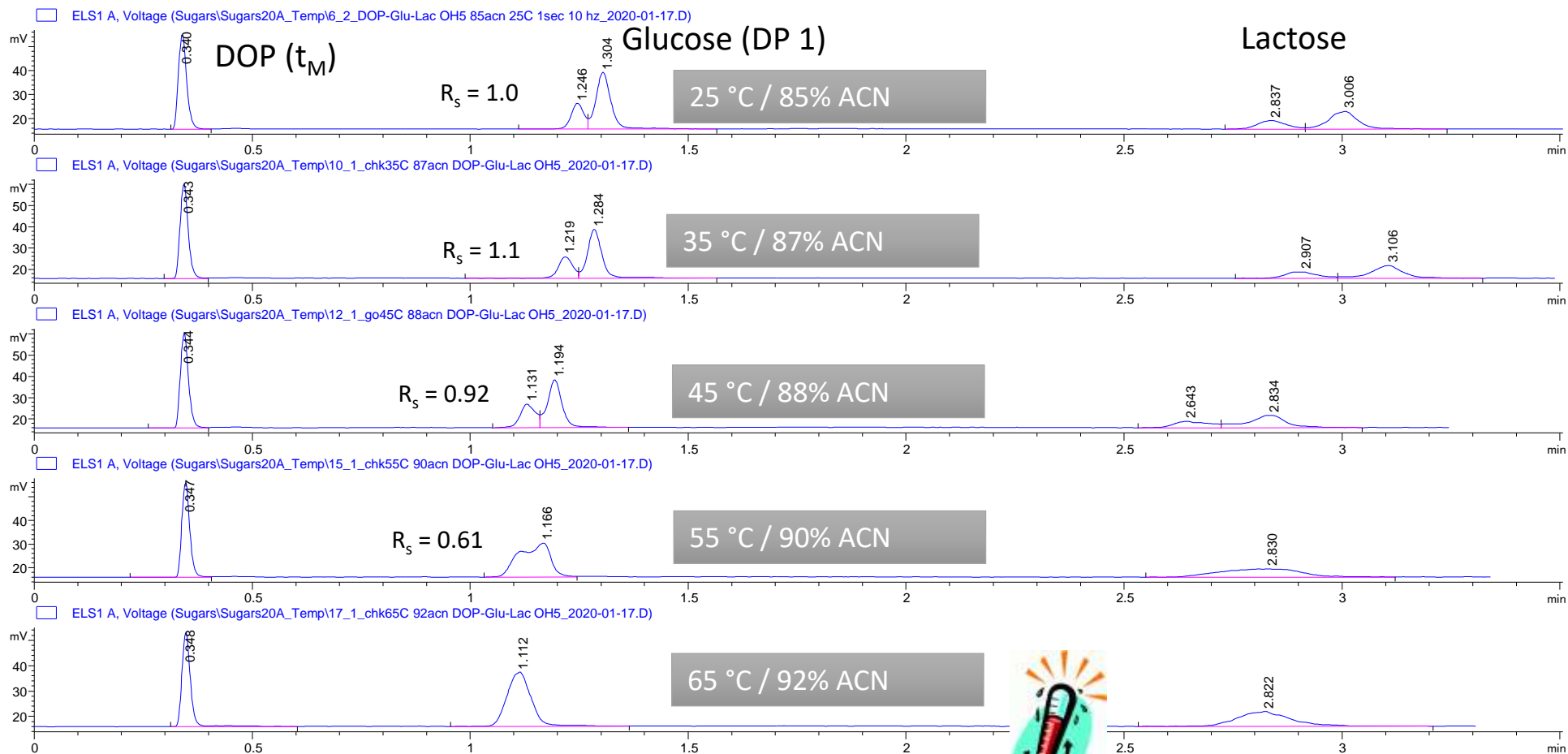
# Improving the Separation

- Anomer splitting increases peak width and decreases separation
- Retention times are longer
- Complete elution from the column is always a concern
- Many other sugar methods operate at higher temperatures
- Possible Solutions
  - Evaluate higher temperatures
  - Use short column
  - Use smaller diameter column to save solvent and improve detectability





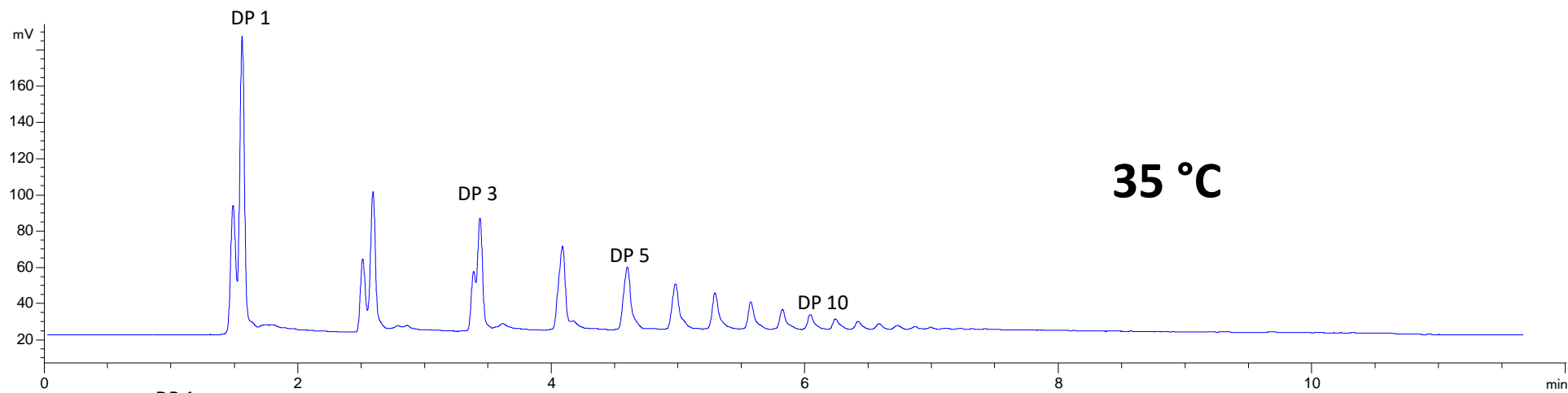
# Peak Shape Improves at Higher Temperatures



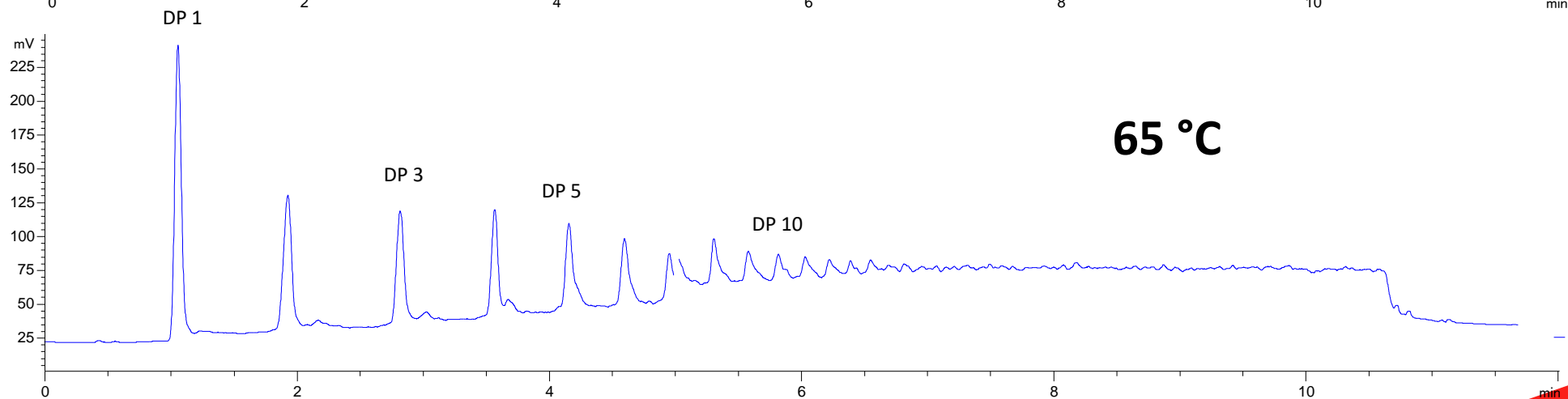


# DE Sugars at Higher Temperature

Column: 4.6X 50 mm, 2.7  $\mu$ m  
Mobile Phase: H<sub>2</sub>O/ACN (90-40% ACN/10 min.)  
Flow: 1.5 mL/min  
Injection: 1  $\mu$ L  
Column Temperature: 35 C  
Detector: ELSD [40 °C, 45 psi]  
Sample: 36 DE, Filtered



35 °C



65 °C





# Final Experimental Details

- Agilent 1290 HPLC with Diode Array Detection (DAD) and Evaporative Light Scattering Detector (ELSD)
- Column
  - AMT Halo Pentahilic
    - 3.0 X 50 mm, 2.7  $\mu$ m
- Mobile Phase: water (A)/acetonitrile (B)
  - Gradient 1: **92 – 42 % B** in 10 minutes
  - Gradient 2: **92 – 52 % B** in 8 minutes
- Flow: 0.75 mL/min
- Injection: 2  $\mu$ L
- Column Temperature: 65 C
- ELSD:
  - 40 C, 45 psi
  - 10 Hz Data Rate, 2 sec Filter



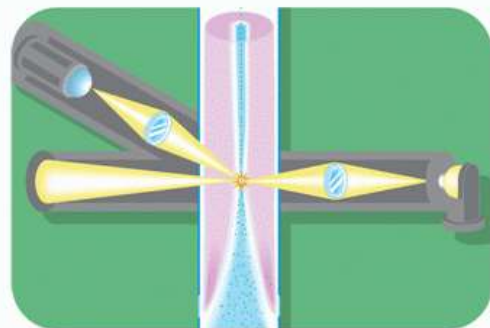


# Evaporative Light Scattering Detection (ELSD)

## Evaporation

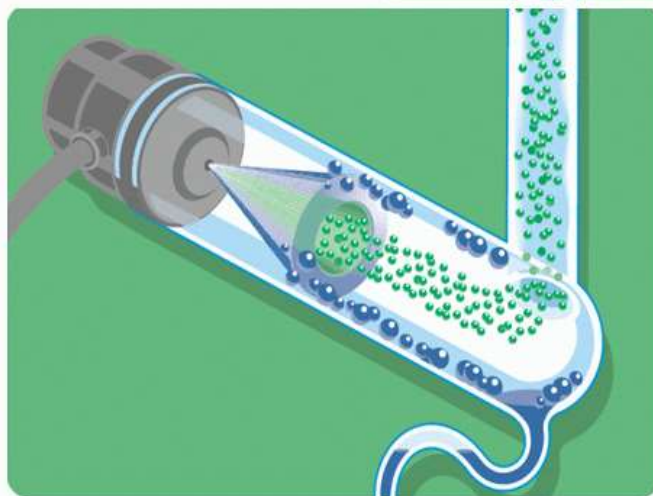
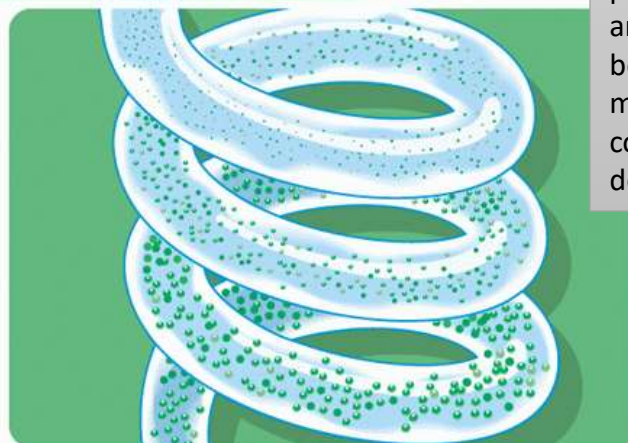
A heated tube is used to evaporate the solvent. The exit of the heated tube leads directly into the detector cell.

- The ELSD is a universal detector like refractive index (RID).
- Only non-volatile components are detected.
- **Gradient elution can be used to improve analysis time and sensitivity.**



## Detection

The detector chamber contains a light emitting diode (LED) and a photomultiplier that is positioned at an angle of  $120^\circ$  with respect to the light beam. When the carrier gas contains microparticles, (produced by eluting compounds) the light is scattered and is detected by the photomultiplier.



## Nebulization

The eluent from the chromatograph is nebulized by the inlet gas (typically nitrogen). The fine mist moves to the evaporation tube.





And now, the real world!

## 3RD ACT CRAFT BREWERY





# Mashing Samples – Double IPA



Sample	Conditions	Comments
1/1A	Mash at start	
2/2A	Mash – 129 °F / 20 minutes	Initial heating
3/3A	Mash – Heat to 147 °F / 0 minutes	Activate beta amylase
4/4A	Mash – 147 °F / 45 minutes	
5/5A	Mash – Heat to 158 °F / 0 minutes	Activate alpha amylase
6/6A	Mash – 158 °F / 15 minutes	
7/7A	Mash – Heat to 180 °F / 0 minutes	Deactivate amylase
8/8A	Kettle	Filter

Note: "A" indicates sample was acidified to ~ pH 2 using phosphoric acid







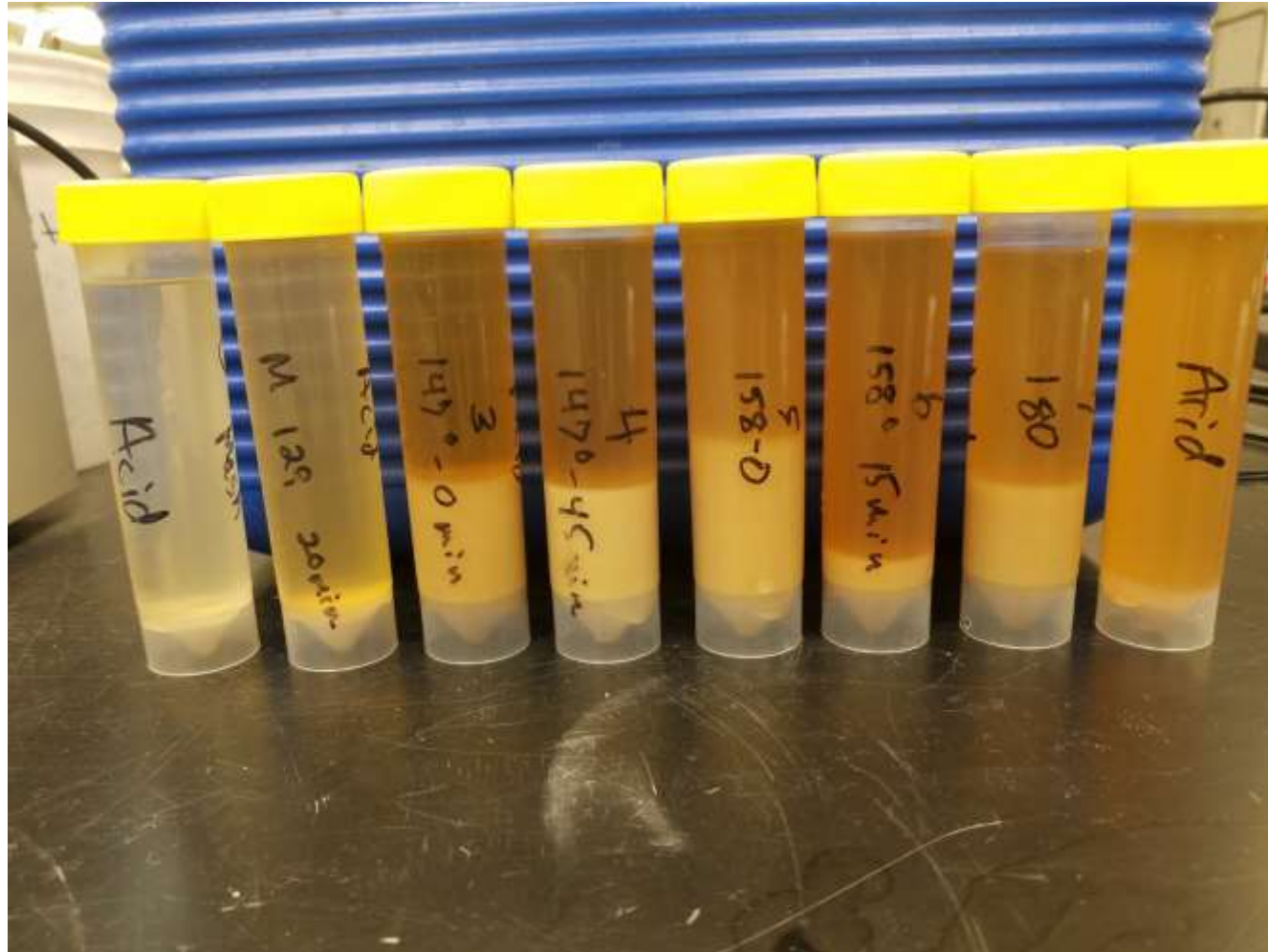
# Sample Collection for Mashing Samples

- Collection Conditions
  - Raw or acidified to ~ pH 2
    - 50 – 100  $\mu\text{L}$   $\text{H}_3\text{PO}_4$
  - Store cold
- Allow to settle or centrifuge, remove supernatant
- HPLC Sample Requirements
  - No particulates (filtered,  $< 0.5 \mu\text{m}$ )
  - Minimize interferences
  - Aqueous or aqueous/organic mixture





# Mashing Samples

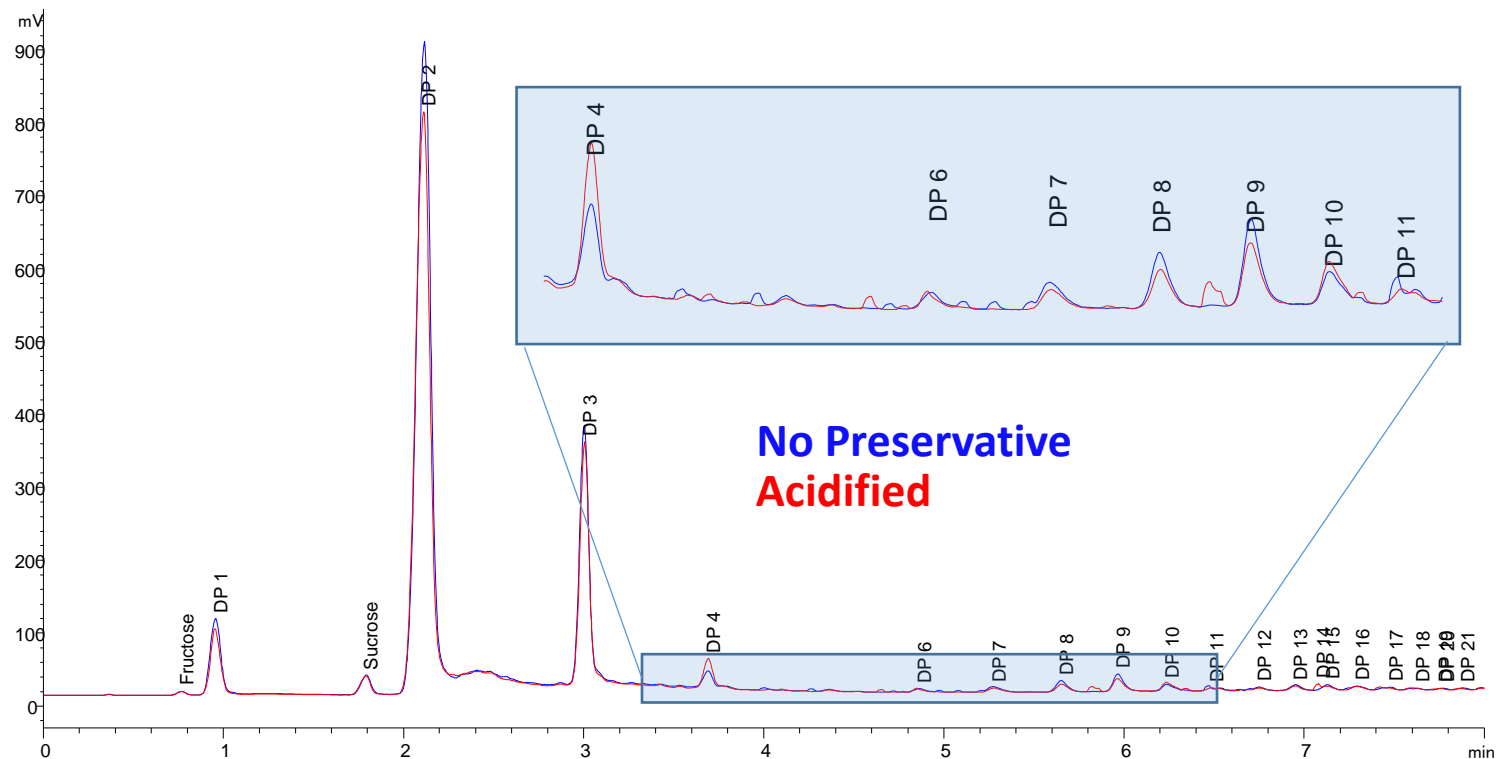




# Sample Stability – 2 days at 4 °C

- Acidified samples showed less fermentable sugars after two days of cold storage.
  - Some enzyme activity was still present in unpreserved samples
- Oligosaccharides (DP 4 – 10) showed some minor changes
- Samples after inactivation (7 and 8) were nearly identical.

ELS1 A, Voltage (Sugars21\_PrepA\25\_Sugars21\_3rd\_25X 2021-01-08\008-47-3rd 3.D)  
ELS1 A, Voltage (Sugars21\_PrepA\25\_Sugars21\_3rd\_25X 2021-01-08\009-48-3rd 3A.D)



Sample Preparation:

Sample diluted 1:25 with deionized water.

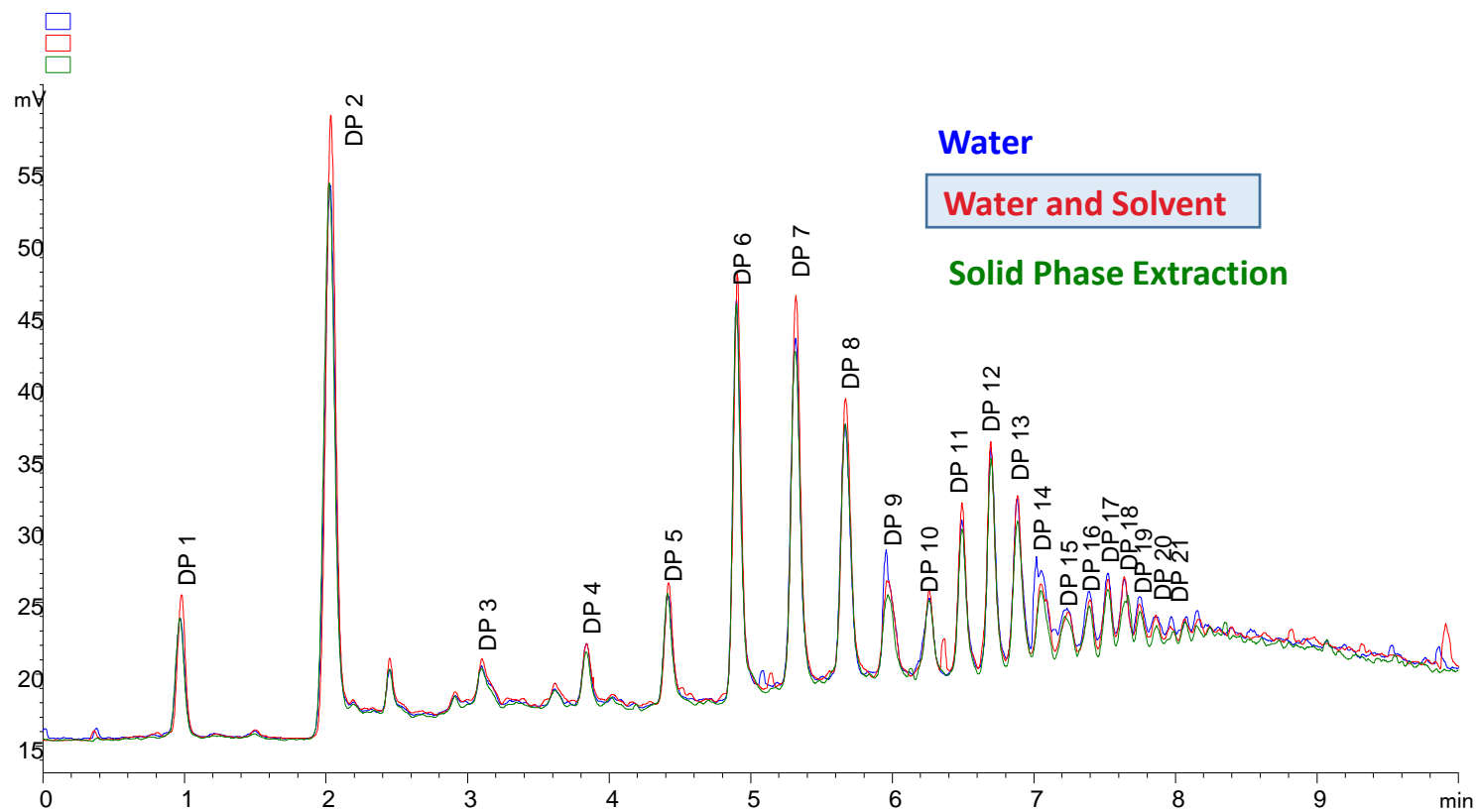
Filtered (0.22 um)





# Preparing Samples for HPLC

- Options (1:25 Dilutions)
  - Dilution with water
  - Dilution with water and 40 % organic solvent (1:1 acetonitrile:methanol)
  - Solid Phase Extraction





# General Dilution Levels for Beer

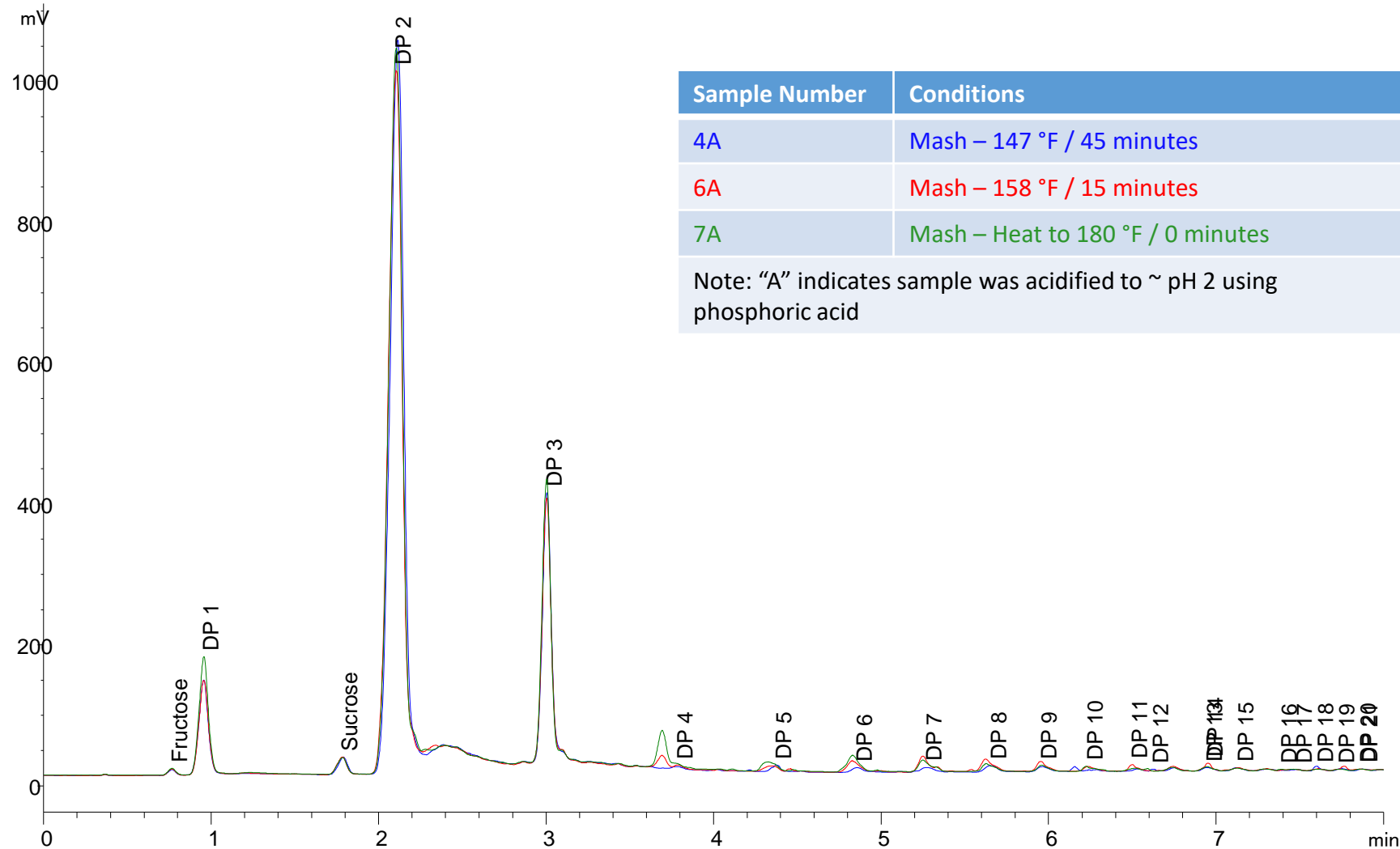


Sample Type	Recommended Dilution	Comments
Mash	1:25	Include organic solvent to precipitate proteins and other interferences
Fermentation	1:10	Maltose will be off scale for early samples but allows assessment of other sugars
	1:5	Allows better review of larger sugars, maltose off scale
Finished Product	1:10	For general screening
	1:5	Allows review of larger sugars
	Undiluted (filtered only)	Most sensitivity to minor components



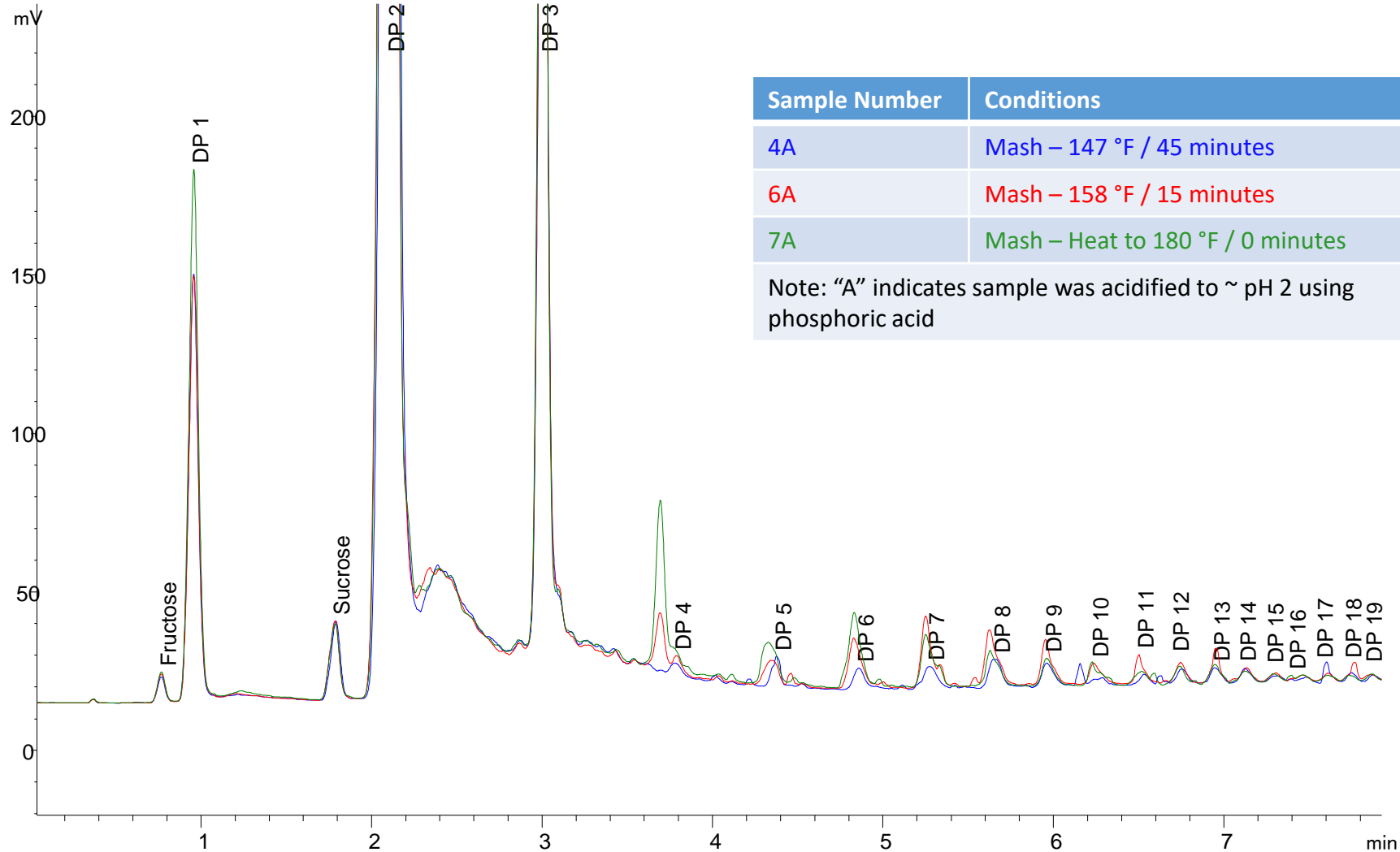


# Mash Sample Summary (Full Scale)





# Mash Sample Summary (Expanded)



Sample Number	Conditions
4A	Mash – 147 °F / 45 minutes
6A	Mash – 158 °F / 15 minutes
7A	Mash – Heat to 180 °F / 0 minutes

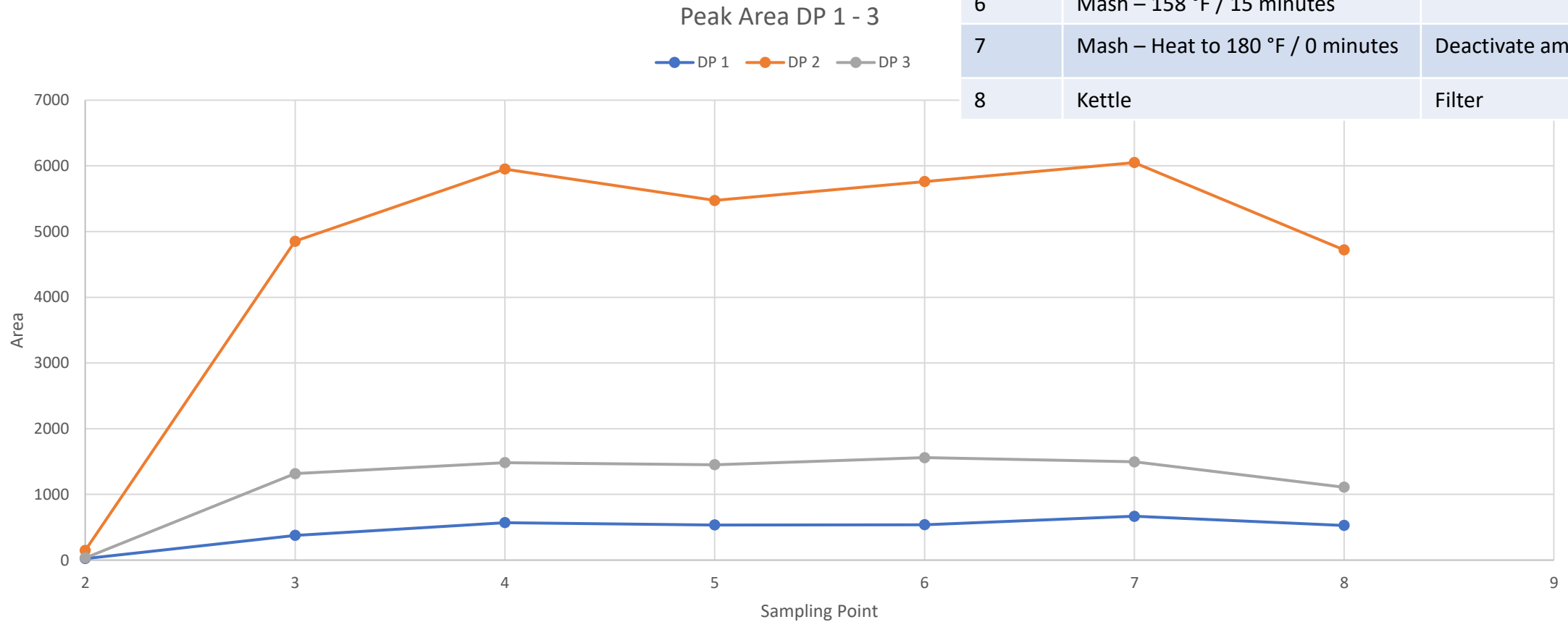
Note: "A" indicates sample was acidified to ~ pH 2 using phosphoric acid





# Mash Samples: Fermentable Sugars

Sample	Conditions	Comments
1	Mash at start	
2	Mash – 129 °F / 20 minutes	Initial heating
3	Mash – Heat to 147 °F/0 minutes	Activate beta amylase
4	Mash – 147 °F / 45 minutes	
5	Mash – Heat to 158 °F / 0 minutes	Activate alpha amylase
6	Mash – 158 °F / 15 minutes	
7	Mash – Heat to 180 °F / 0 minutes	Deactivate amylase
8	Kettle	Filter

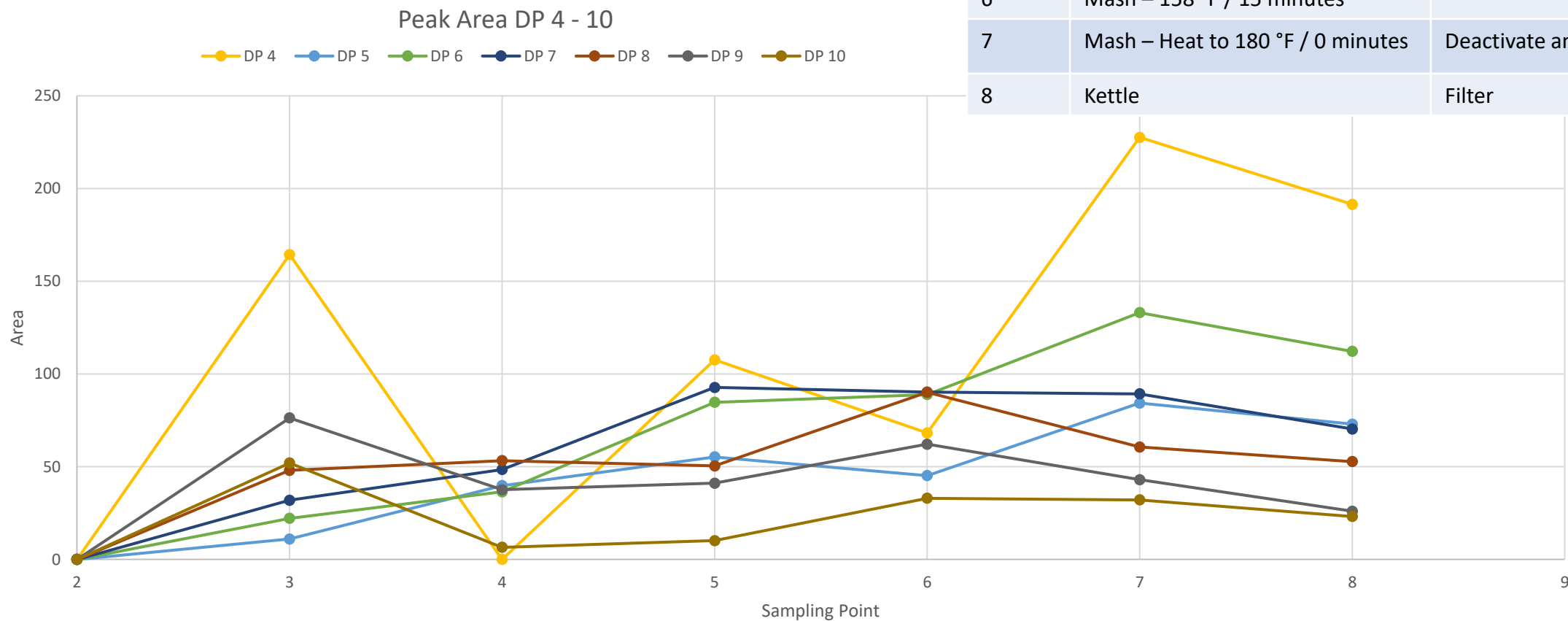






# Mash Samples: Non-Fermentable Sugars

Sample	Conditions	Comments
1	Mash at start	
2	Mash – 129 °F / 20 minutes	Initial heating
3	Mash – Heat to 147 °F/0 minutes	Activate beta amylase
4	Mash – 147 °F / 45 minutes	
5	Mash – Heat to 158 °F / 0 minutes	Activate alpha amylase
6	Mash – 158 °F / 15 minutes	
7	Mash – Heat to 180 °F / 0 minutes	Deactivate amylase
8	Kettle	Filter





1A Ferm No Yeast
2A Ferm 30 min post yeast
3A Ferm first yeast activity
4A Ferm day 1
5A Ferm day 2
6A Ferm day 3
7A Ferm day 7
8A Ferm day 8
9A Ferm day 10 end

# Fermentation Samples

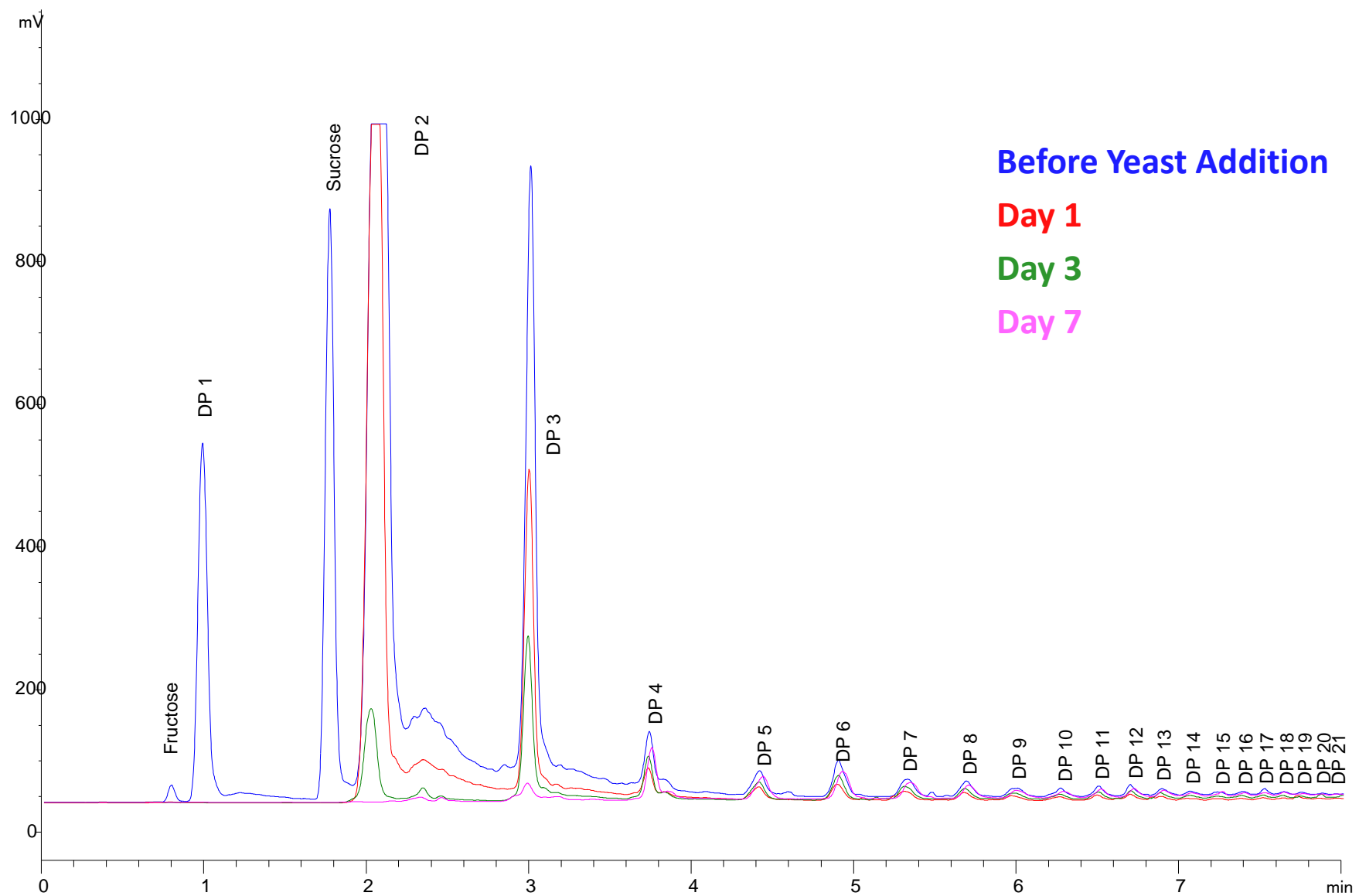
Belgian Ale

**All samples diluted 1:10**



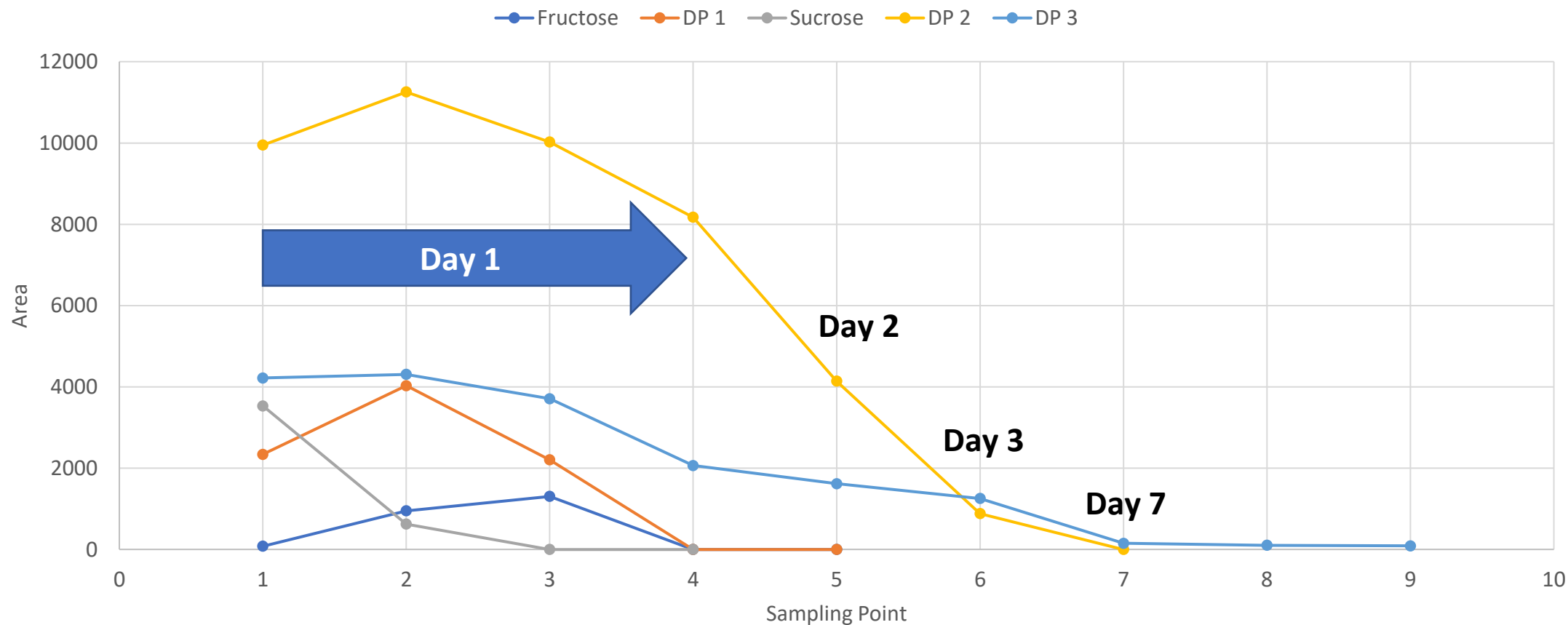


# Fermentation Summary – Belgian Ale





# Fermentable Sugars During Fermentation





# Maltose Concentration

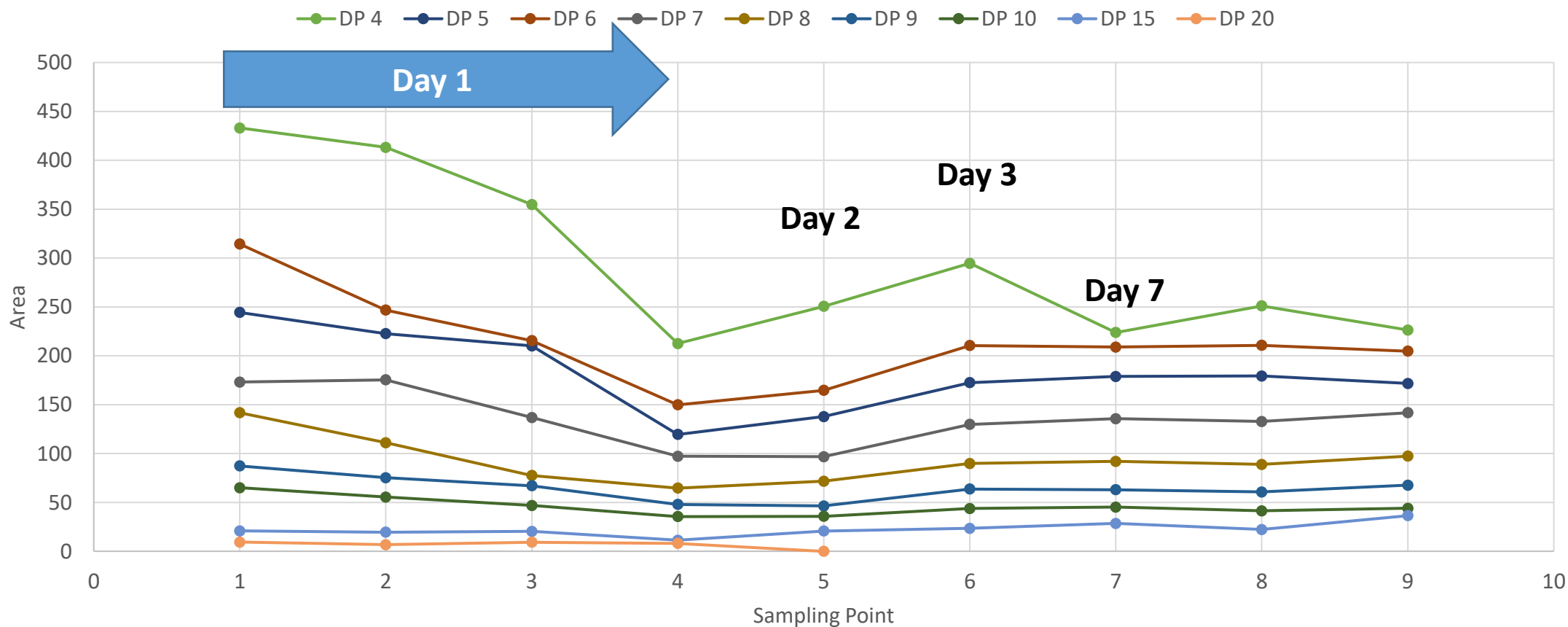
Sample Name	RT	Area	Amount, mg/mL	Amount, %
1A Before Yeast Addition	2.096	9950.308	55.5*	5.5*
2A 30 min After Yeast Addition	2.092	11256.36	60.0*	6.0*
3A First Evidence of Yeast Activity	2.079	10025.14	55.8*	5.6*
4A Day 1	2.056	8175.833	48.8	4.9
5A Day 2	2.026	4142.898	30.9	3.1
6A Day 3	2.014	882.6907	9.8	0.1

\* Amounts above 50 mg/mL are estimated as they are outside the calibration range.





# Large Sugars (Peak Area)





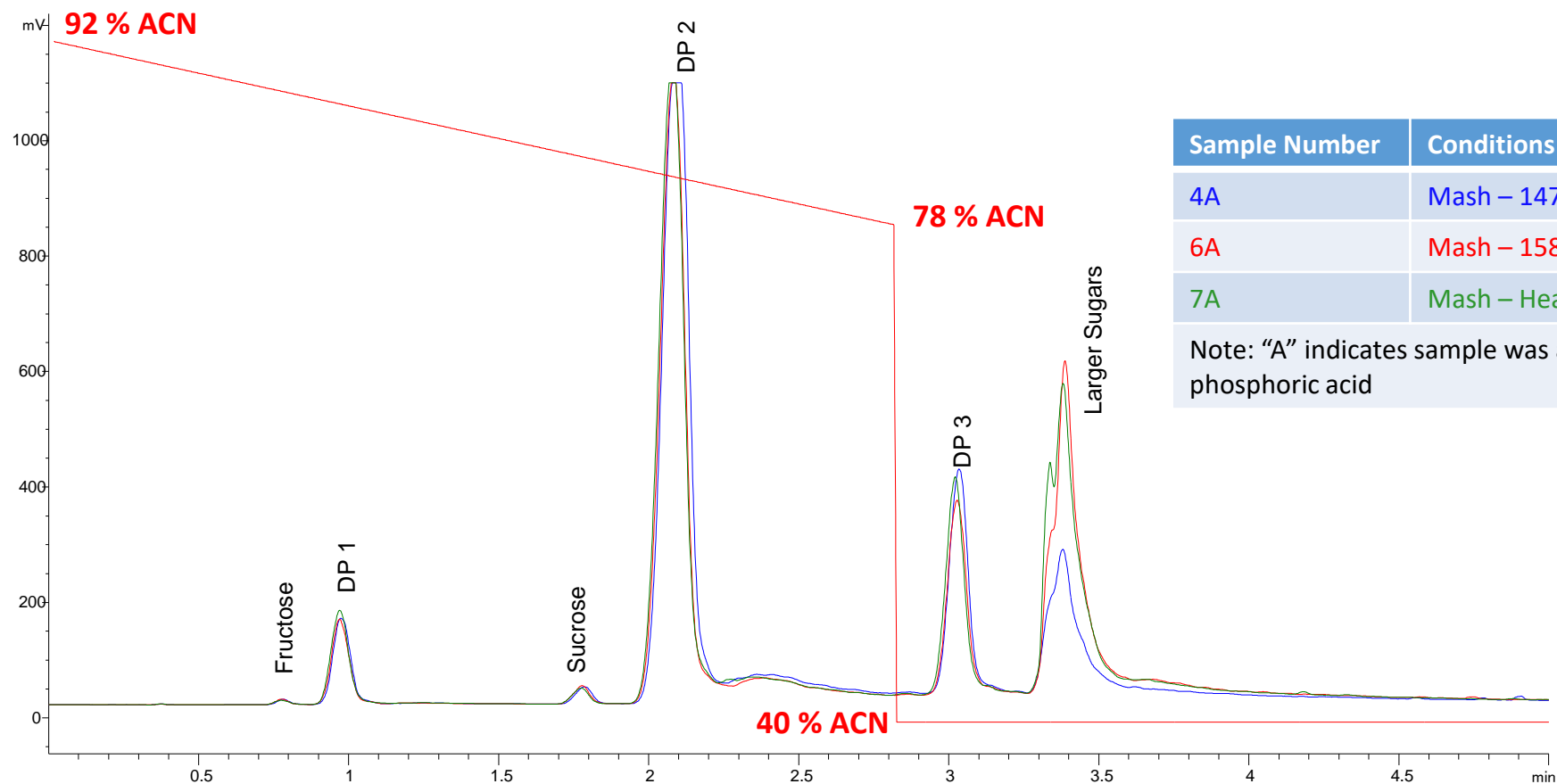
# Other Possibilities





# Group Separations

- A simple adjustment of operating conditions can combine all the larger sugars (DP4+) into one group.
- Produces a faster separation and is easier to see relative amounts.



Sample Number	Conditions
4A	Mash – 147 °F / 45 minutes
6A	Mash – 158 °F / 15 minutes
7A	Mash – Heat to 180 °F / 0 minutes

Note: "A" indicates sample was acidified to ~ pH 2 using phosphoric acid

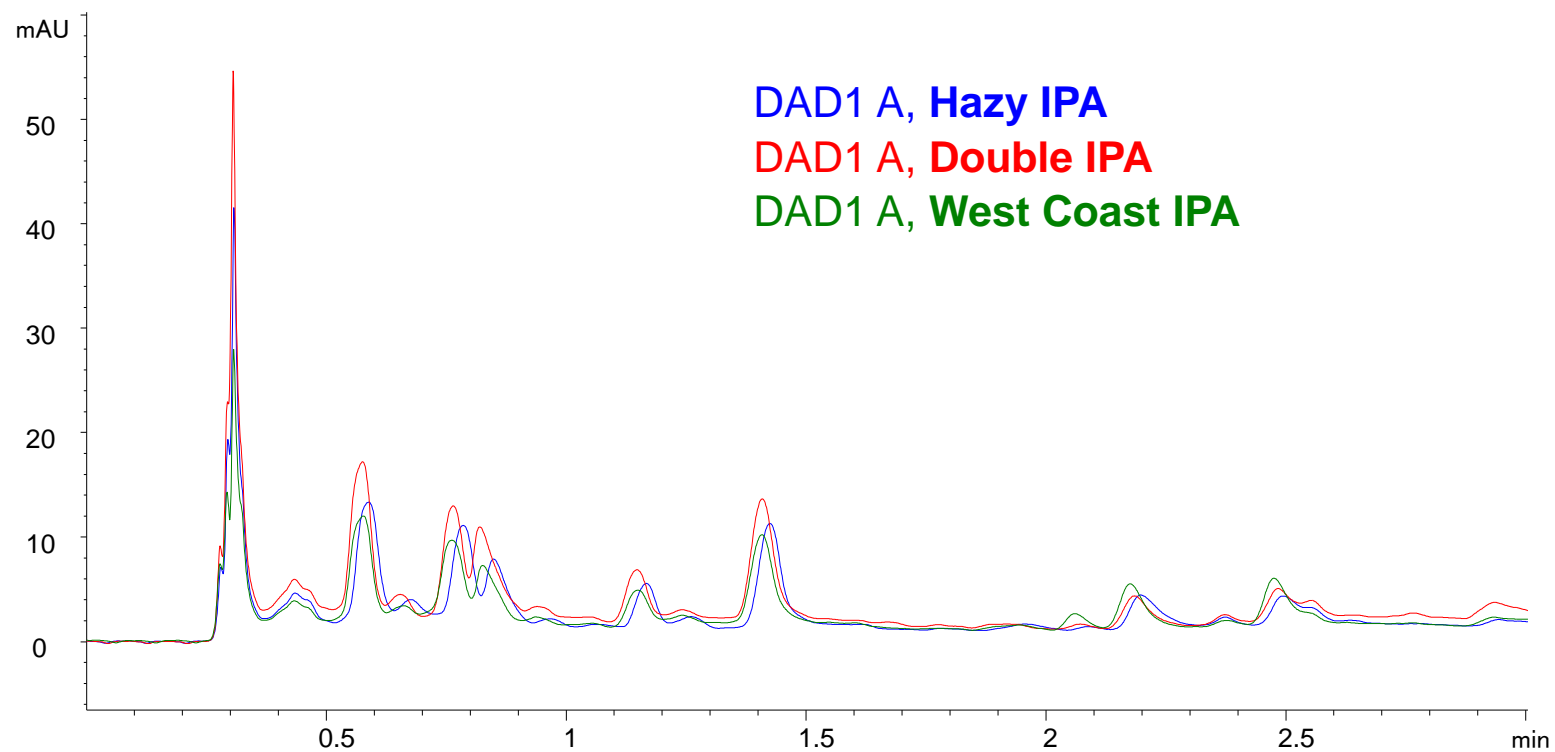






# What About the Diode Array?

- The absorbance detector is also collecting data and there are numerous common peaks that appear.
- What are they?





# Finished Beer Samples

All Samples Diluted 1:10



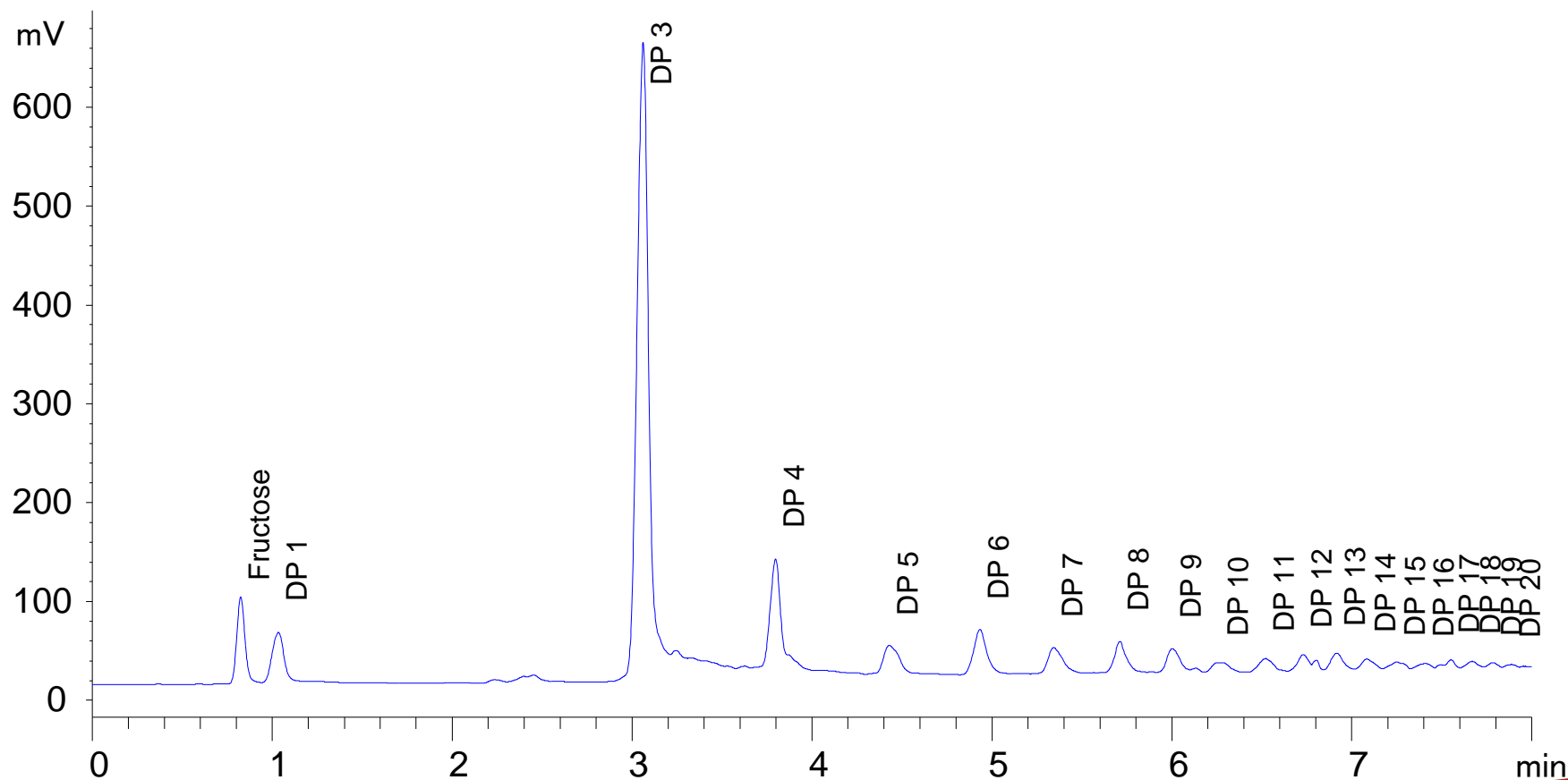


# Blueberry Cream Ale



- 6.3% ABV, 19 IBU's
- *Light but smooth cream ale infused with real blueberries.*

ELS1 A, Voltage (Sugars21\_PrepD\8\_Sugars21\_Blue\_AM 2021-01-24\001-51-Blueberry 1-5-2-2 5 cm.D)



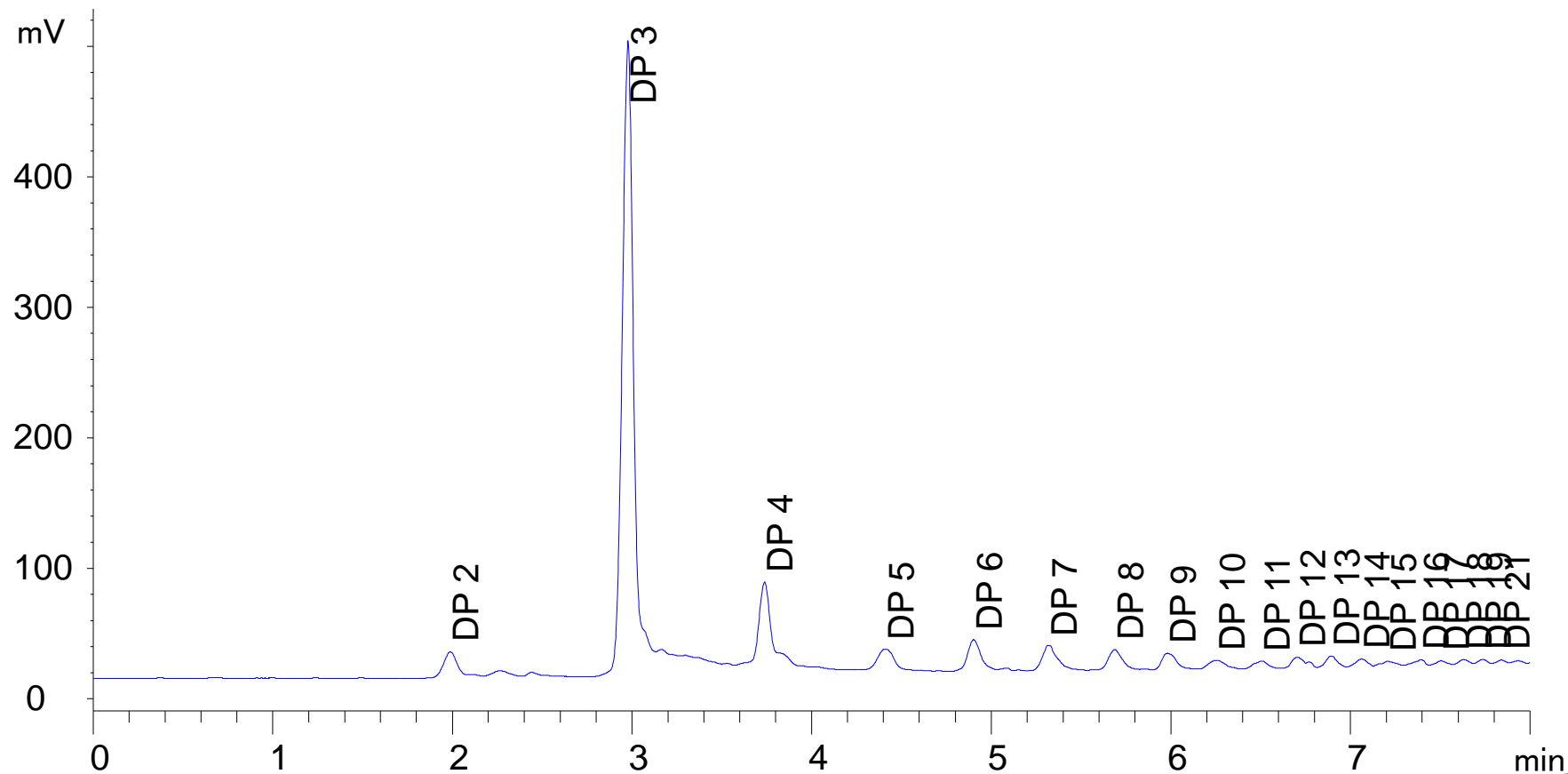


# East Coast Hazy IPA



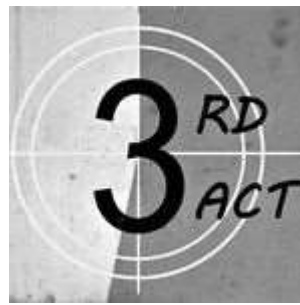
- 5.4% ABV, 85 IBU's
- *This New England version of IPA is a hop lover's dream. This beer is only partially filtered.*

ELS1 A, Voltage (Sugars21\_PrepE\13X\_Finished IPA 2021-02-02\\_004\_004-52-10 Hazy IPA 1-10.D)



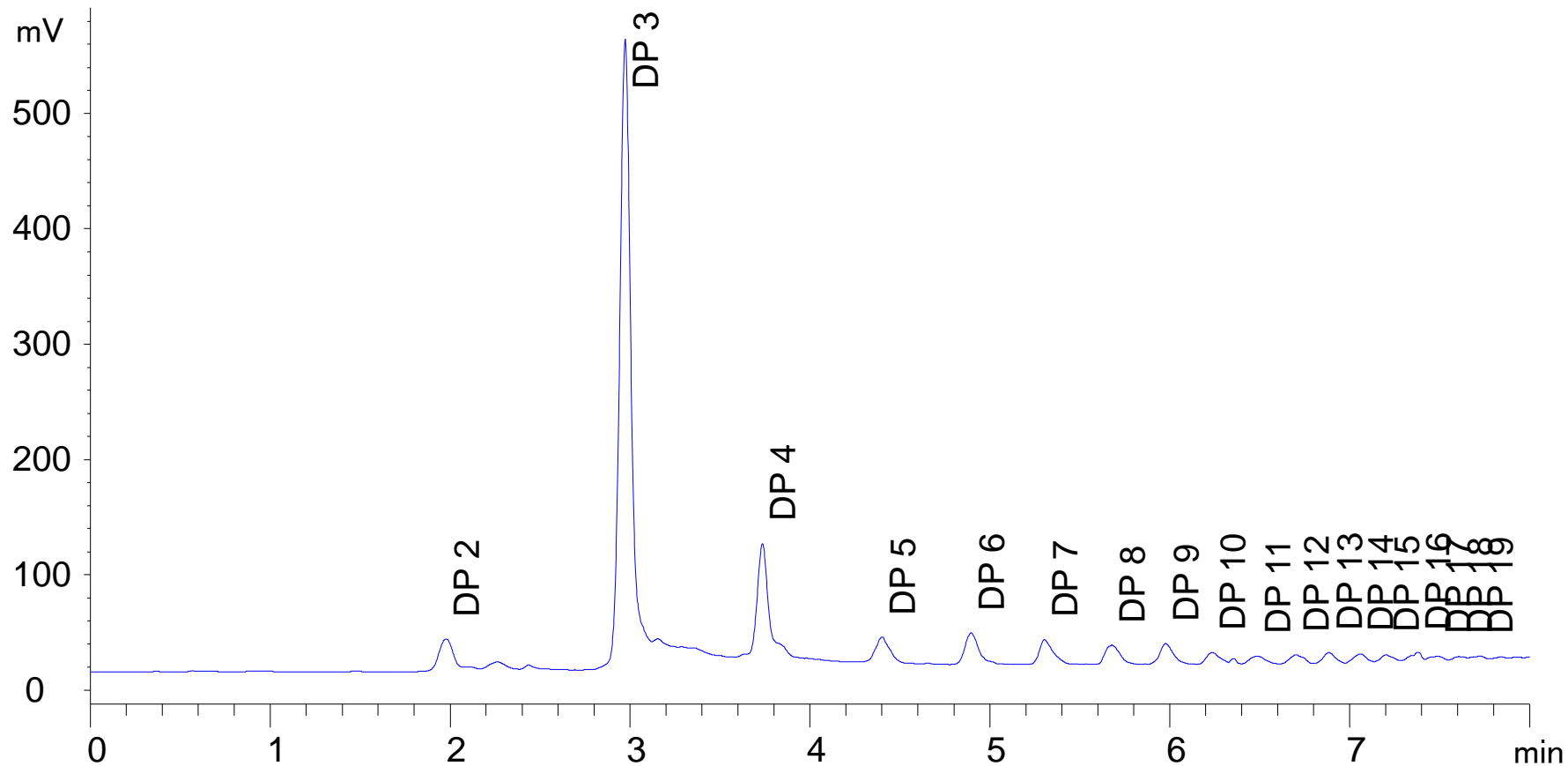


# Double IPA



- 8% ABV, 77 IBU's
- *Big double IPA that has been dry hopped twice. It carries both citrus and piney aspects with a bold start and smooth finish.*

ELS1 A, Voltage (Sugars21\_PrepE\13X\_Finished IPA 2021-02-02\\_006\_006-54-11 DIPA 1-10.D)



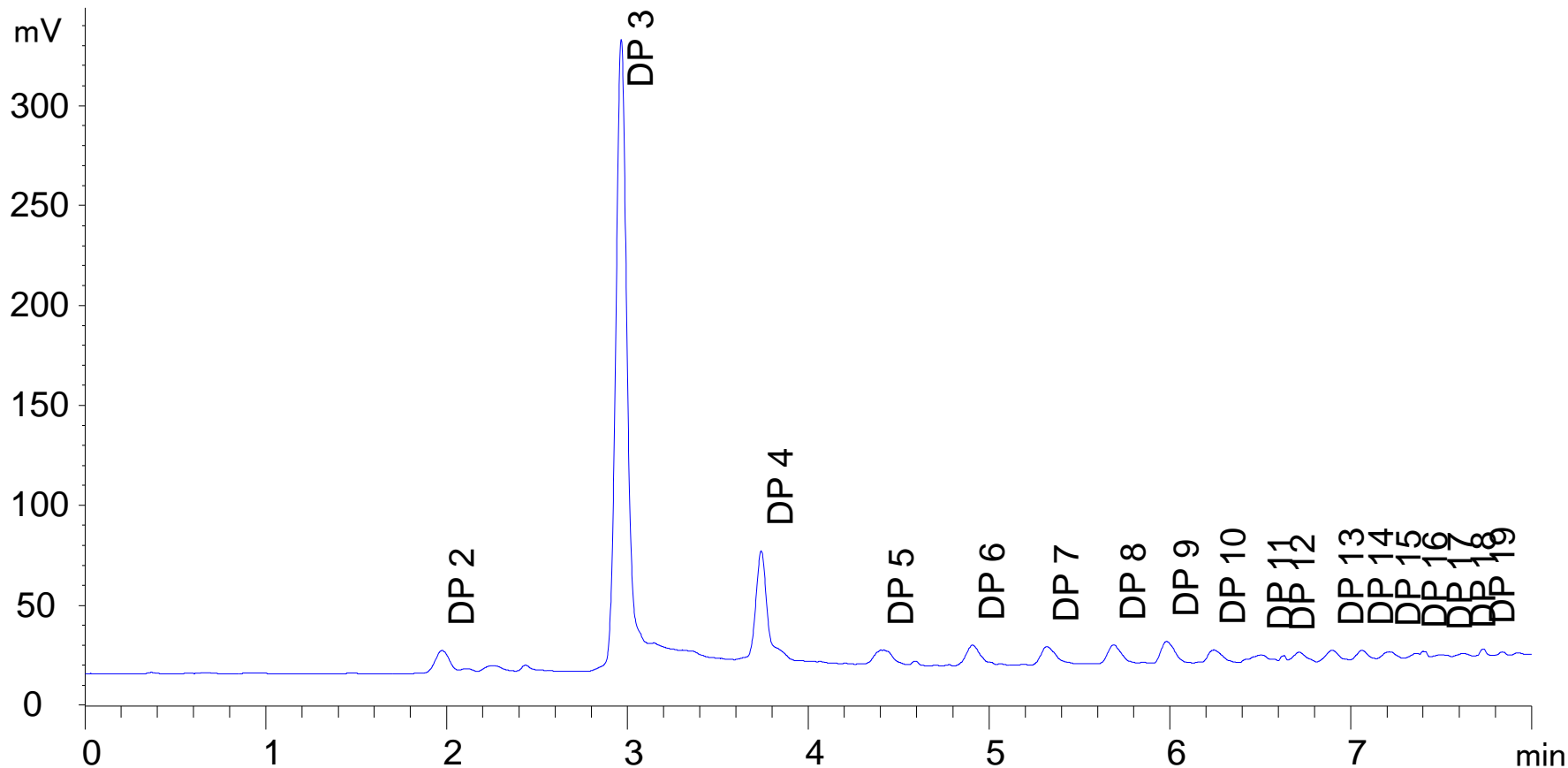


# West Coast IPA



- 4.7% ABV, 65 IBU's
- A red IPA that is malt forward enough to couch the heavily hopped IPA showcasing all-American hops.

ELS1 A, Voltage (Sugars21\_PrepE113X\_Finished IPA 2021-02-02\ 008\_008-56-12 West IPA 1-10.D)





# IPA Overlay

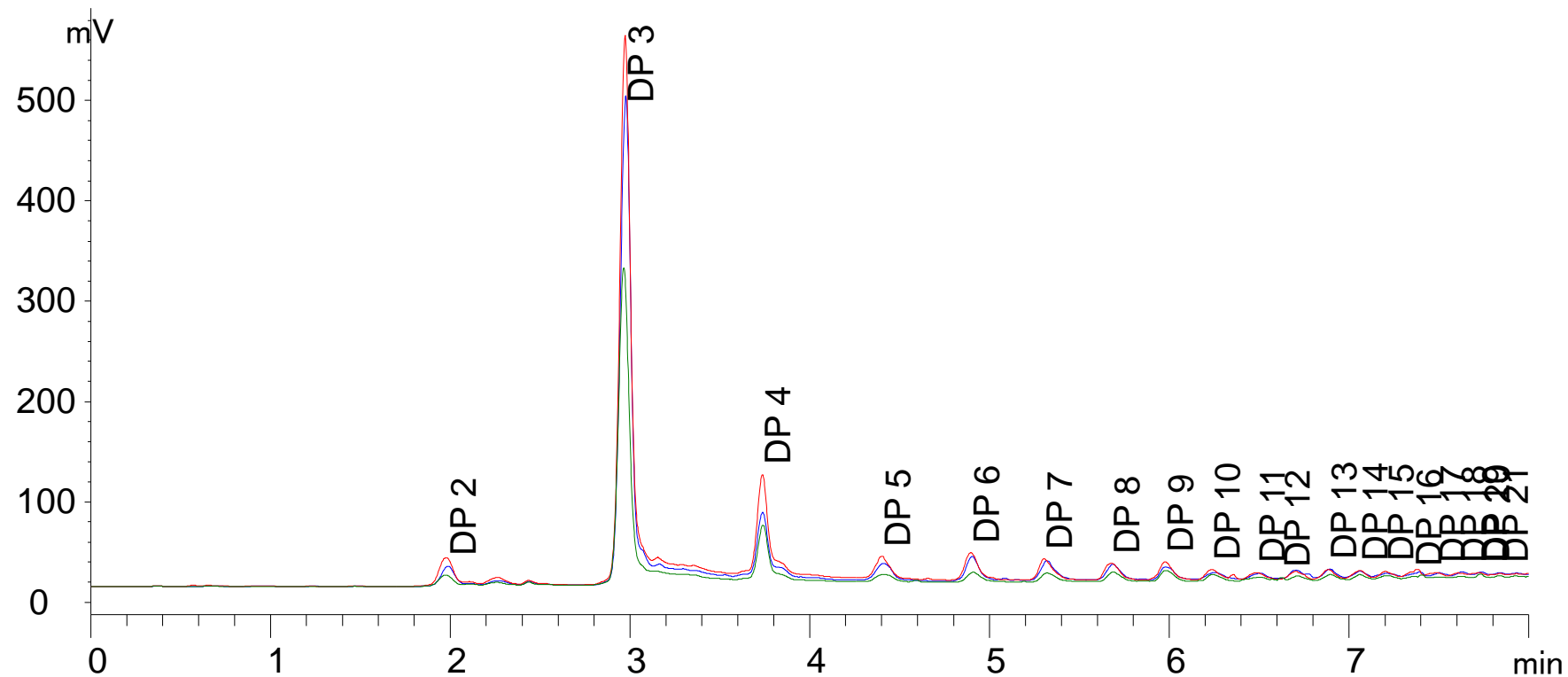


- These three IPAs used different grains but the same yeast.

ELS1 A, Voltage ([Hazy IPA 1-10.D](#))

ELS1 A, Voltage ([DIPA 1-10.D](#))

ELS1 A, Voltage ([West IPA 1-10.D](#))

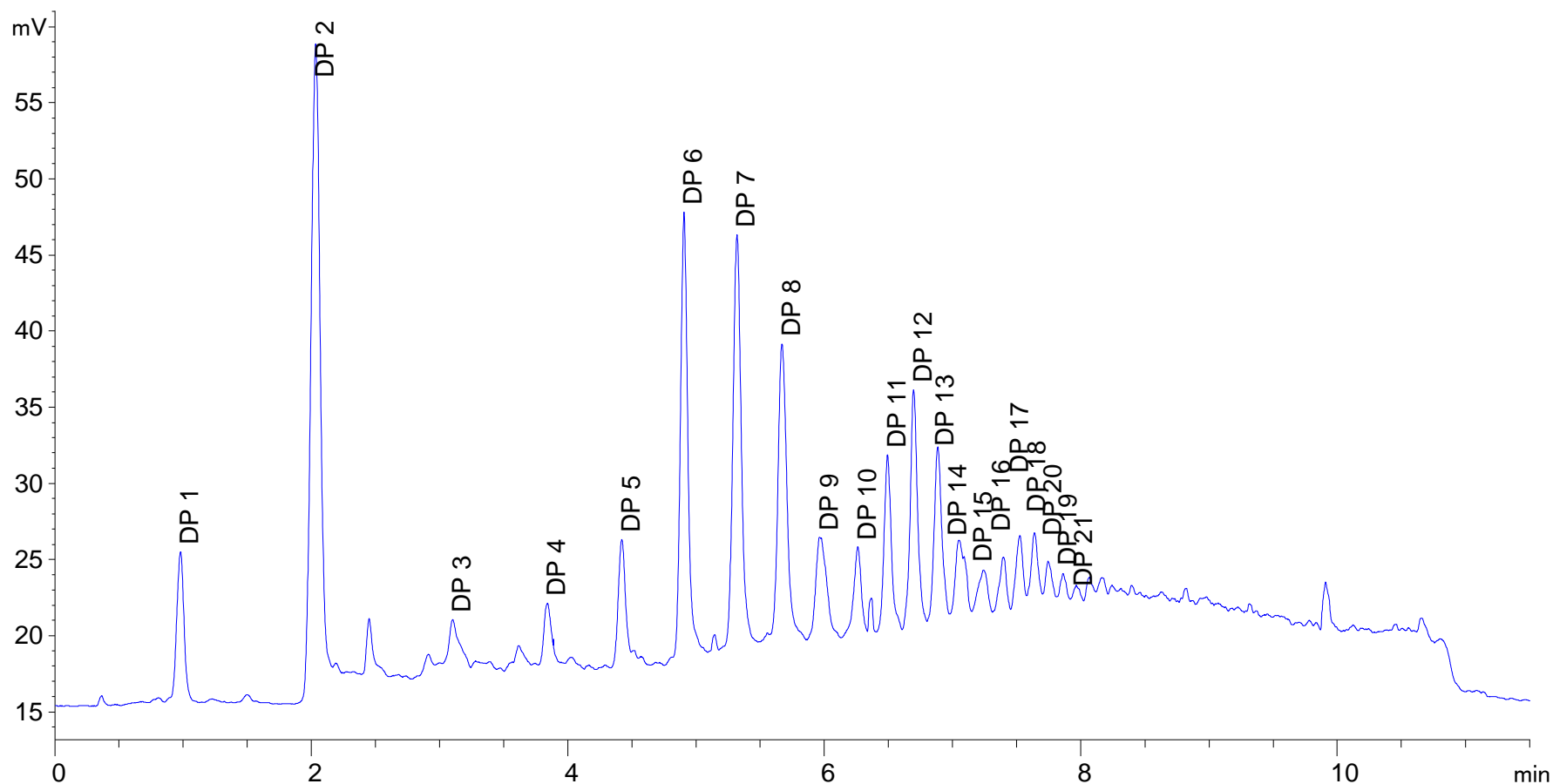




# Lift Bridge Hop Dish IPA



- ABV: 6.5% IBU: 75 Color: 13.5
- Aggressively hopped IPA with aromas of citrus, fruit, pine. A subtle malt sweetness with notes of caramel.





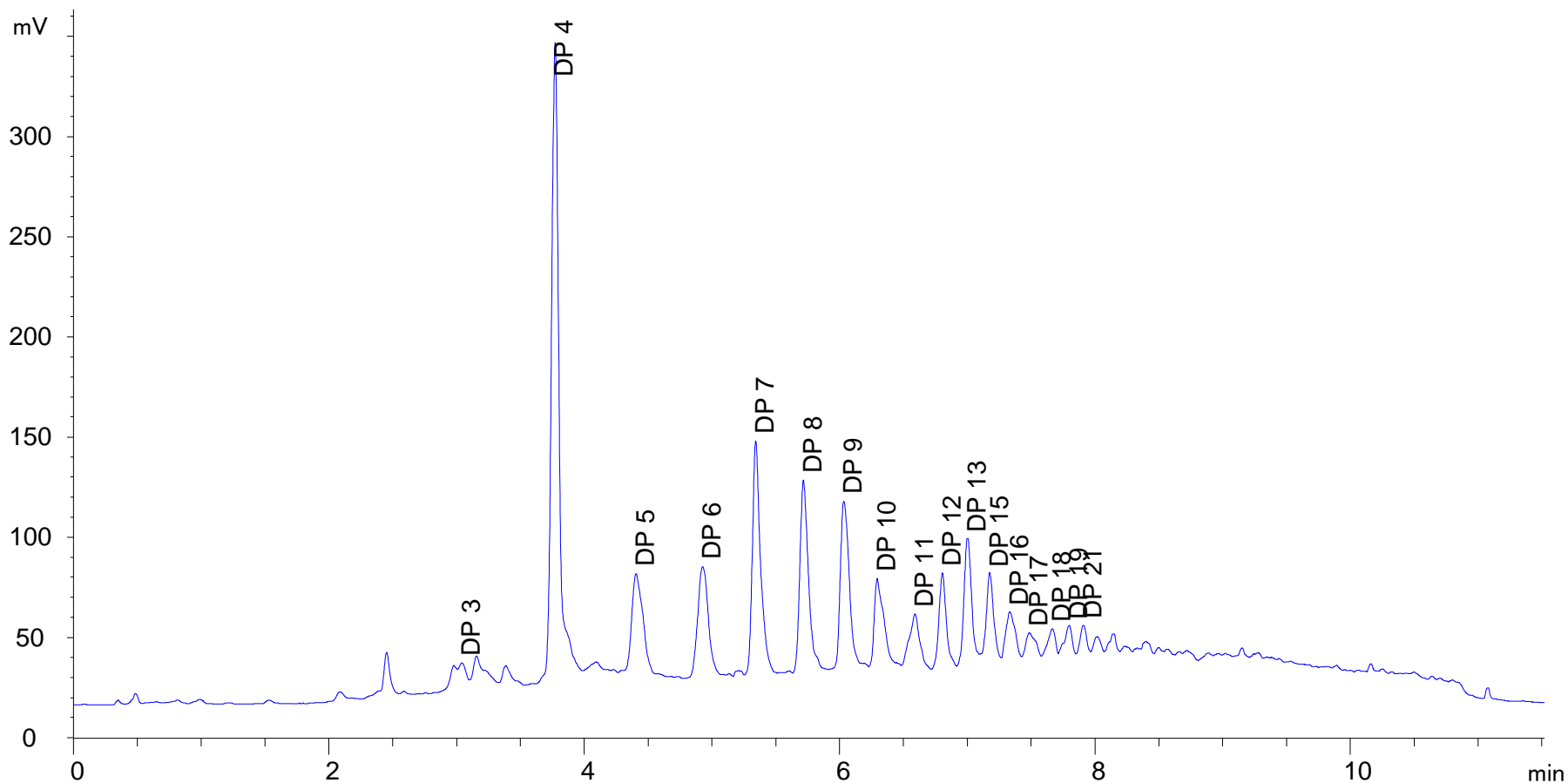


# Indeed Stir Crazy Porter



- 6.50% ABV, 50 IBU
- Malts: Rahr Pale, Munich II, Simpson's DRC, Brown Malt, Chocolate, Flaked Oats. Hops: HBC 472. Yeast: A15 Independence

[ELS1 A, Voltage \(Sugars21\\_PrepA\26\\_Sugars21\\_PrepB\\_MeOHPPT 2021-01-07\005-56-Porter 1-2-2 BWM.D\)](#)





# Summary



- Fast screening is possible with this system, and provides information on fermentable sugars, oligosaccharides (DP4 – DP 9), and poly saccharides, up to about DP 20.
  - Analysis time is less than 10 minutes.
- Cold sample storage at pH 2 preserves both mash and fermentation samples for later analysis.
  - 100  $\mu\text{L}$  of  $\text{H}_3\text{PO}_4$  for each 50 mL of sample
- Multiple sample preparation options are available, but dilution in aqueous-organic mixtures is recommended.
- The complete sugar profile pattern may be useful for more diagnostic and aesthetic purposes.
  - More information is needed.





# Thank You!



Stephanie Schuster, Ph. D.



**Richard A. Henry, Ph. D.**  
**(Consultant)**



Thomas J. Waeghe, Ph.D.



Michael Woodman

