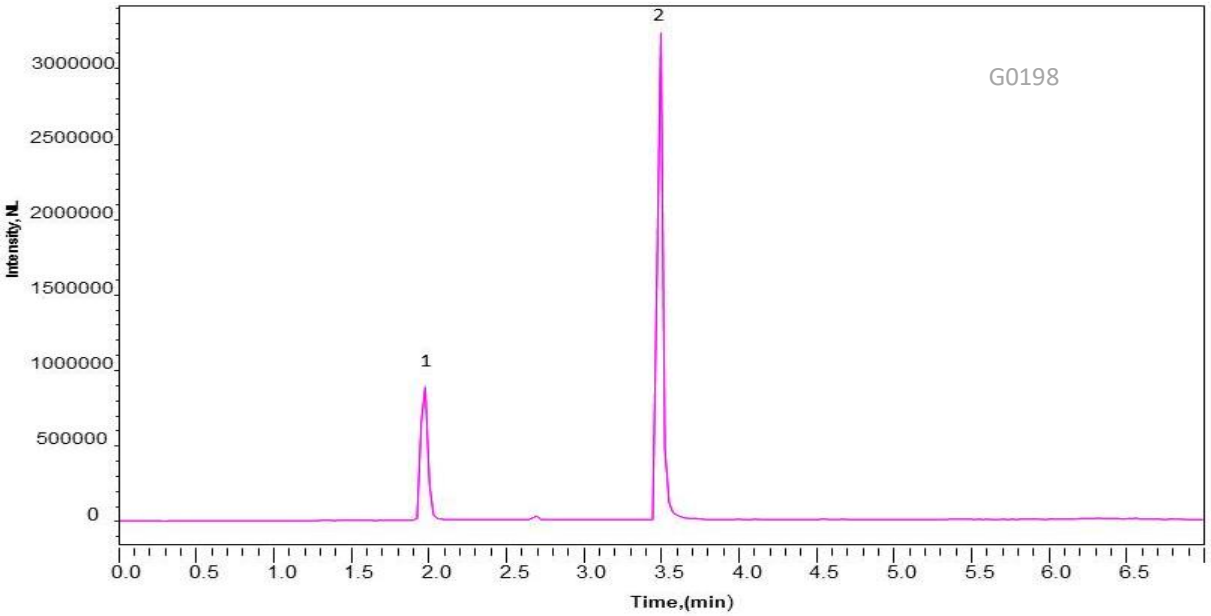
HALO® | Fused-Core® Particle Technology

Application Note: 204-TOX

LC-MS Separation of Kratom and its Metabolite on HALO® C18, 2 µm



TEST CONDITIONS:

Column: HALO 90 Å C18, 2 µm, 2.1 x 50mm
Part Number: 91812-402
Mobile Phase A: Water/0.1% Formic acid
Mobile Phase B: ACN/0.1% Formic acid
Gradient:

Time	%B
0.0	10
4.00	95
5.00	95
5.01	10
7.00	END

Flow Rate: 0.4 mL/min
Initial Pressure: 315 bar
Temperature: ambient
Injection Volume: 2 µL
Sample Solvent: 95/5 ACN/Water

PEAK IDENTITIES:

1. 7-OH Mitragynine (MH^+=415.502 g/mol)
2. Mitragynine (MH^+=399.453 g/mol)

MS CONDITIONS:

LCMS system: Shimadzu LCMS-2020
Detection: +ESI MS
Spray voltage: 4.50 kV
Drying line temp: 300 °C
Heat Block: 450 °C

The 2 µm HALO C18 is an ideal choice for analysis of kratom and its metabolite. Kratom is an herbal extract that comes from the leaves of an evergreen tree (Mitragyna speciosa) grown in Southeast Asia. Believed to act on opioid receptors, kratom has been used by people to mitigate the symptoms of opioid withdraw. However, studies on the effects of kratom have identified many safety concerns and no clear benefits, and kratom is not currently regulated by the United States.

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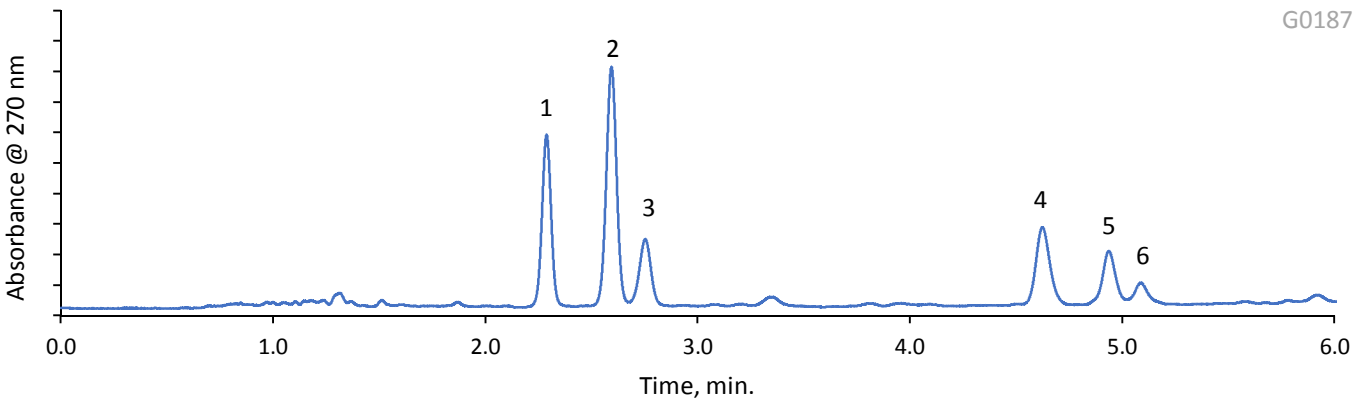
Application Note: 193-OA

Separation of Hop Acids on HALO® 5 µm Biphenyl



TEST CONDITIONS:

Columns: HALO 90 Å Biphenyl, 5 µm, 4.6 x 150mm
Part Number: 95812-611
Mobile Phase A: Water, 0.1% Formic acid
Mobile Phase B: Acetonitrile, 0.1% Formic acid
Gradient:

Time	% B
0.0	60
3.0	60
6.0	80

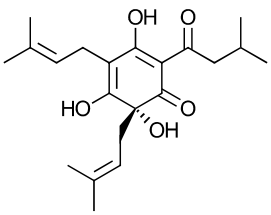
Flow Rate: 2.0 mL/min
Initial Pressure: 236 bar
Temperature: 30°C
Detection: 270 nm, PDA
Injection Volume: 5 µL
Sample Solvent: Acetonitrile
Data Rate: 100 Hz
Response Time: 0.025 sec.
Flow Cell: 1 µL
LC System: Shimadzu Nexera X2

Hops are primarily made up of essential oils and alpha and beta acids. They have many benefits in the beer brewing process, including their antiseptic nature and bitterness flavor they give to the beer. Alpha and beta acids from the International Calibration Standard Extract (ICE-3) are separated on a HALO® Biphenyl column.

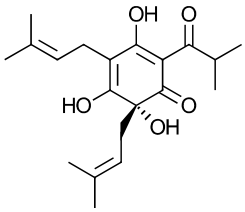
PEAK IDENTITIES:

Alpha Acids	Beta Acids
1. Cohumulone	4. Colupulone
2. Humulone	5. Lupulone
3. Adhumulone	6. Adlupulone

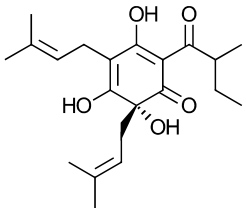
STRUCTURES:



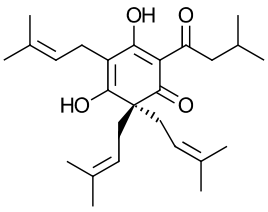
Cohumulone



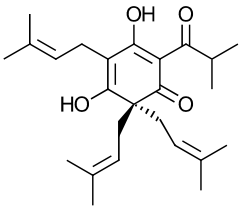
Humulone



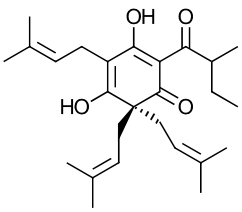
Adhumulone



Colupulone



Lupulone



Adlupulone

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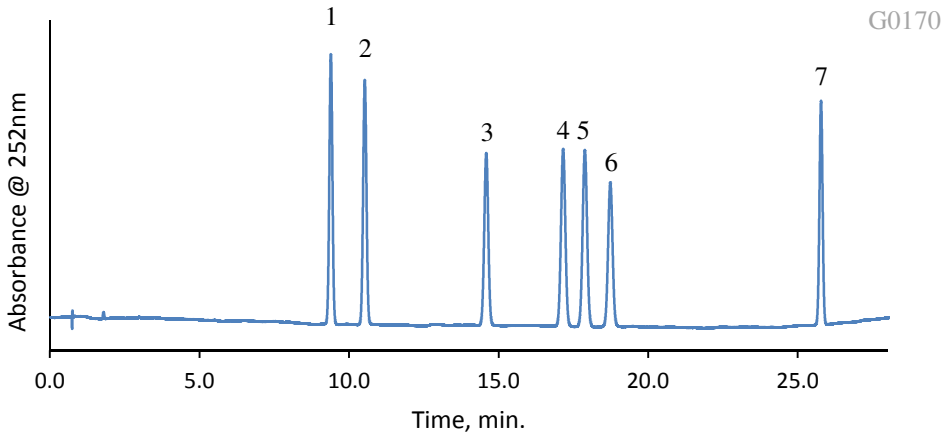
Application Note: 177-P

Chinese Pharmacopeia Separation of Parabens on HALO C18, 2.7µm



PEAK IDENTITIES:

1. Isopropyl paraben
2. Propyl paraben
3. Phenyl paraben
4. Isobutyl paraben
5. Butyl paraben
6. Benzyl paraben
7. Pentyl paraben

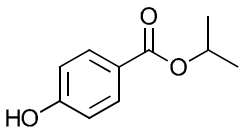
TEST CONDITIONS:

Column: HALO 90Å C18, 2.7 µm, 4.6 x 100mm
Part Number: 92814-602
Mobile Phase A: Water
Mobile Phase B: Methanol
Gradient:

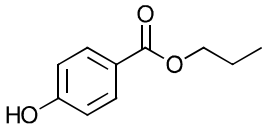
Time	%B
0.0	40
23.0	55
28.0	70

Flow Rate: 1.2 mL/min
Initial Pressure: 403 bar
Temperature: 30°C
Detection: UV 252 nm, PDA
Injection Volume: 1.5 µL
Sample Solvent: 50-50 Methanol-Water
Data Rate: 40 Hz
Response Time: 0.025 sec.
Flow Cell: 1 µL
LC System: Shimadzu Nexera X2

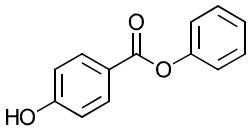
STRUCTURES:



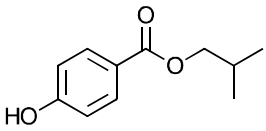
Isopropyl paraben



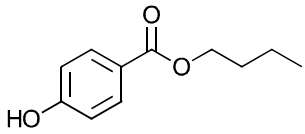
Propyl paraben



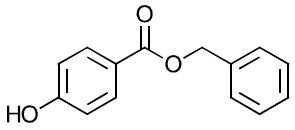
Phenyl paraben



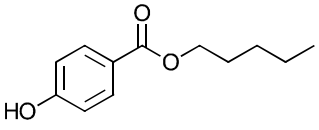
Isobutyl paraben



Butyl paraben



Benzyl paraben



Pentyl paraben

A separation of parabens is performed on a HALO C18 column showing high resolution between critical pairs using a Chinese Pharmacopeia method. Parabens are esters of para-hydroxybenzoic acid and have many varieties. Parabens are widely used in a variety of cosmetics as a preservative. This can include many things such as shampoos, moisturizers, makeup, and shaving gels.

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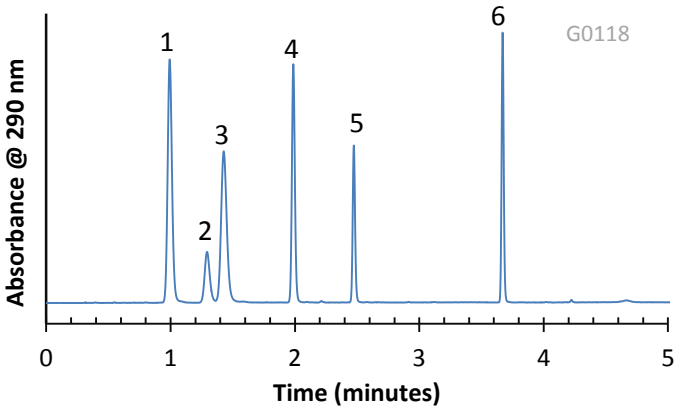
Application Note: 133-P

Separation of Resveratrols and Related Compounds on HALO 5 C18



PEAK IDENTITIES:

1. *trans*-Polydatin
2. Piceatannol
3. *trans*-Oxyresveratrol
4. *trans*-Resveratrol
5. *cis*-Resveratrol
6. Pterostilbene

TEST CONDITIONS:

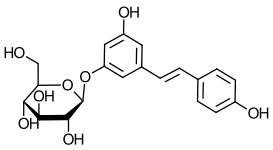
Column: 3.0 x 100 mm, HALO 5 C18, 5 µm
Part Number: 95813-602
Mobile Phase:
A= Water
B= Methanol
Gradient:

Time	%B
0.0	32
1.0	32
4.0	90
5.0	90

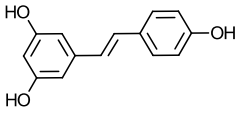
Flow Rate: 1.2 mL/min.
Pressure: 245 Bar
Temperature: 35°C
Detection: UV 290 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: 50/50: Acetonitrile/water
Response Time: 0.02 sec.
Data rate: 25 Hz.
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
ECV: ~14 µL

These naturally occurring compounds can be found in grapes and grape vines and other plants and are claimed to have health benefits. Resveratrol and these related compounds can be analyzed in less than 5 minutes using a HALO 5 C18 column.

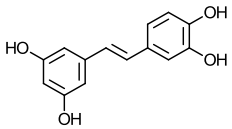
STRUCTURES:



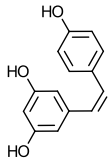
trans-Polydatin



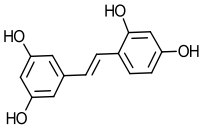
trans-Resveratrol



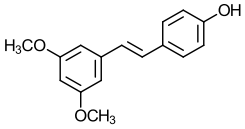
Piceatannol



cis-Resveratrol



trans-Oxyresveratrol



Pterostilbene

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Session Number: S23-05
Analytical Chemistry of Beer and Brewing
Tuesday, March 09, 2021 11:05 AM - 11:40 AM

Fast Screening of Oligo- and Poly-saccharides in Beer

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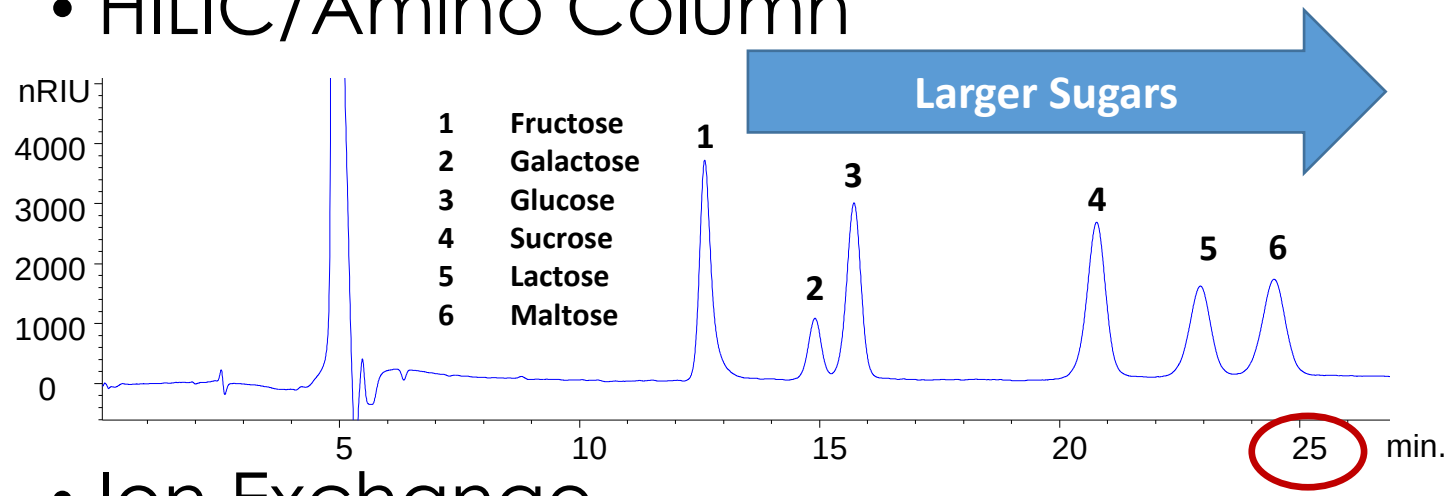
Overview

- 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

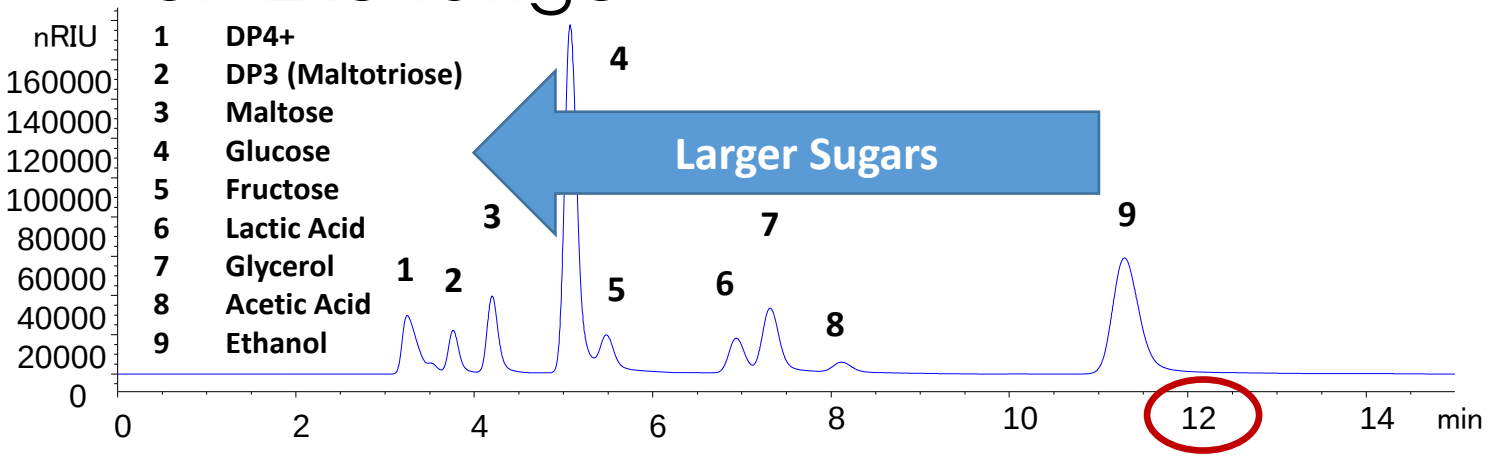
- HILIC/Amino Column



2X 4.6 X 150 mm APHera NH2 (2 columns in series)

75/25 ACN/Water

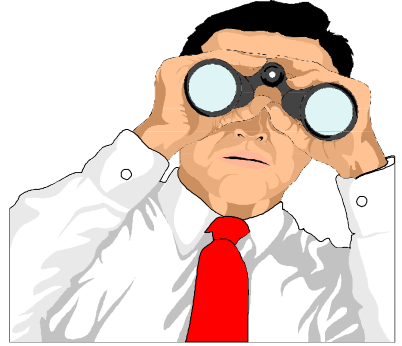
- Ion Exchange

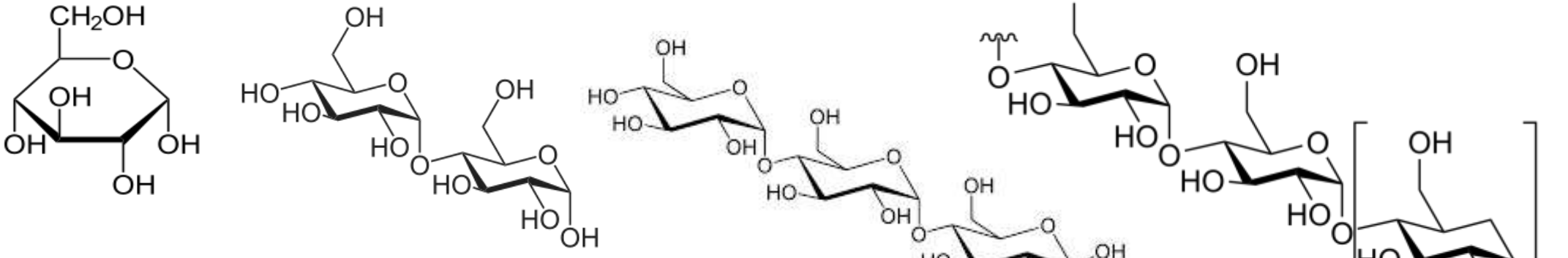


4.6 X 150 mm Ion Exchange (H+)

0.005 N H_2SO_4

Looking For a Better Option for Larger Sugars





DP 1 (Glucose)

Log P = -3.2

DP 2 (Maltose)

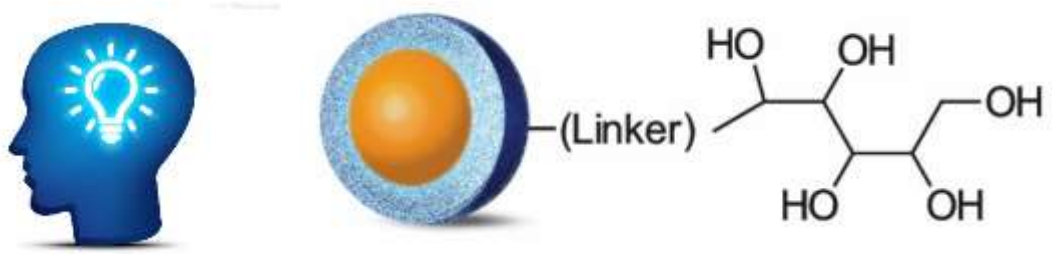
Log P = -4.7 (calc.)

DP 3 (Maltotriose)

Log P = -5.6

DP n+2

Halo Pentahilic



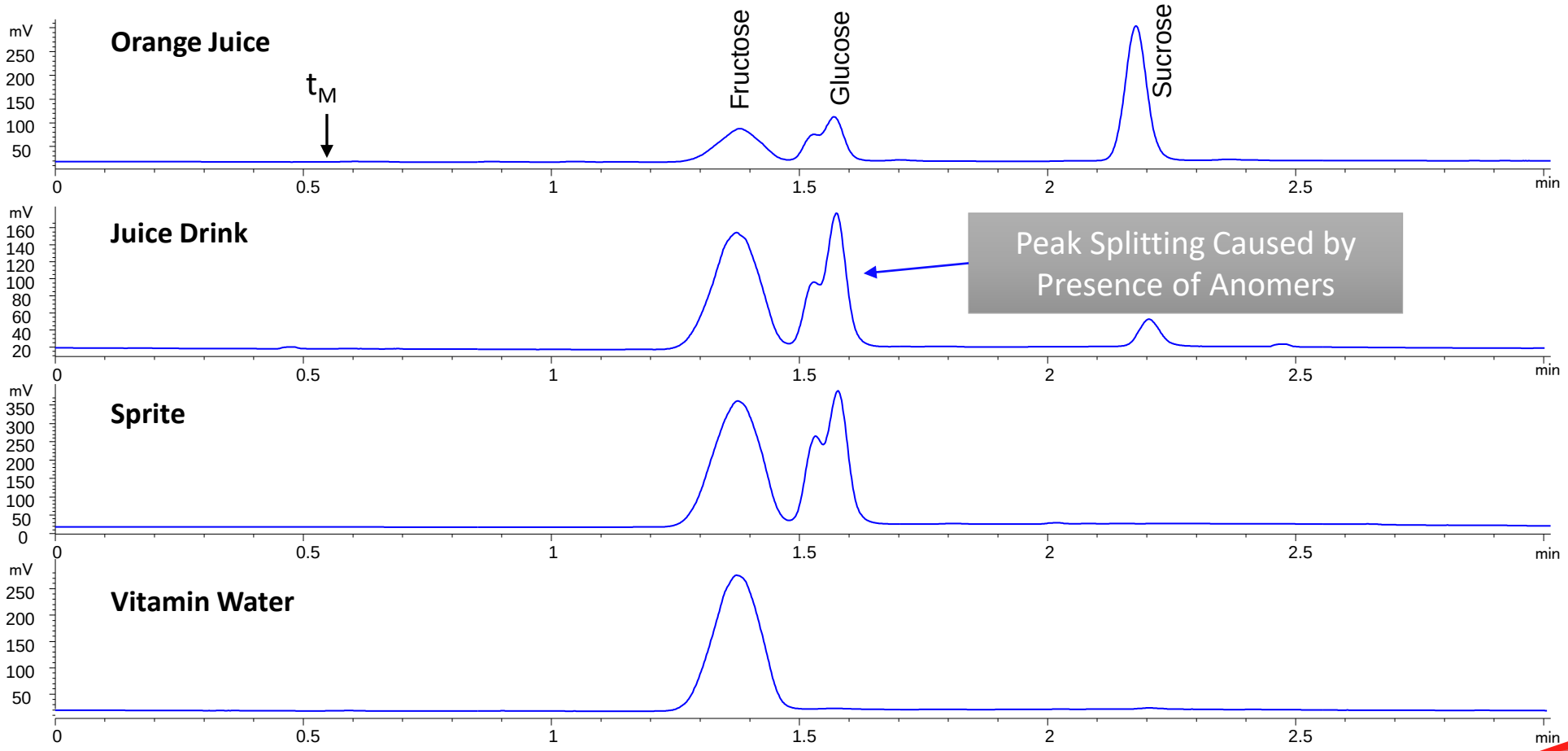
Courtesy: AMT

A "glycan" stationary phase! (HILIC Mode)

Fruit Drinks (Fast Isocratic Method)

Column:3.0X 100 mm, 2.7 µm
Mobile Phase: H_2O/ACN (20/80)
Flow: 0.75 mL/min
Injection: 2 µL
Column Temperature: 35 C
Detector: ELSD [40 °C, 45 psi]
Sample: Diluted 1:10 with water/acn

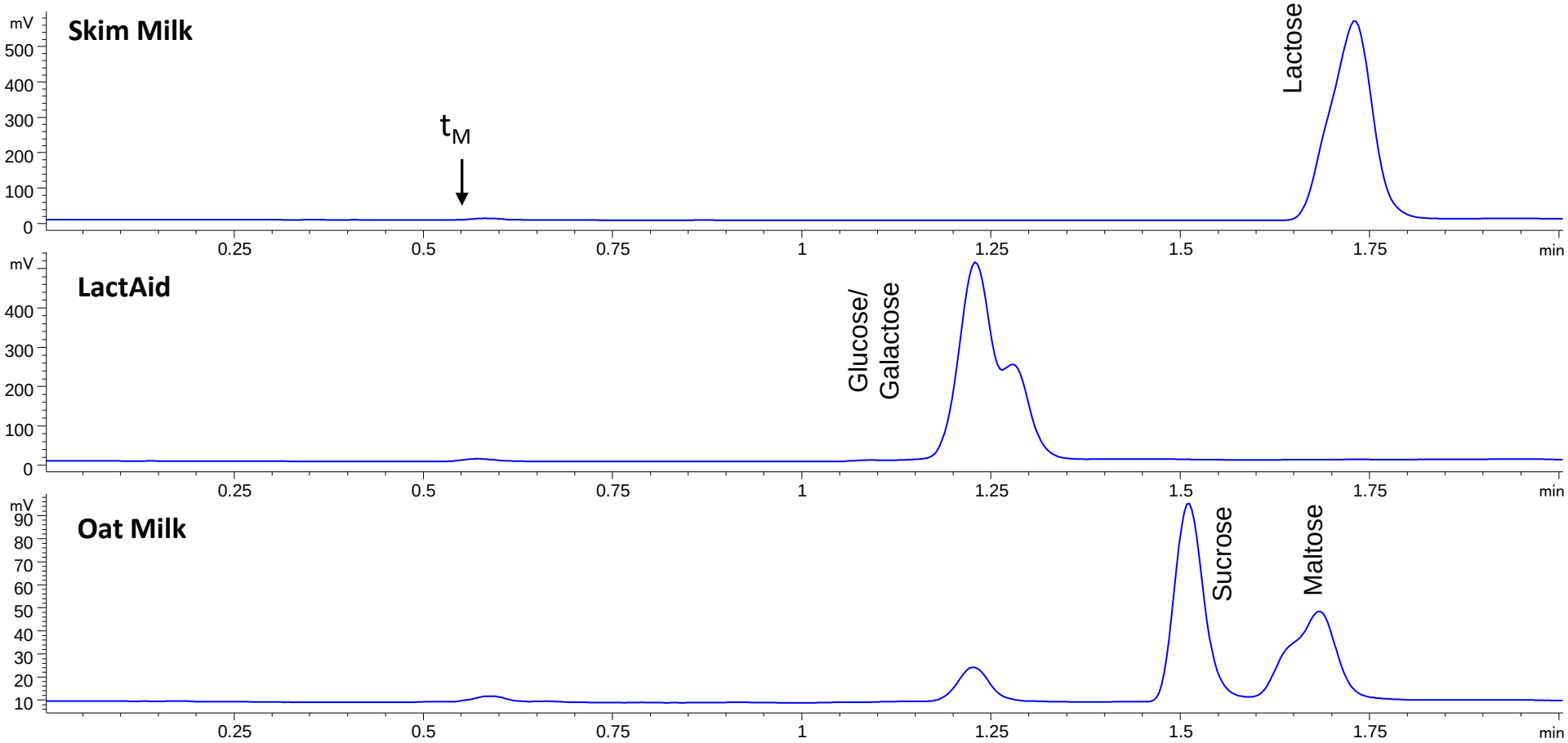
- Analysis time less than 2.5 minutes!



Dairy/Plant Drinks (Fast Isocratic Method)

Column:3.0X 100 mm, 2.7 µm
Mobile Phase: H_2O/ACN (25/75)
Flow: 0.75 mL/min
Injection: 1 µL
Column Temperature: 35 C
Detector: ELSD [40 °C, 45 psi]
Sample: Diluted 1:10 with water/acn

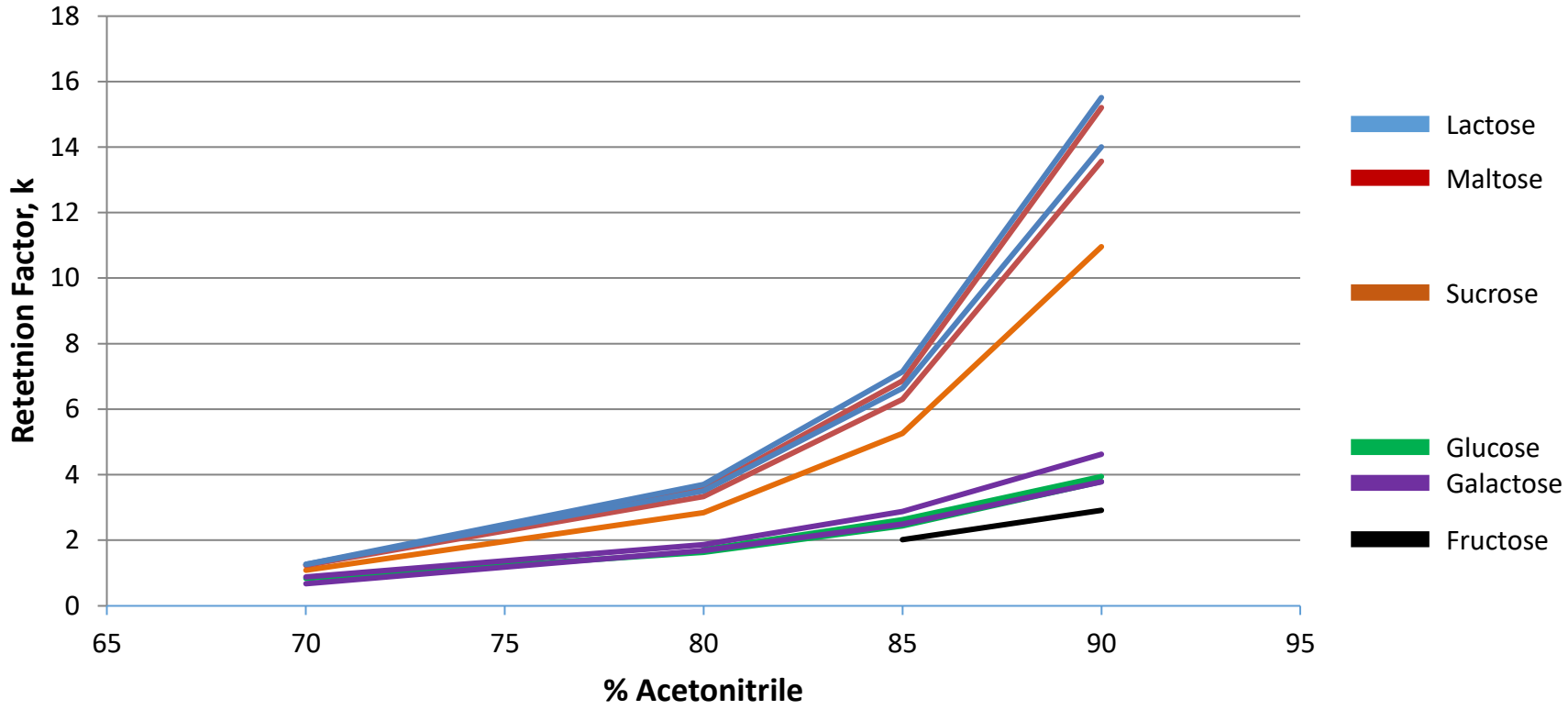
- Analysis time less than 2 minutes!



Column:3.0X 100 mm, 2.7 µm
Mobile Phase: H_2O/ACN
Flow: 0.75 mL/min
Injection: 1 µL
Column Temperature: 35 C
Detector: ELSD [40 °C, 45 psi]
Sample: 1.0 mg/mL

Retention Pattern

- Retention behavior follows a typical HILIC trend

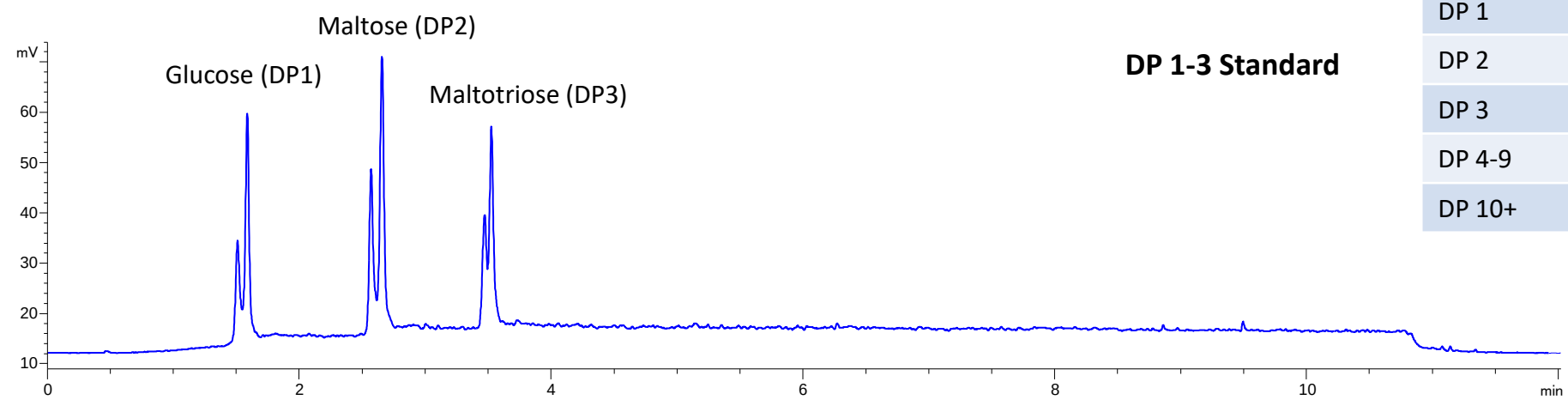


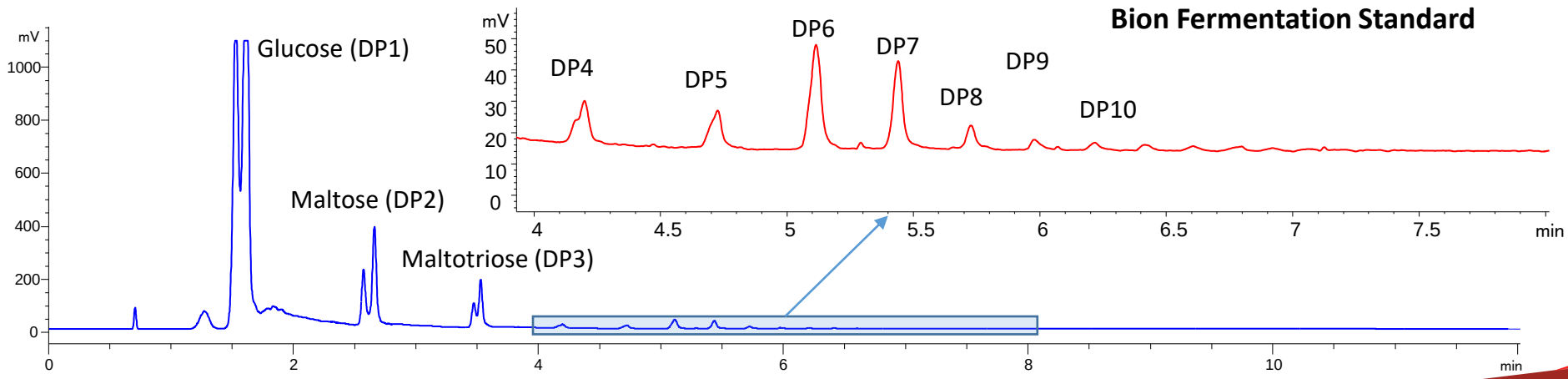
DP Sugars

Column:4.6X 50 mm, 2.7 µm
Mobile Phase: H_2O/ACN (90-40% ACN/10 min.)
Flow: 1.5 mL/min
Injection: 1 µL
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

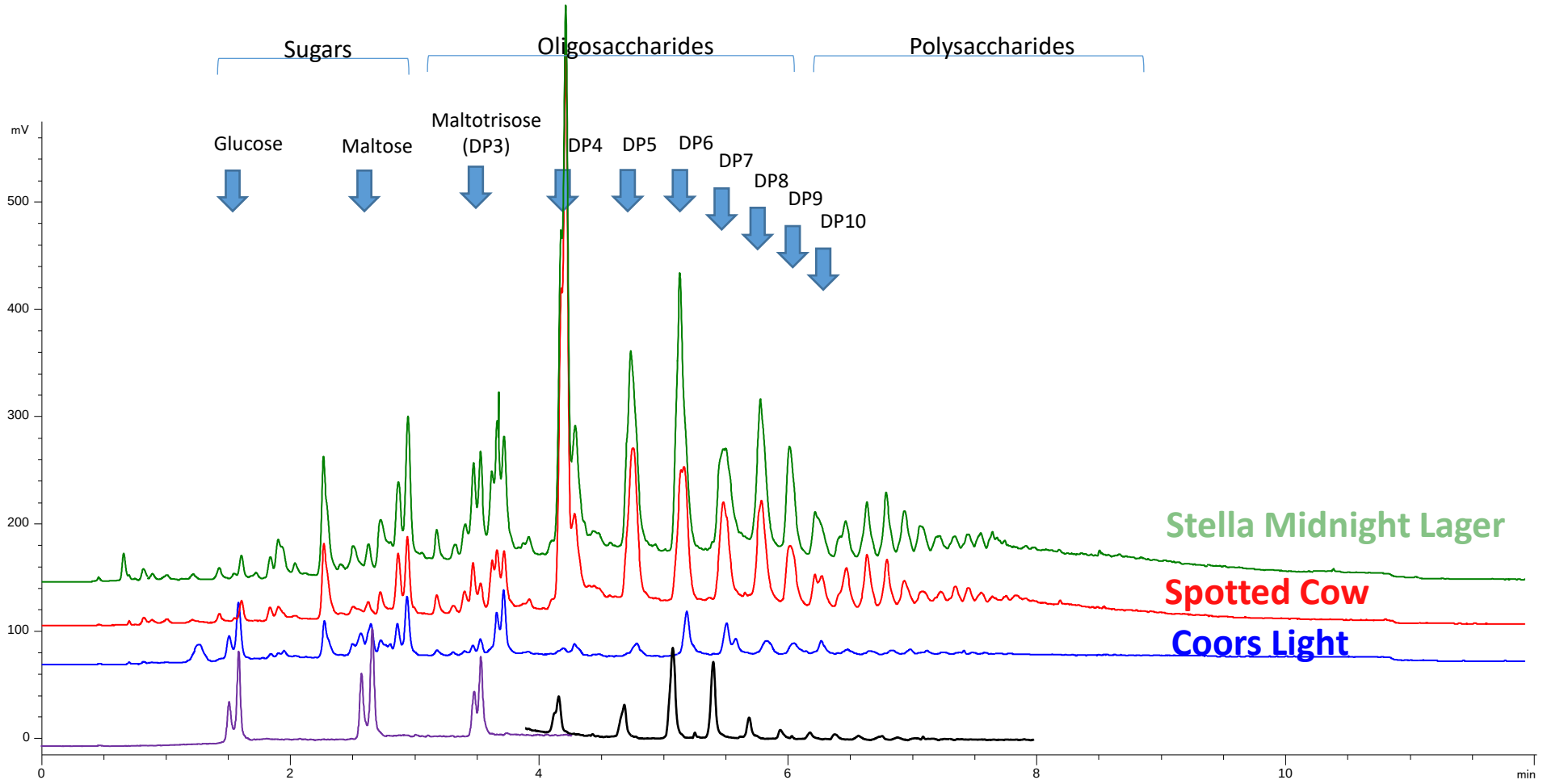
Abbreviation	Common Name
DP 1	Glucose
DP 2	Maltose
DP 3	Maltotriose
DP 4-9	Oligosaccharides
DP 10+	Polysaccarides





Beer Is More Interesting

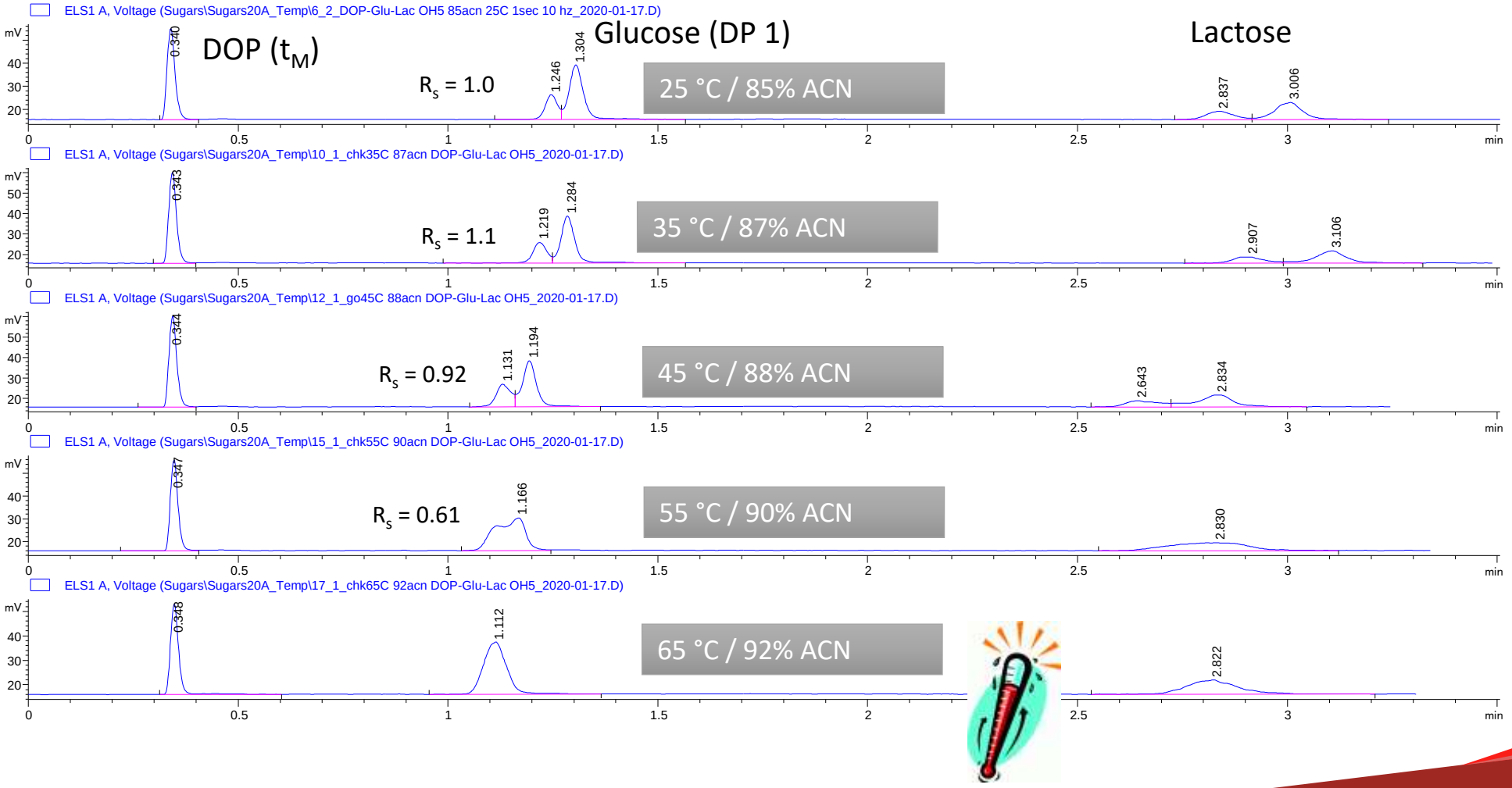
Column:4.6X 50 mm, 2.7 µm
Mobile Phase: H_2O/ACN (90-40% ACN/10 min.)
Flow: 1.5 mL/min
Injection: 1 µL
Column Temperature: 35 C
Detector: ELSD [40 °C, 45 psi]
Sample: Filtered



Improving the Separation

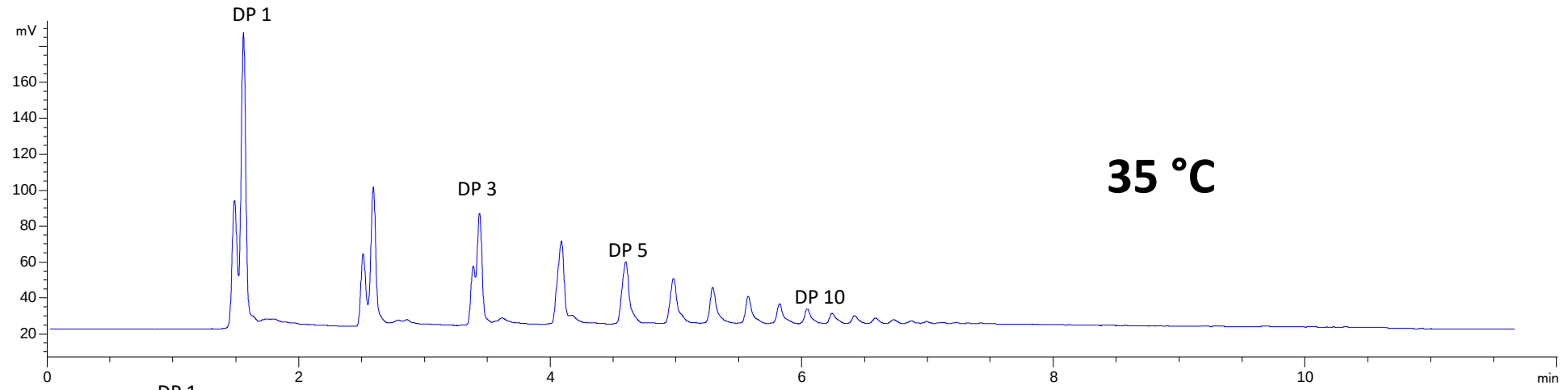
- Anomer splitting increases peak width and decreases separation
- Retention times are longer
- Complete elution from the column is always a concern
- Many other sugar methods operate at higher temperatures
- Possible Solutions
  - Evaluate higher temperatures
  - Use short column
  - Use smaller diameter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

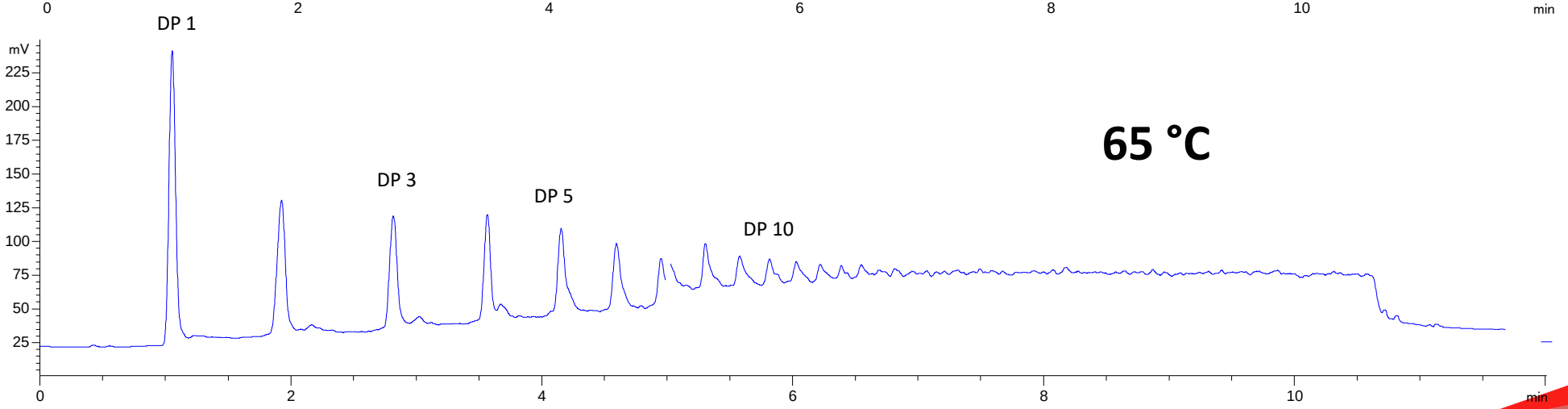
Peak Shape Improves at Higher Temperatures



DE Sugars at Higher Temperature

Column:4.6X 50 mm, 2.7 µm
Mobile Phase: H_2O/ACN (90-40% ACN/10 min.)
Flow: 1.5 mL/min
Injection: 1 µL
Column Temperature: 35 C
Detector: ELSD [40 °C, 45 psi]
Sample: 36 DE, Filtered

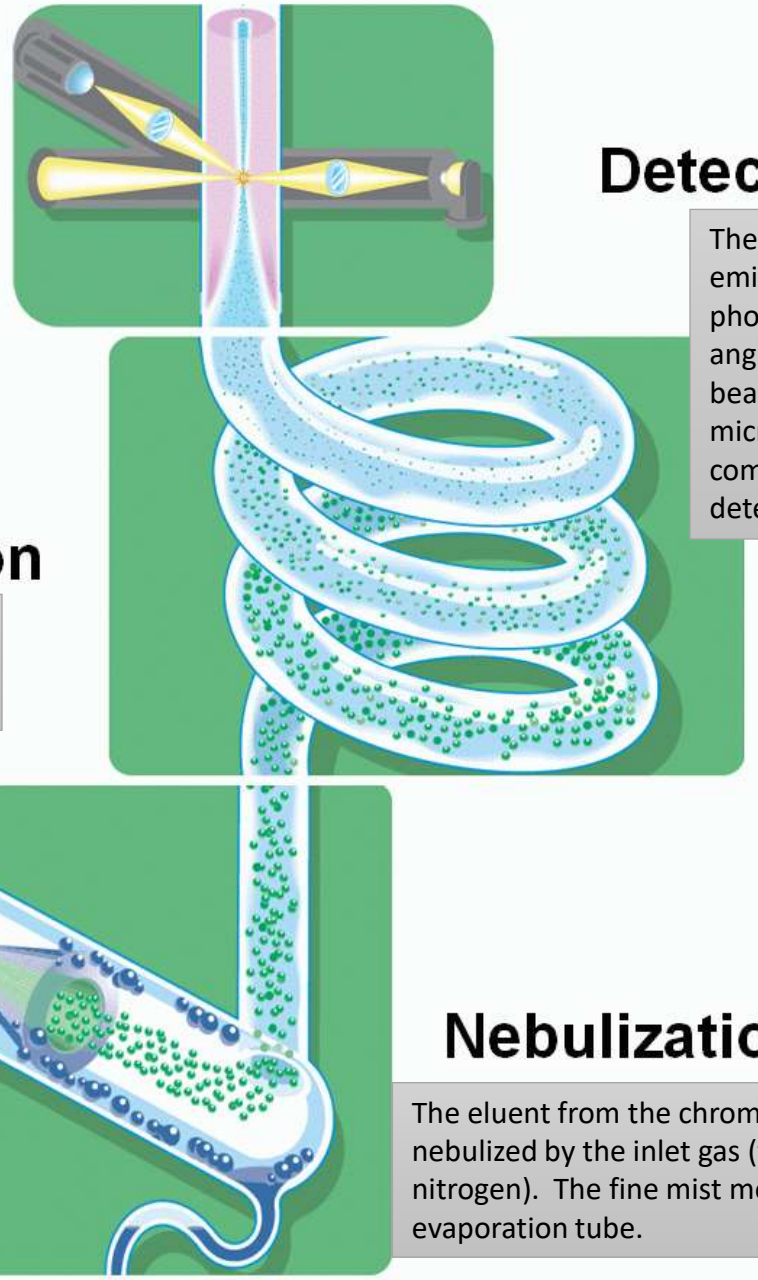




Final Experimental Details

- 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 um
- 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 uL
- Column Temperature: 65 C
- ELSD:
  - 40 C, 45 psi
  - 10 Hz Data Rate, 2 sec Filter

Evaporative Light Scattering Detection (ELSD)



Detection

The detector chamber contains a light emitting diode (LED) and a photomultiplier that is positioned at an angle of 120° 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.

Evaporation

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

Nebulization

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

- 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**.

And now, the real world!

Mashing Samples – Double IPA

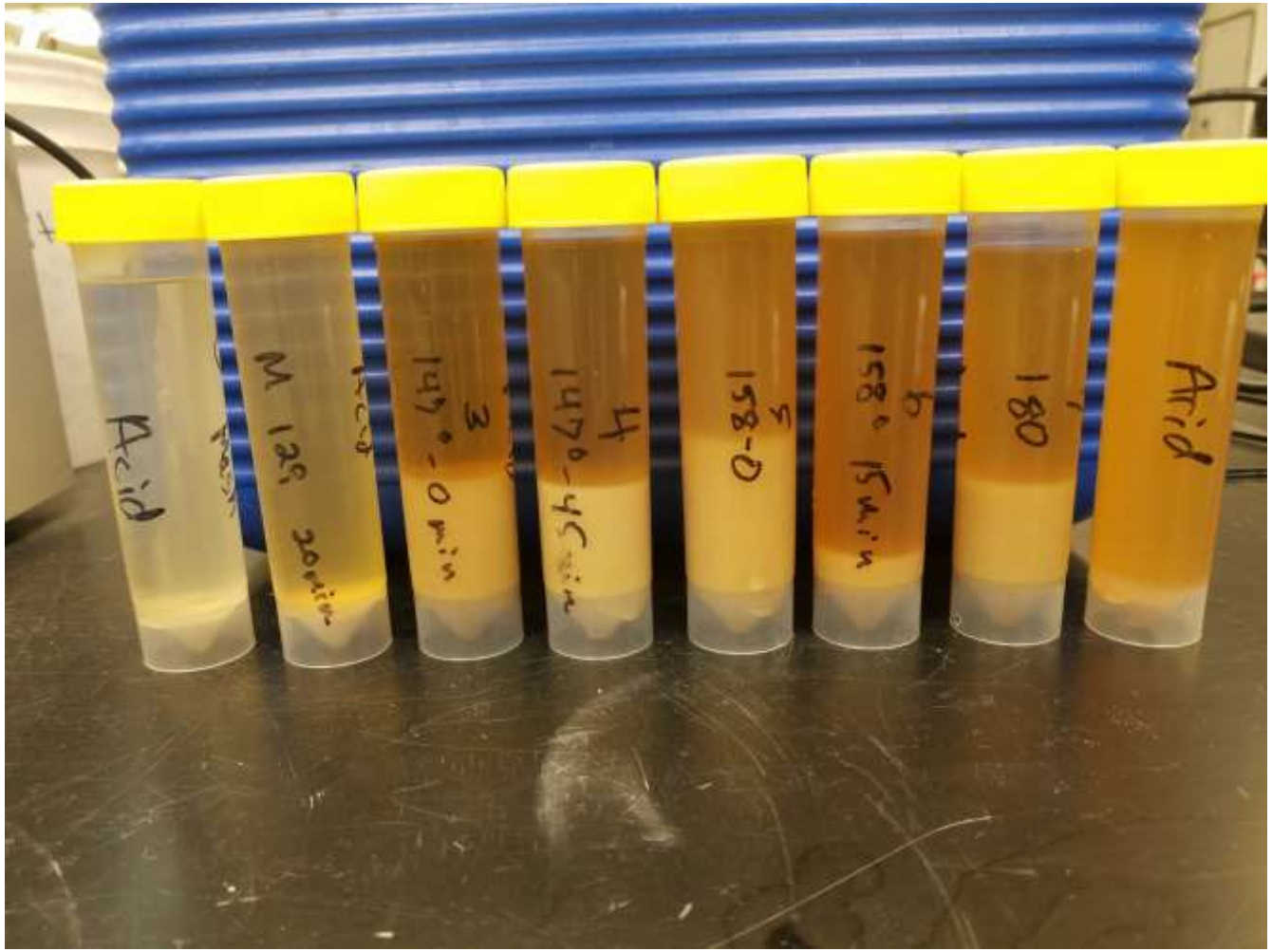
Sample	Conditions	Comments
1/1A	Mash at start	
2/2A	Mash – 129 °F / 20 minutes	Initial heating
3/3A	Mash – Heat to 147 °F/0 minutes	Activate beta amylase
4/4A	Mash – 147 °F / 45 minutes	
5/5A	Mash – Heat to 158 °F / 0 minutes	Activate alpha amylase
6/6A	Mash – 158 °F / 15 minutes	
7/7A	Mash – Heat to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

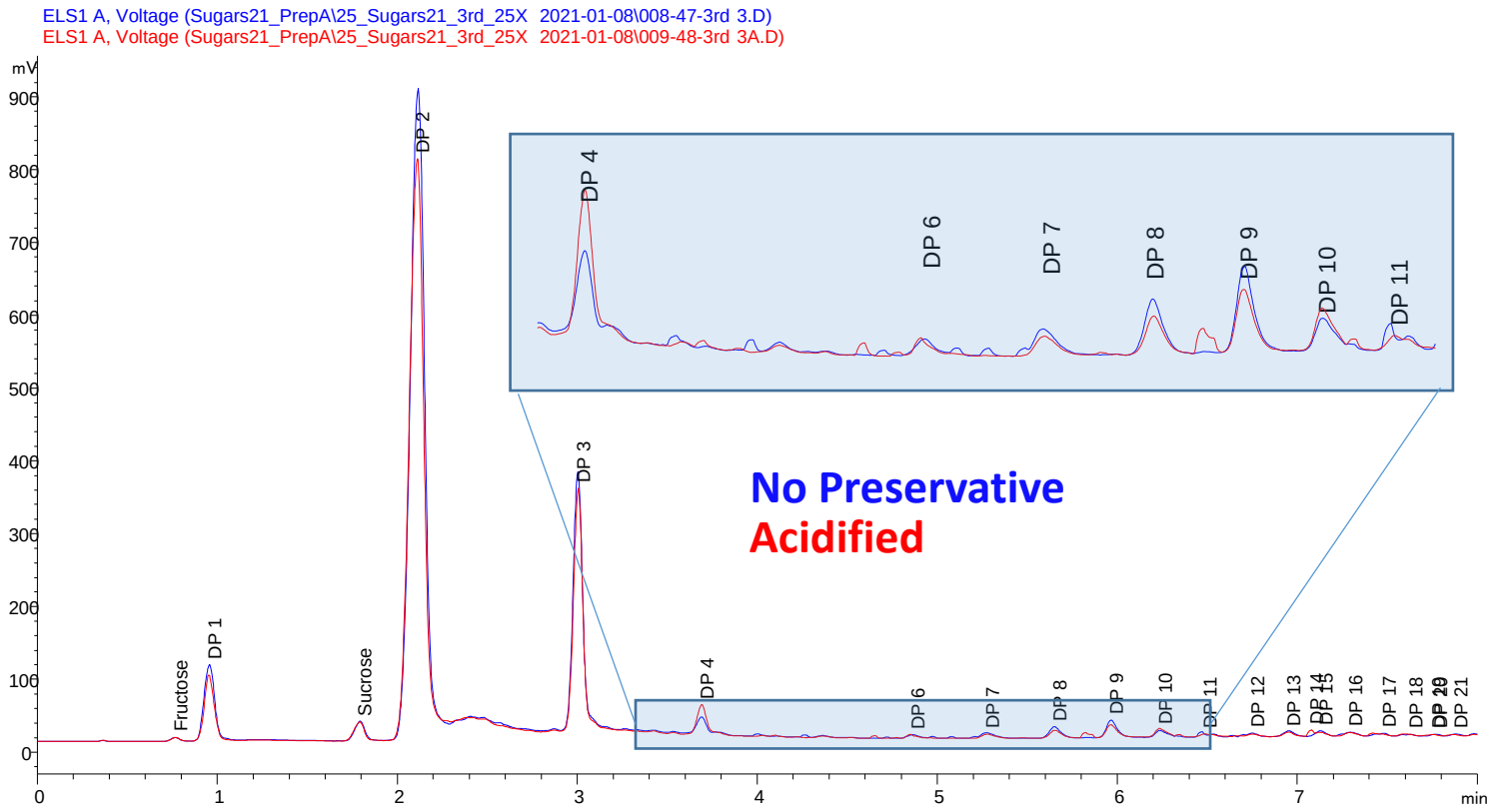
- Collection Conditions
  - Raw or acidified to ~ pH 2
    - 50 – 100 µL H_3PO_4
  - Store cold
- Allow to settle or centrifuge, remove supernatant
- HPLC Sample Requirements
  - No particulates (filtered, < 0.5 µm)
  - Minimize interferences
  - Aqueous or aqueous/organic mixture

Mashing Samples



Sample Stability – 2 days at 4 °C

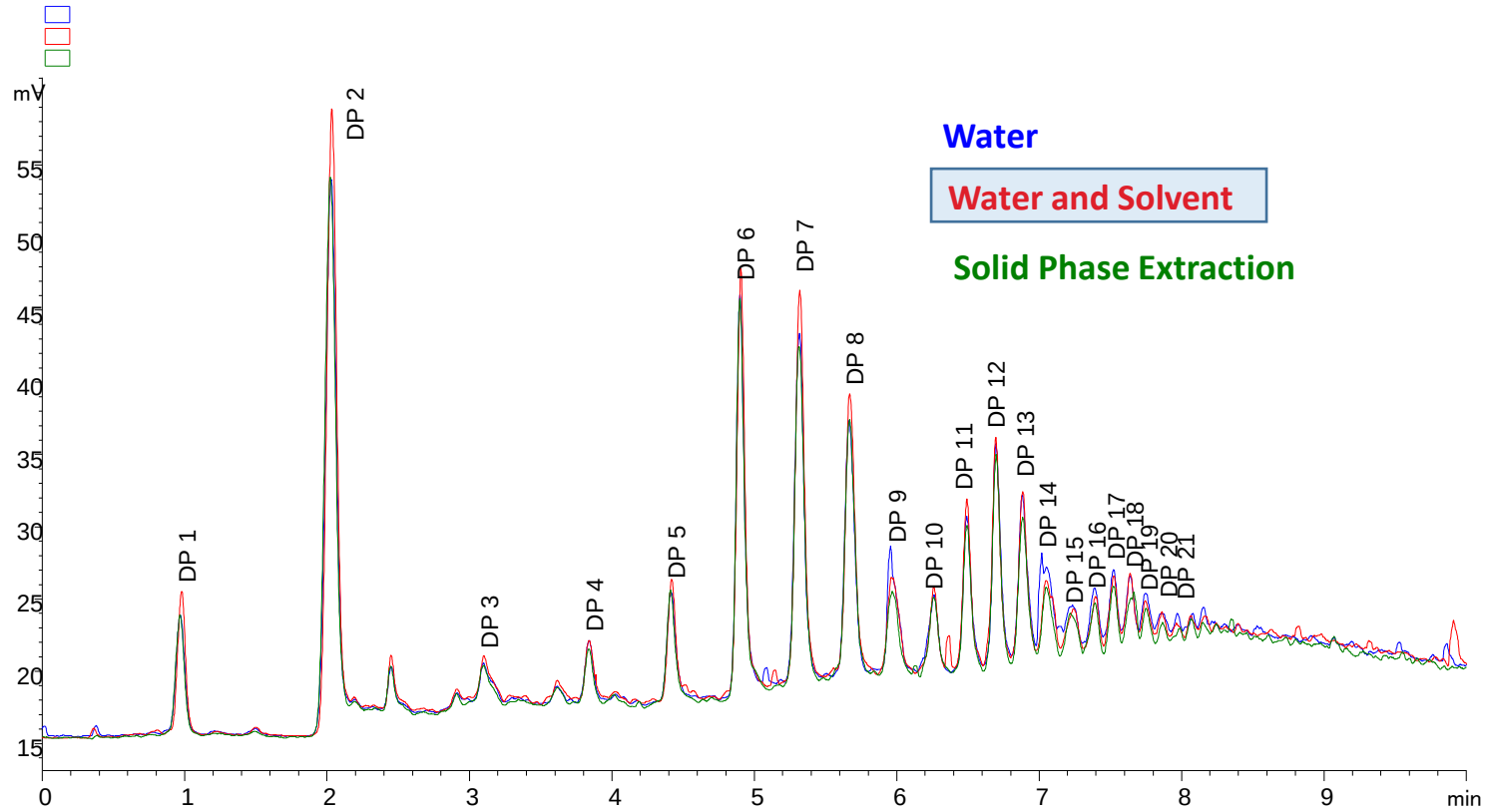
- Acidified samples showed less fermentable sugars after two days of cold storage.
  - Some enzyme activity was still present in unpreserved samples
- Oligosaccharides (DP 4 – 10) showed some minor changes
- Samples after inactivation (7 and 8) were nearly identical.



Sample Preparation:
Sample diluted 1:25 with deionized water.
Filtered (0.22 um)

Preparing Samples for HPLC

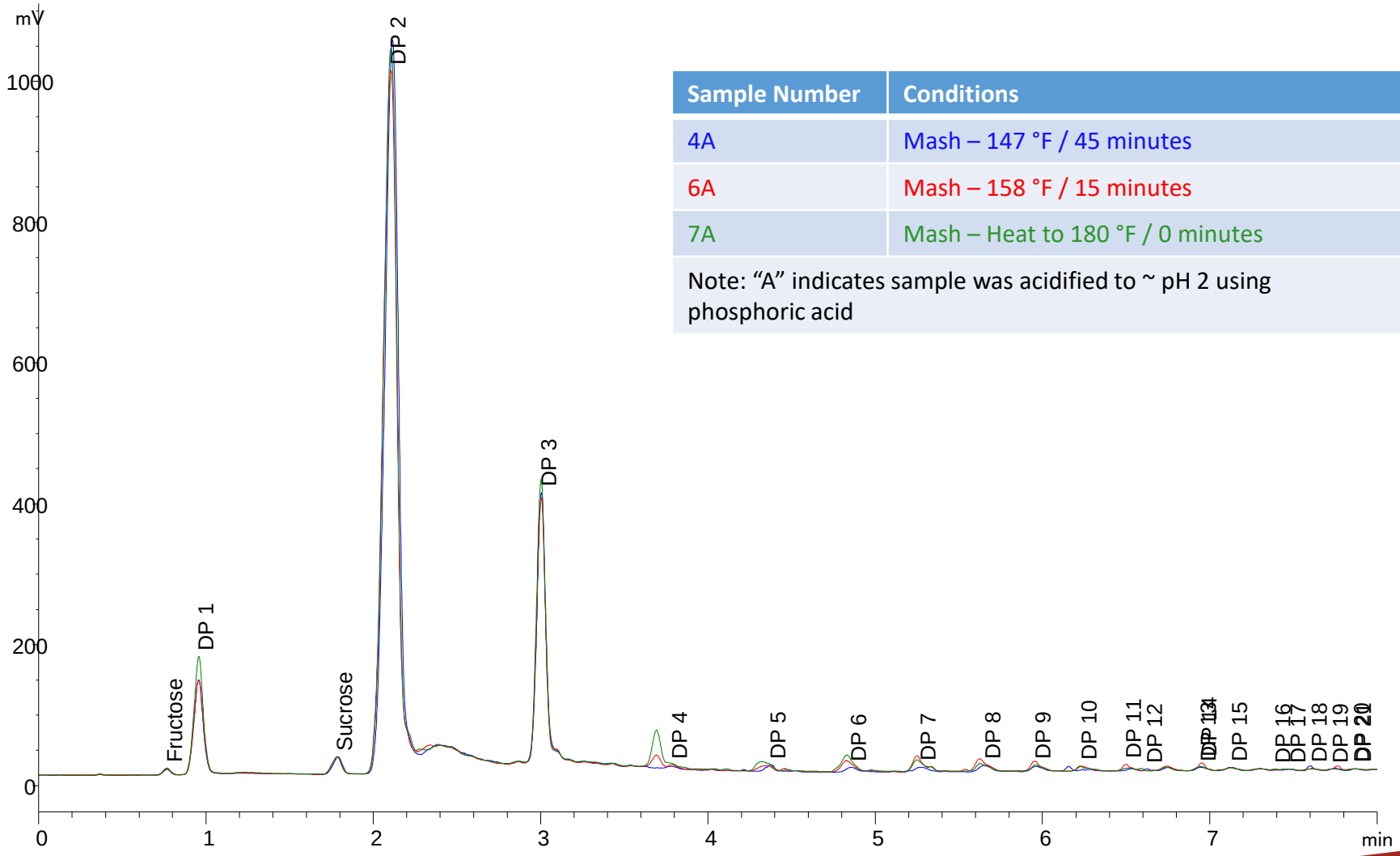
- Options (1:25 Dilutions)
  - Dilution with water
  - Dilution with water and 40 % organic solvent (1:1 acetonitrile:methanol)
  - Solid Phase Extraction



General Dilution Levels for Beer

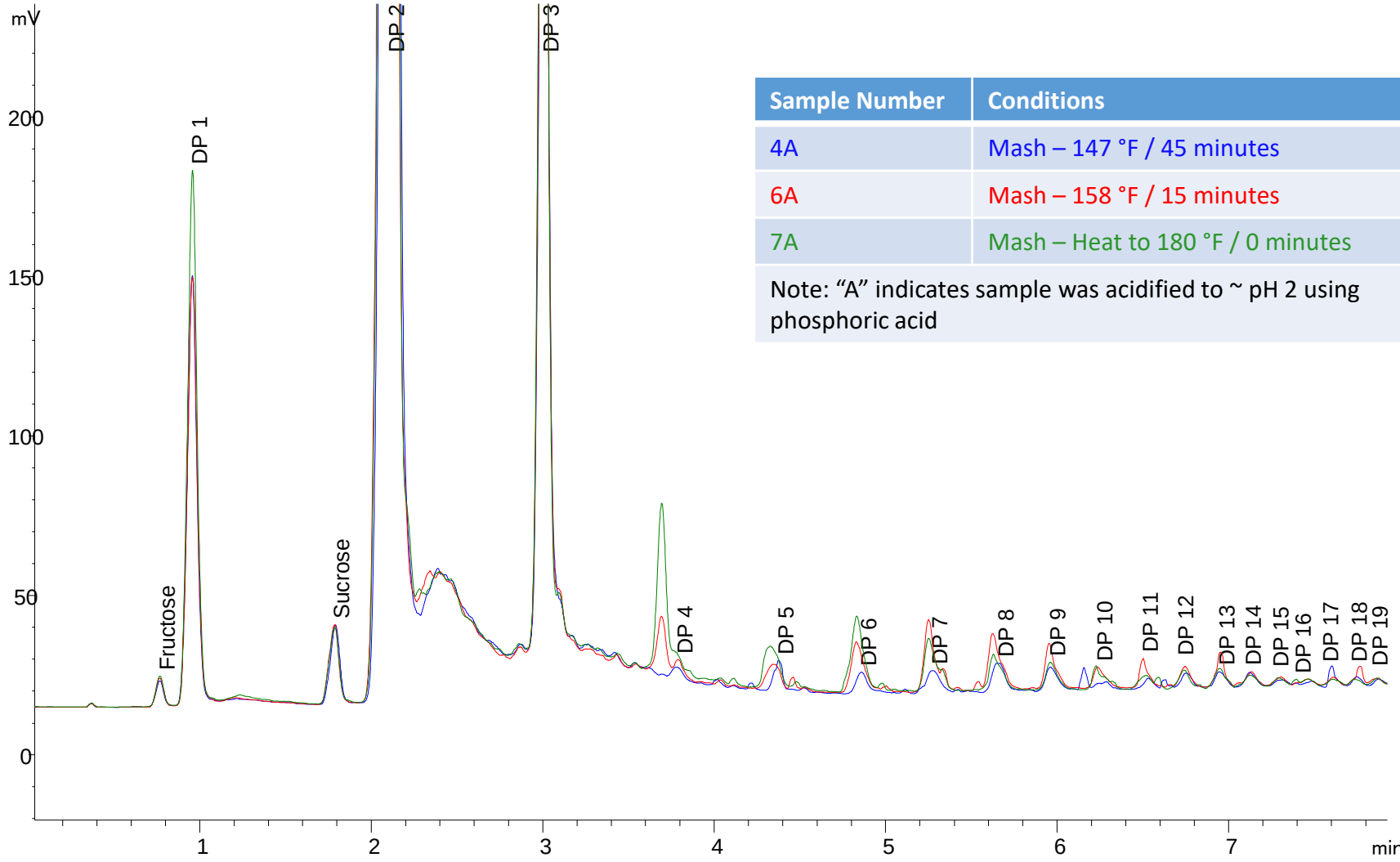
Sample Type	Recommended Dilution	Comments
Mash	1:25	Include organic solvent to precipitate proteins and other interferences
Fermentation	1:10	Maltose will be off scale for early samples but allows assessment of other sugars
	1:5	Allows better review of larger sugars, maltose off scale
Finished Product	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)



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 Sample Summary (Expanded)

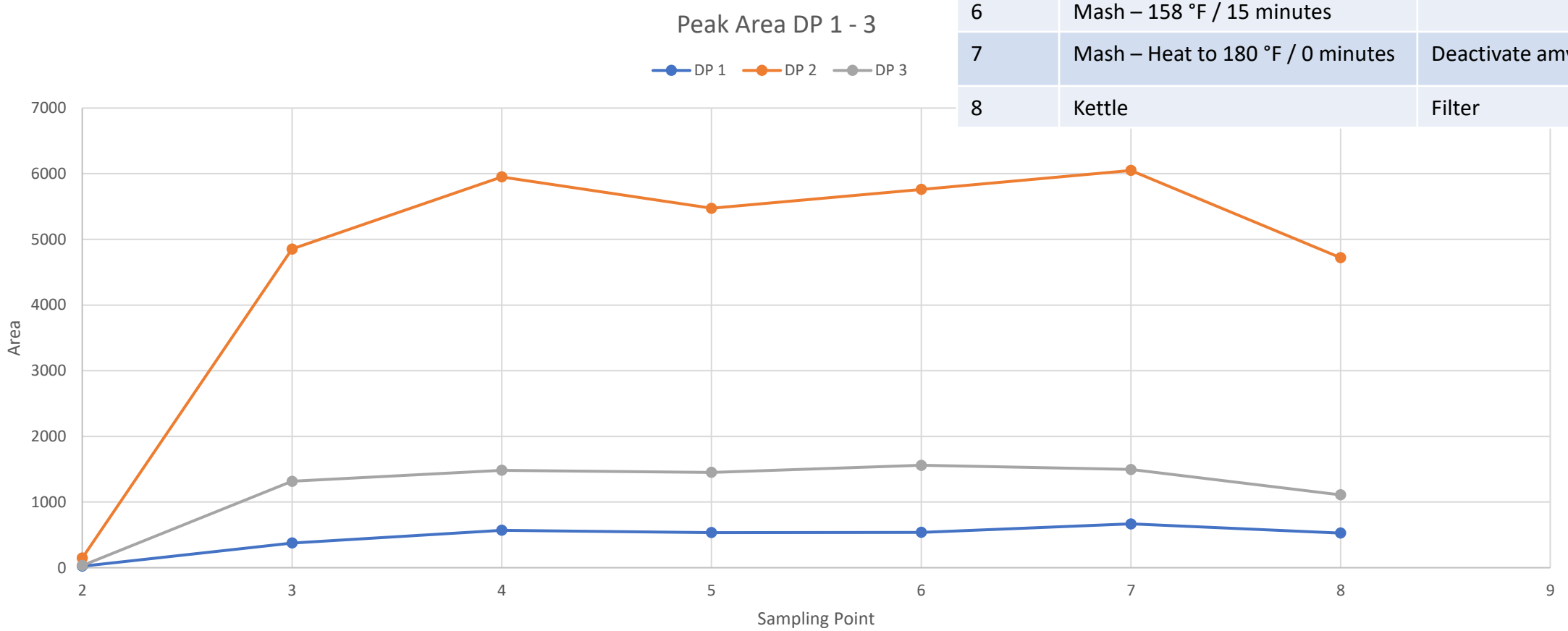


Sample Number	Conditions
4A	Mash – 147 °F / 45 minutes
6A	Mash – 158 °F / 15 minutes
7A	Mash – Heat to 180 °F / 0 minutes

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

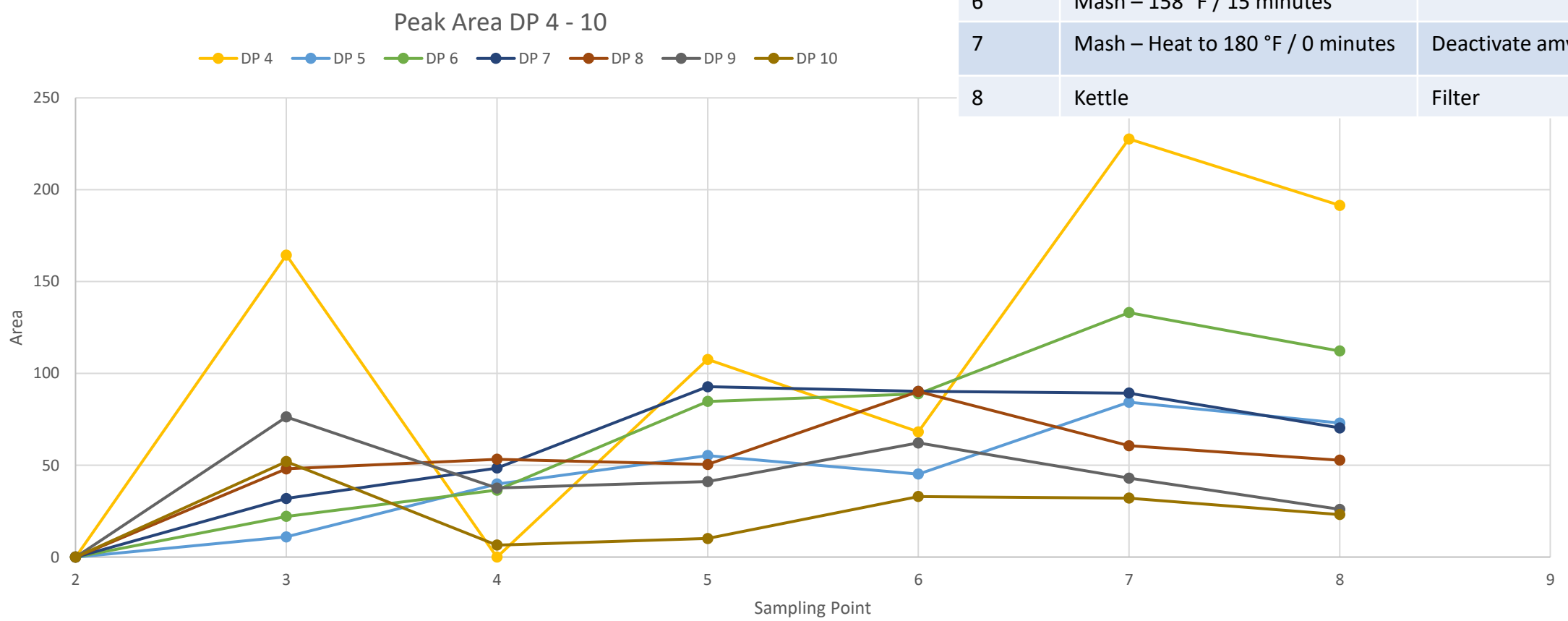
Mash Samples: Fermentable Sugars

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



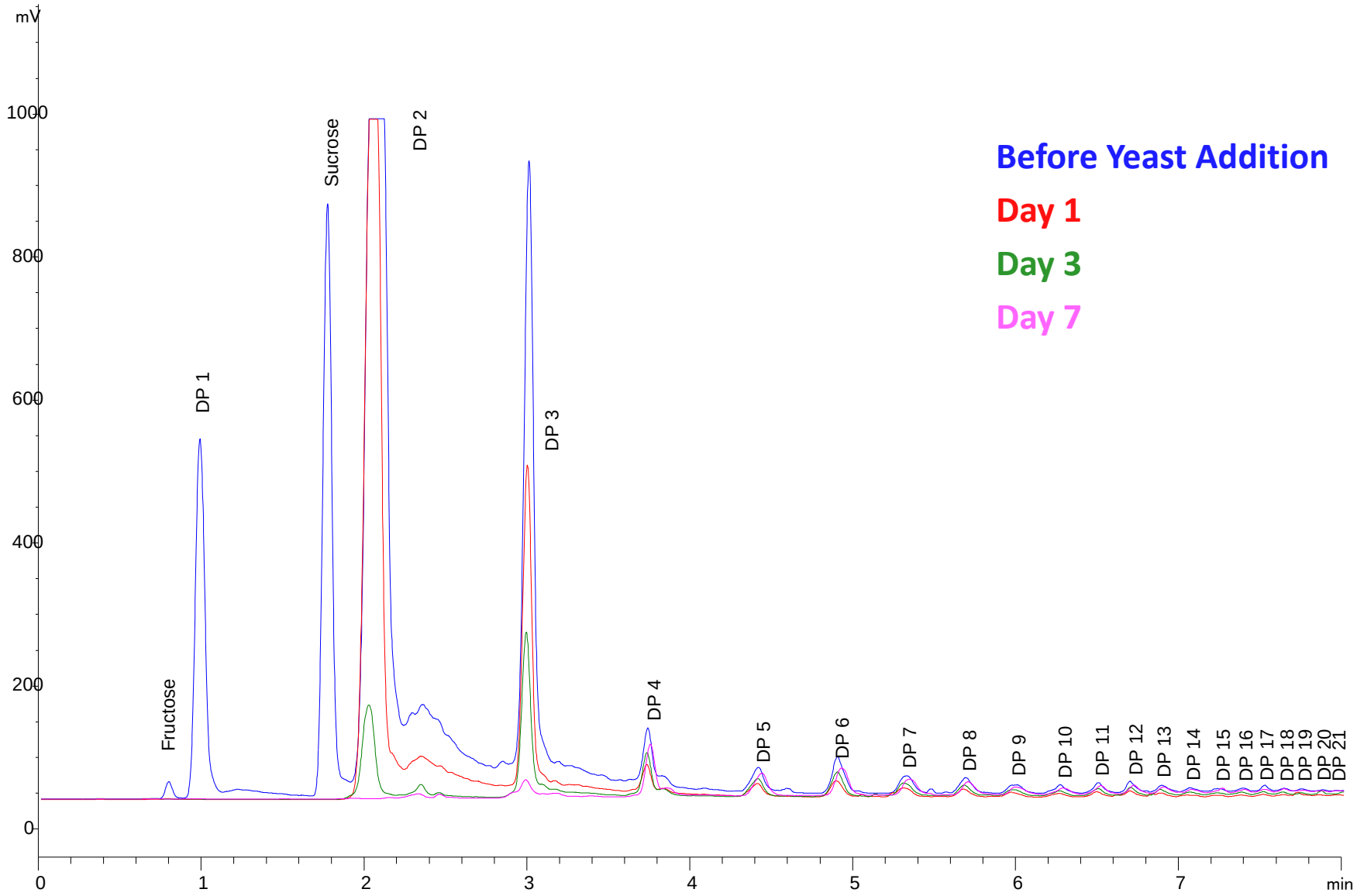
1A Ferm No Yeast
2A Ferm 30 min post yeast
3A Ferm first yeast activity
4A Ferm day 1
5A Ferm day 2
6A Ferm day 3
7A Ferm day 7
8A Ferm day 8
9A Ferm day 10 end

Fermentation Samples

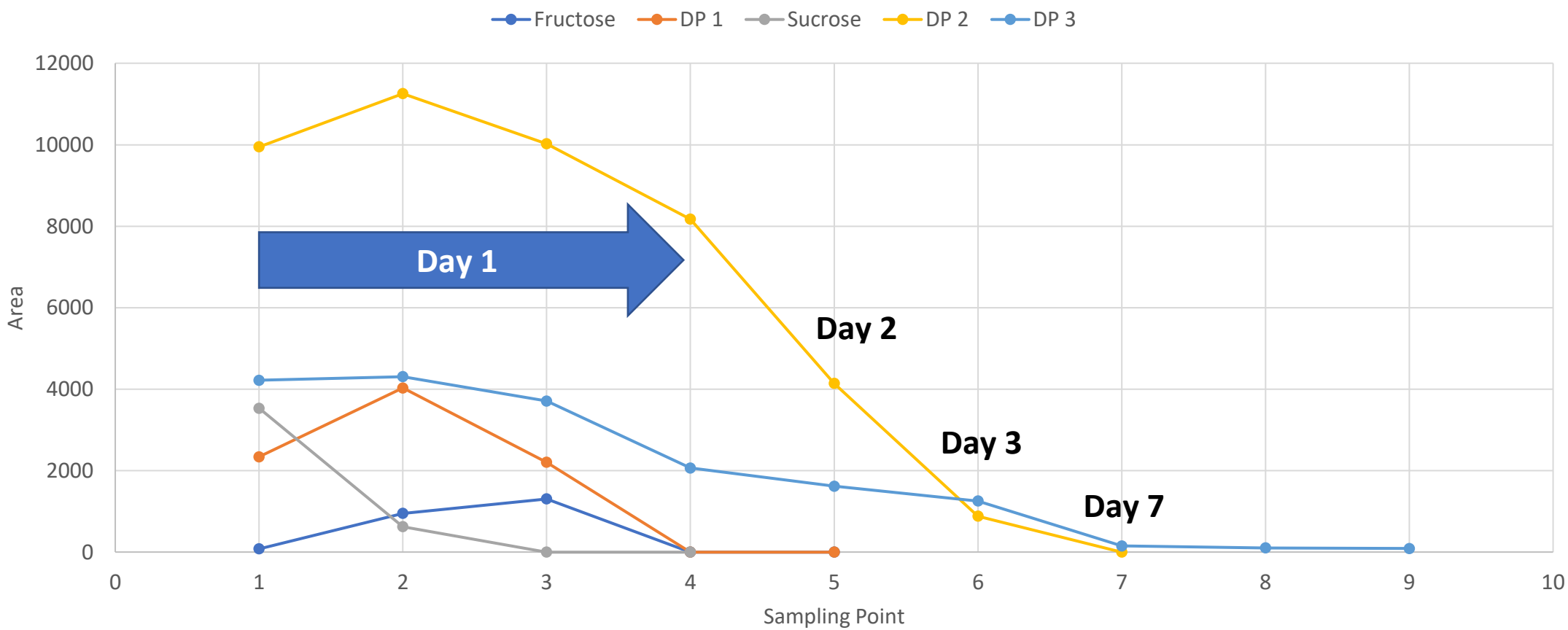
Belgian Ale

All samples diluted 1:10

Fermentation Summary – Belgian Ale



Fermentable Sugars During Fermentation

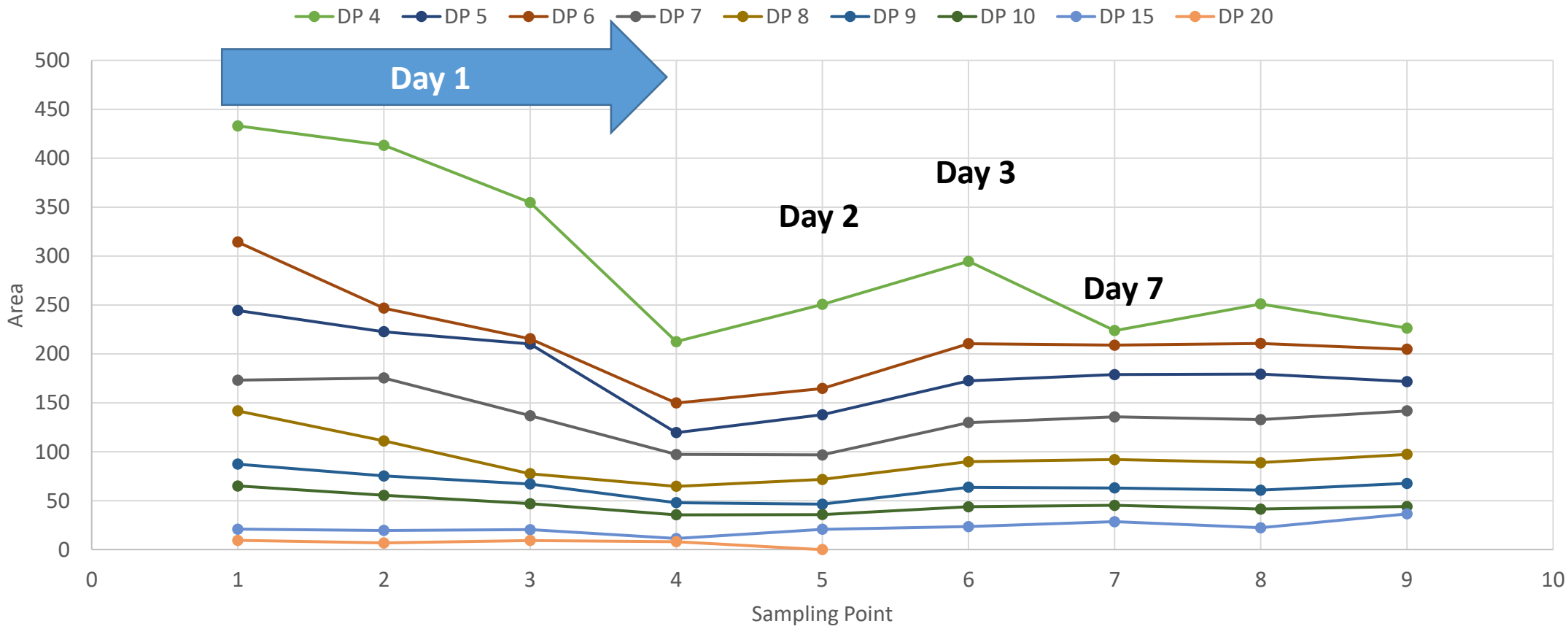


Maltose Concentration

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.

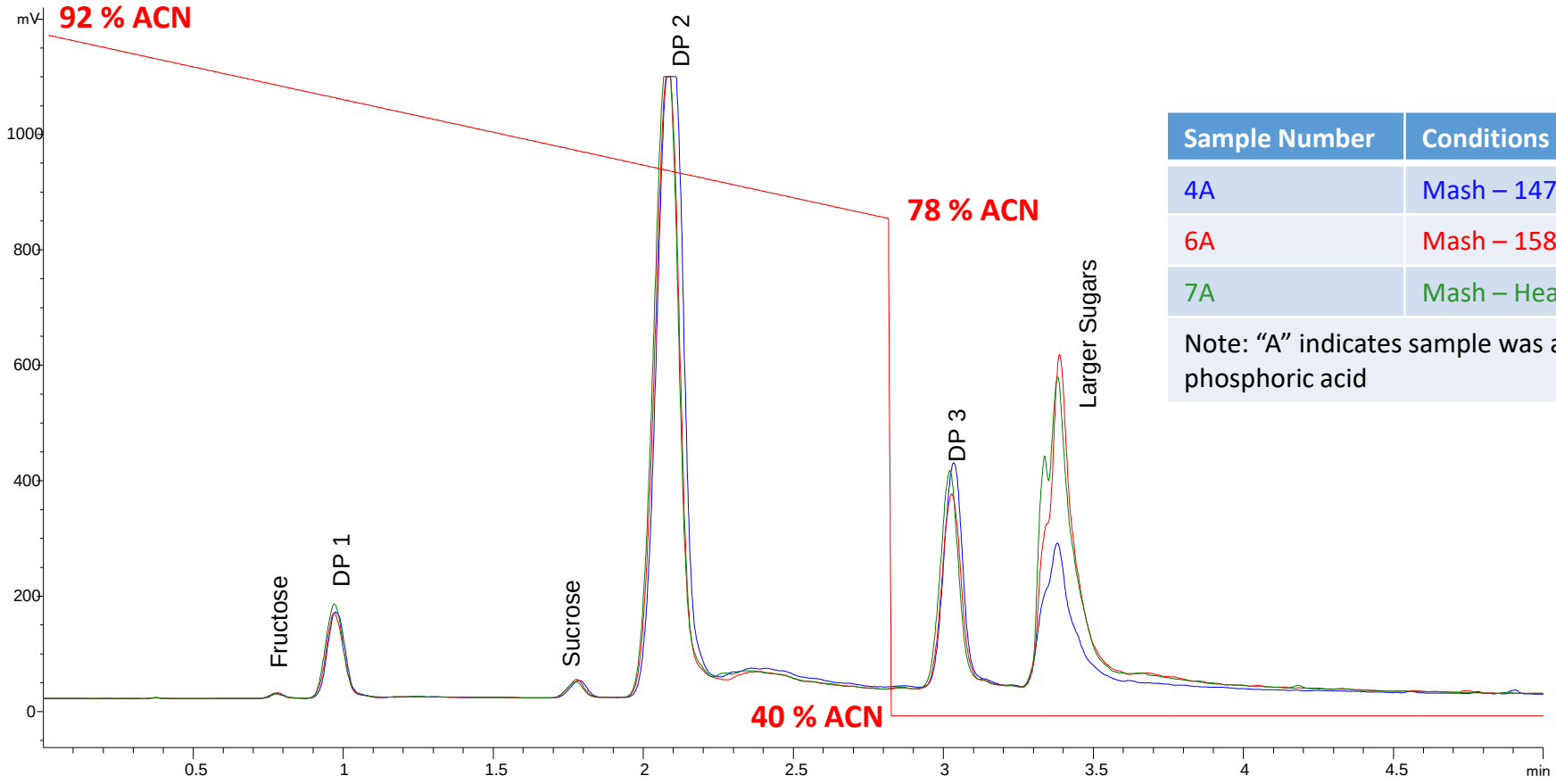
Large Sugars (Peak Area)



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.

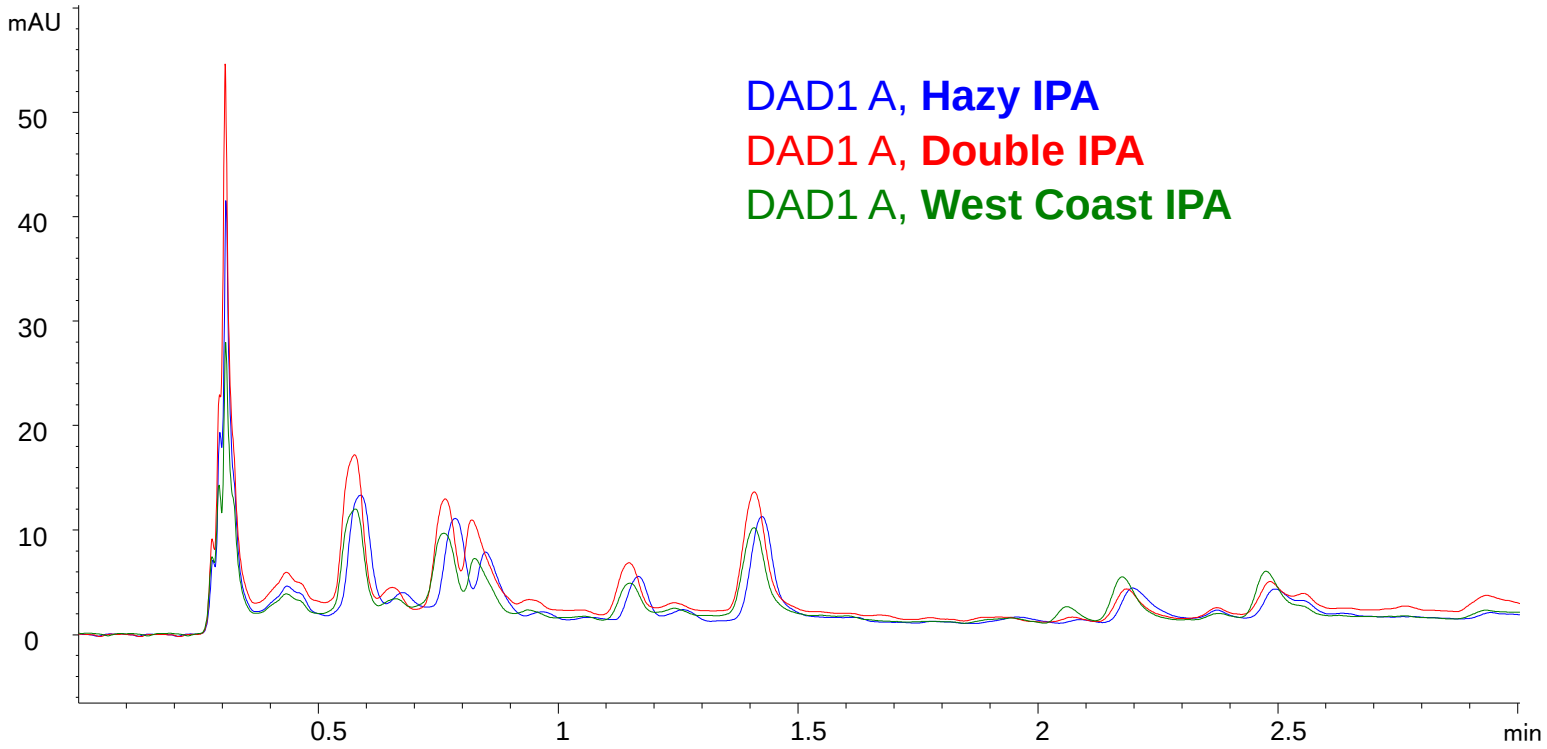


Sample Number	Conditions
4A	Mash – 147 °F / 45 minutes
6A	Mash – 158 °F / 15 minutes
7A	Mash – Heat to 180 °F / 0 minutes

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?

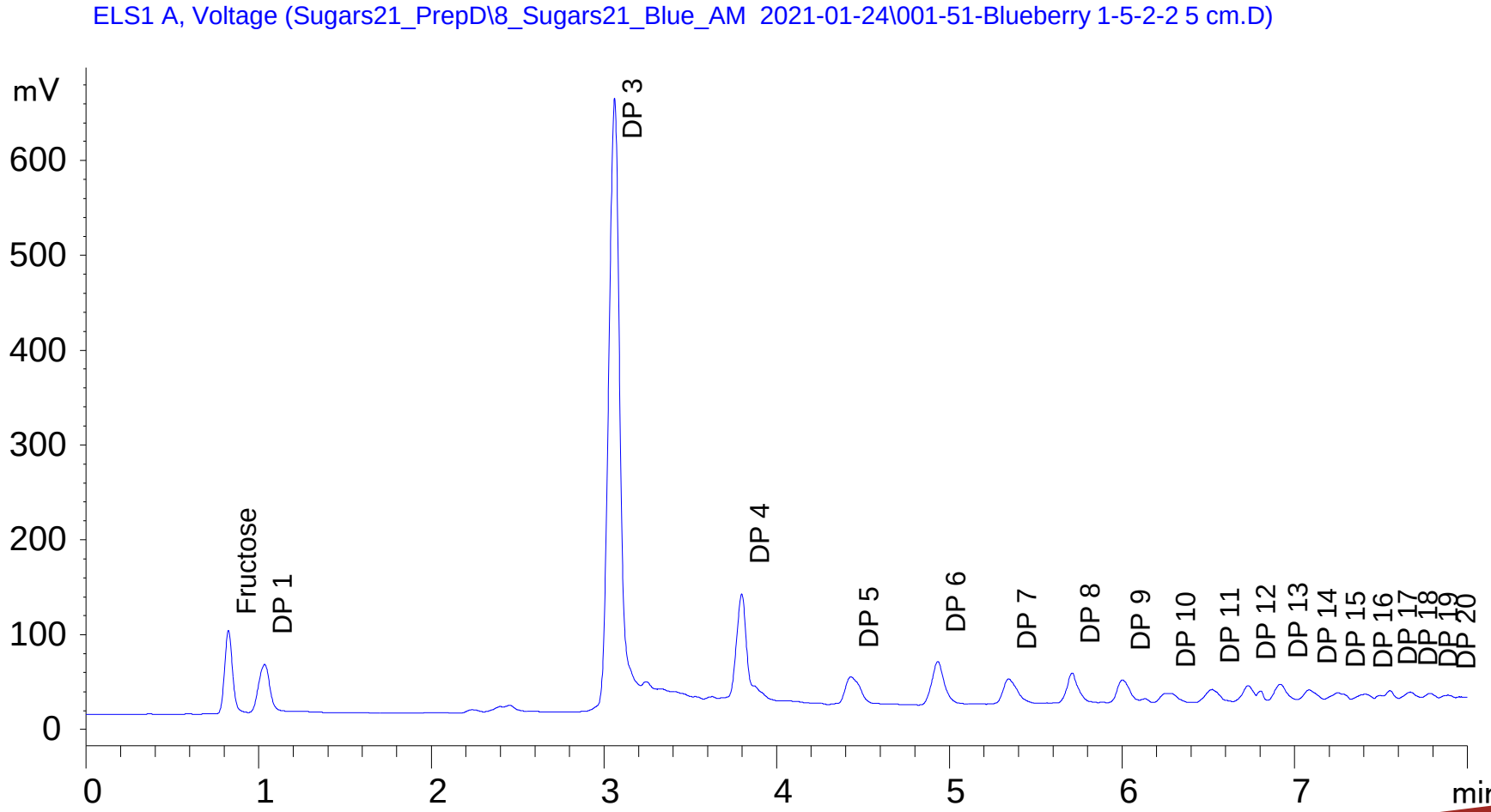


Finished Beer Samples

All Samples Diluted 1:10

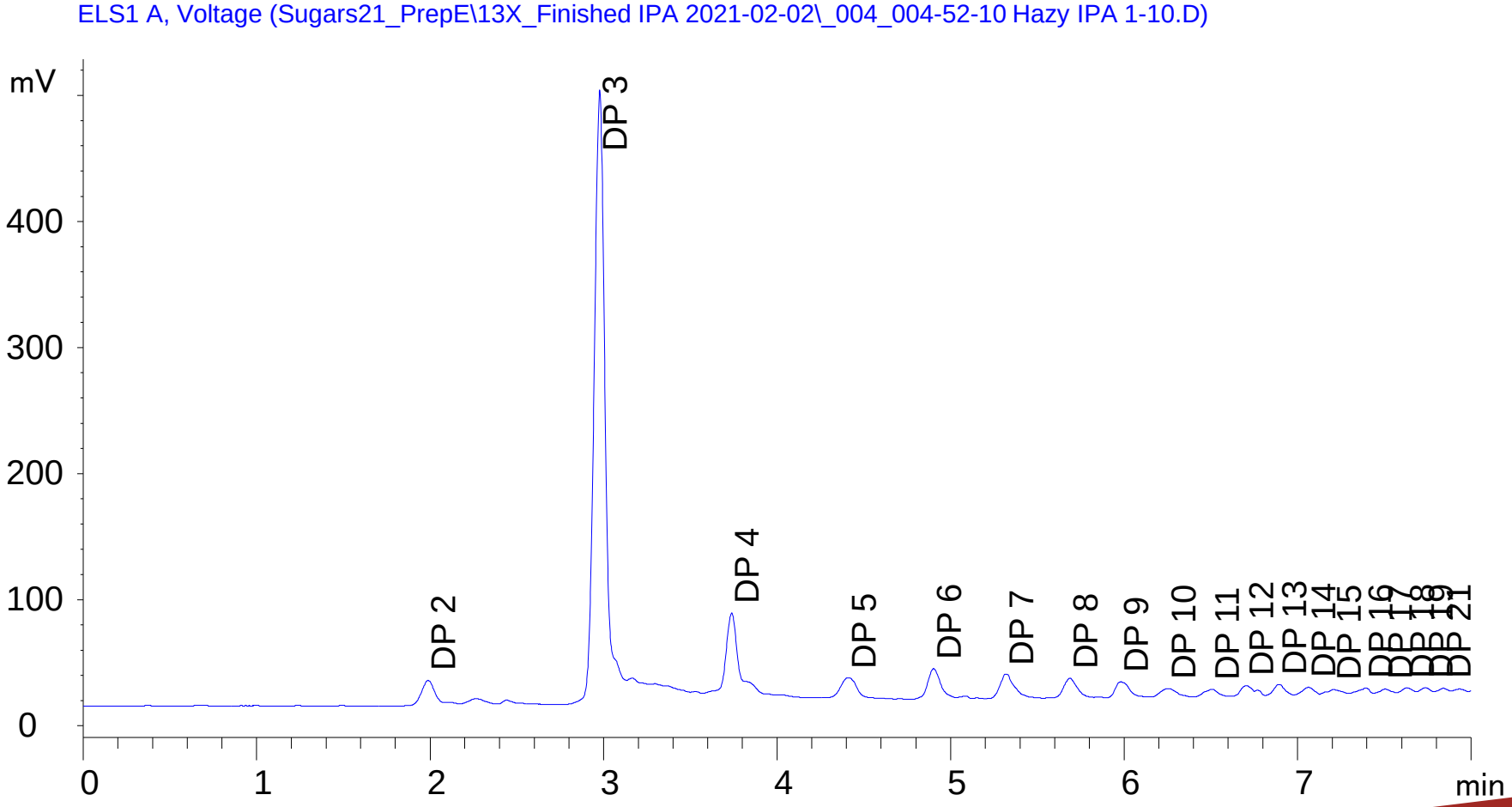
Blueberry Cream Ale

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



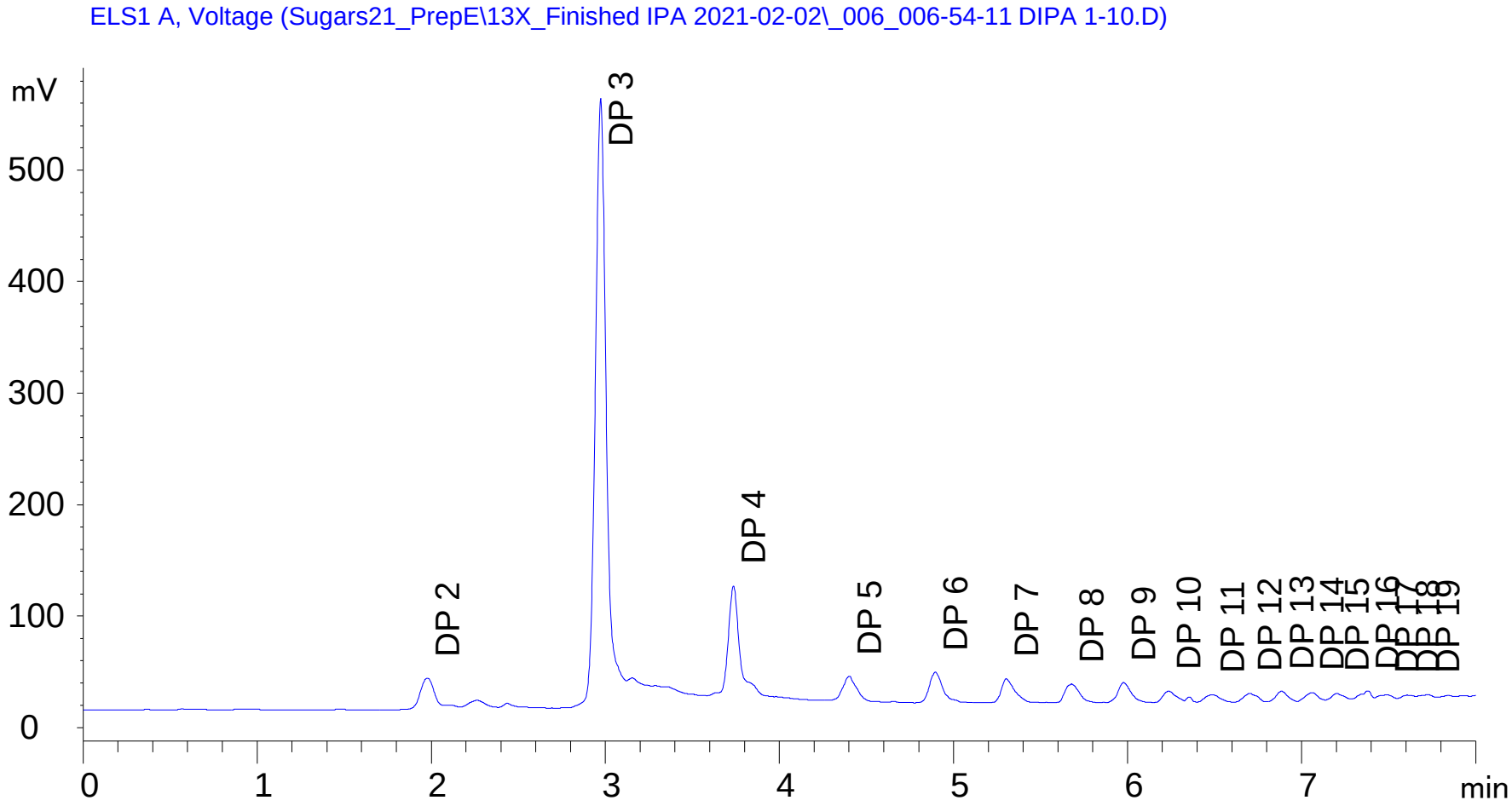
East Coast Hazy IPA

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



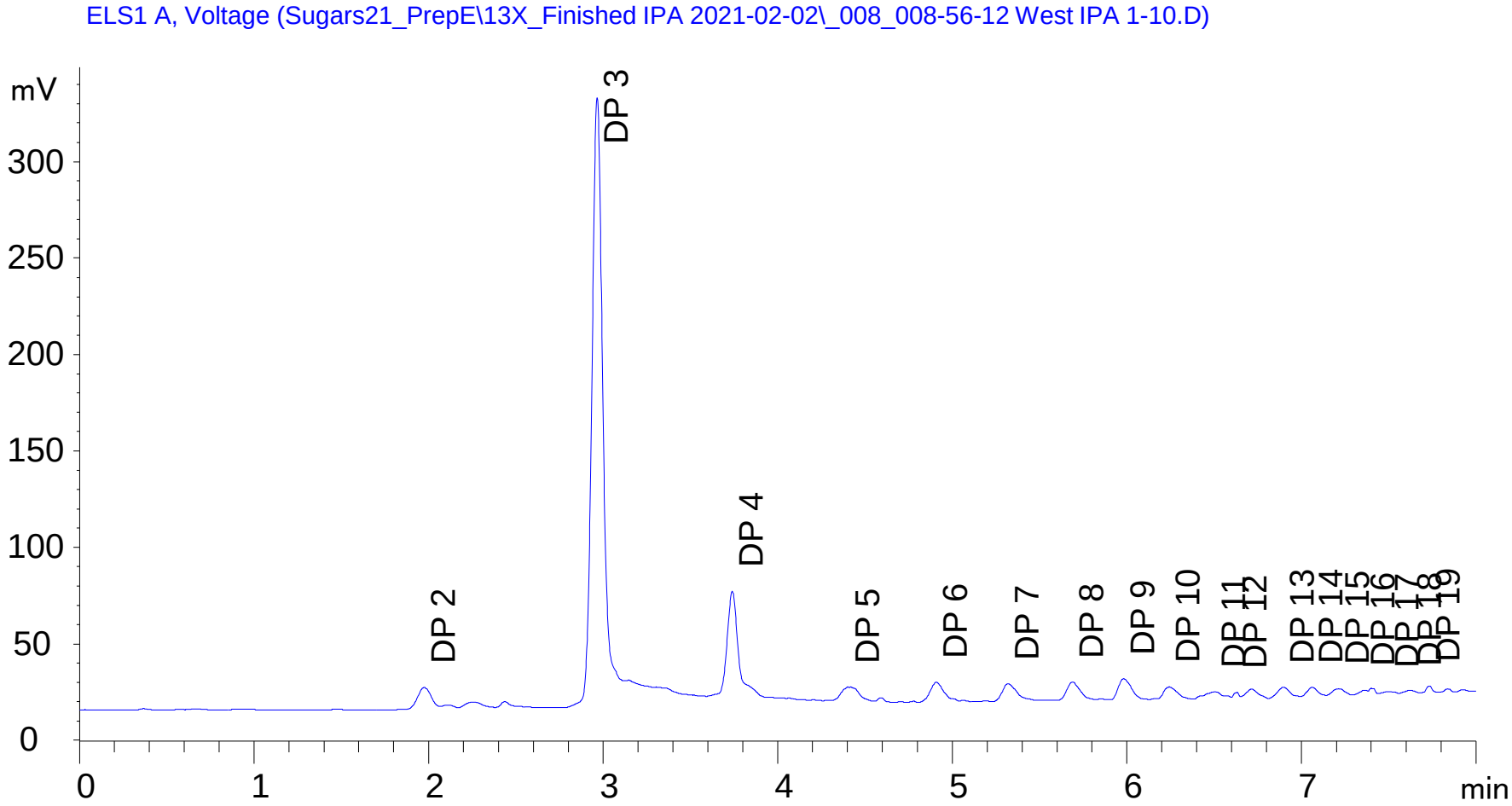
Double IPA

- 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.*



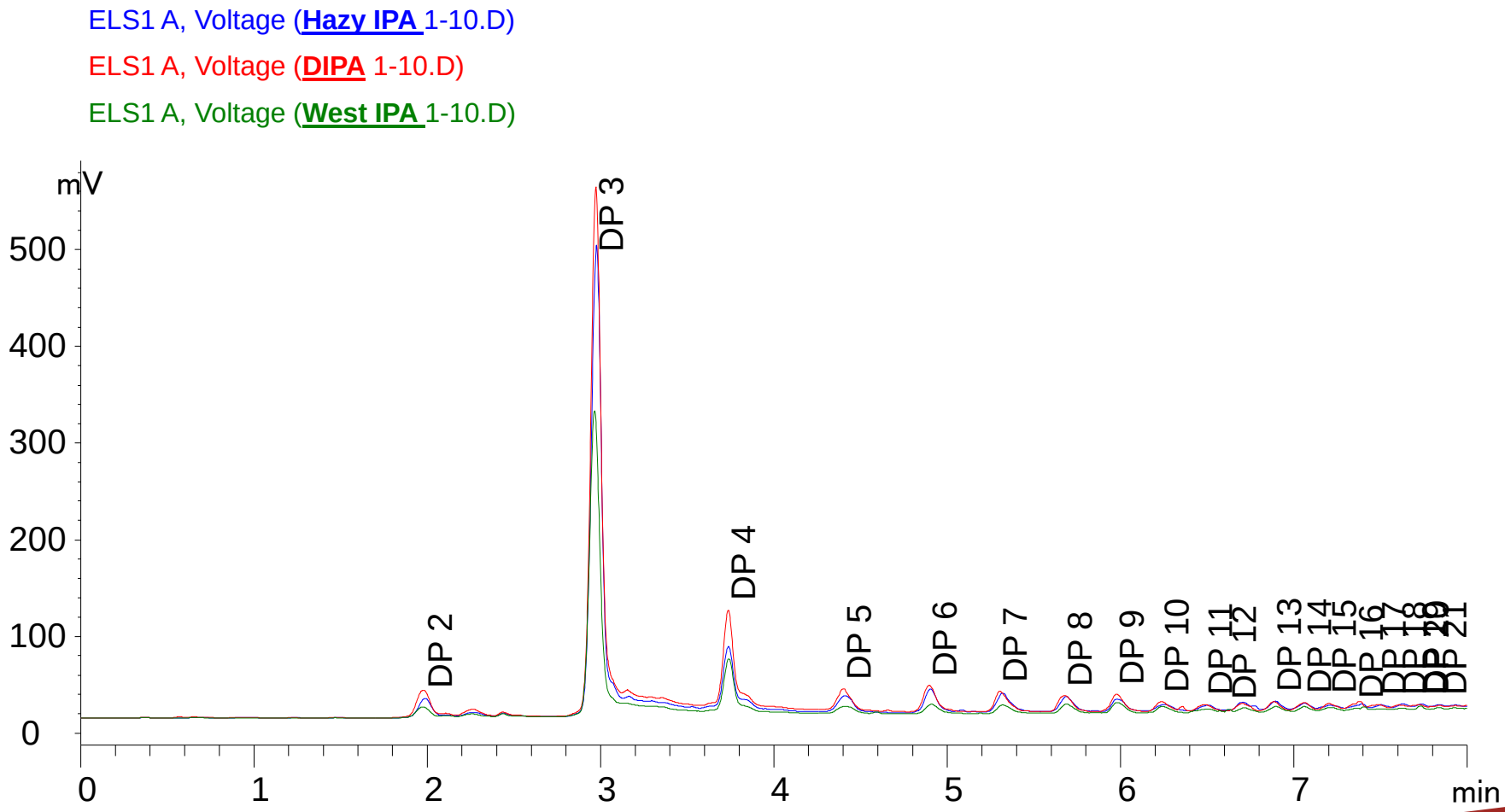
West Coast IPA

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



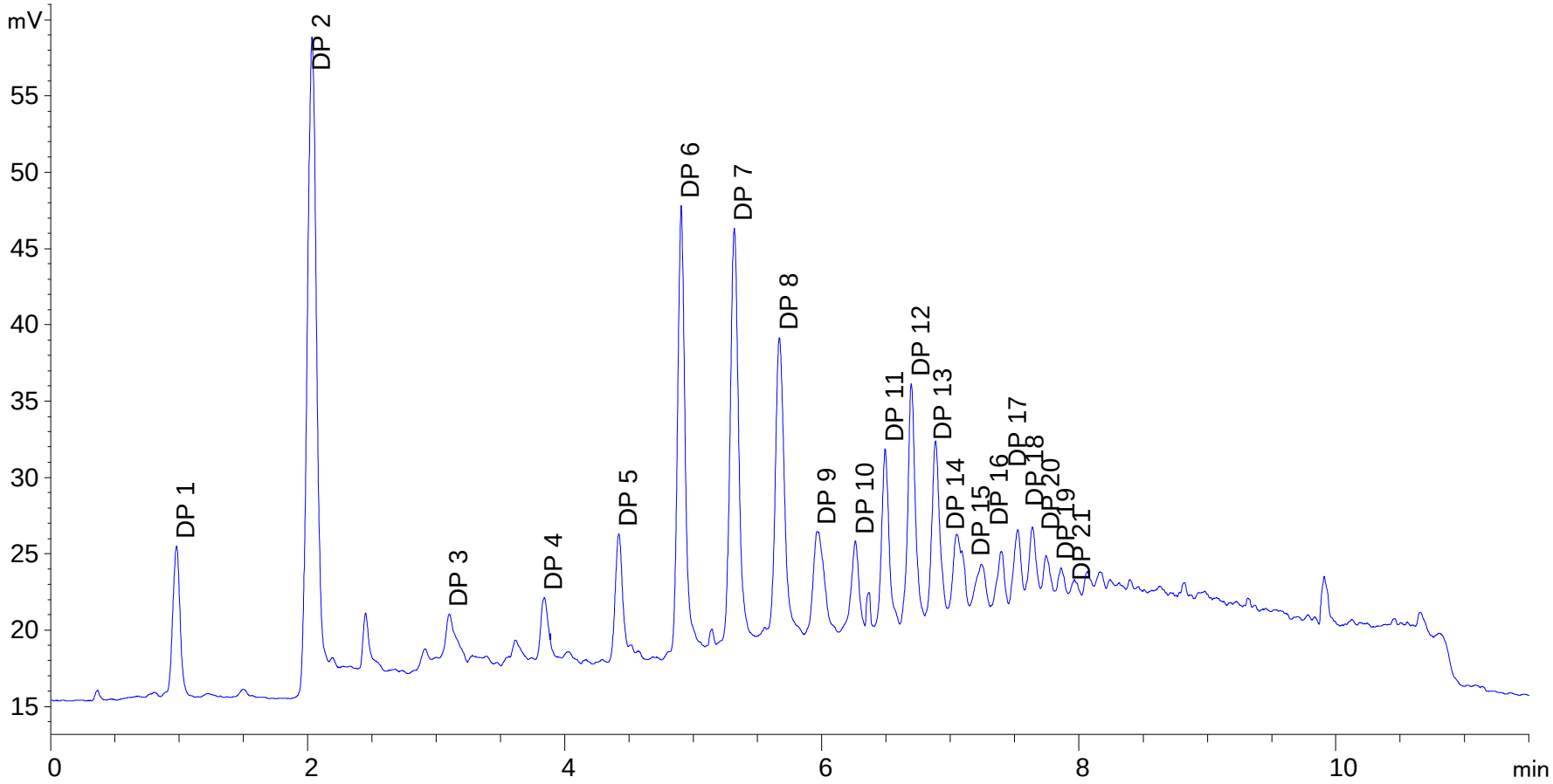
IPA Overlay

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



Lift Bridge Hop Dish IPA

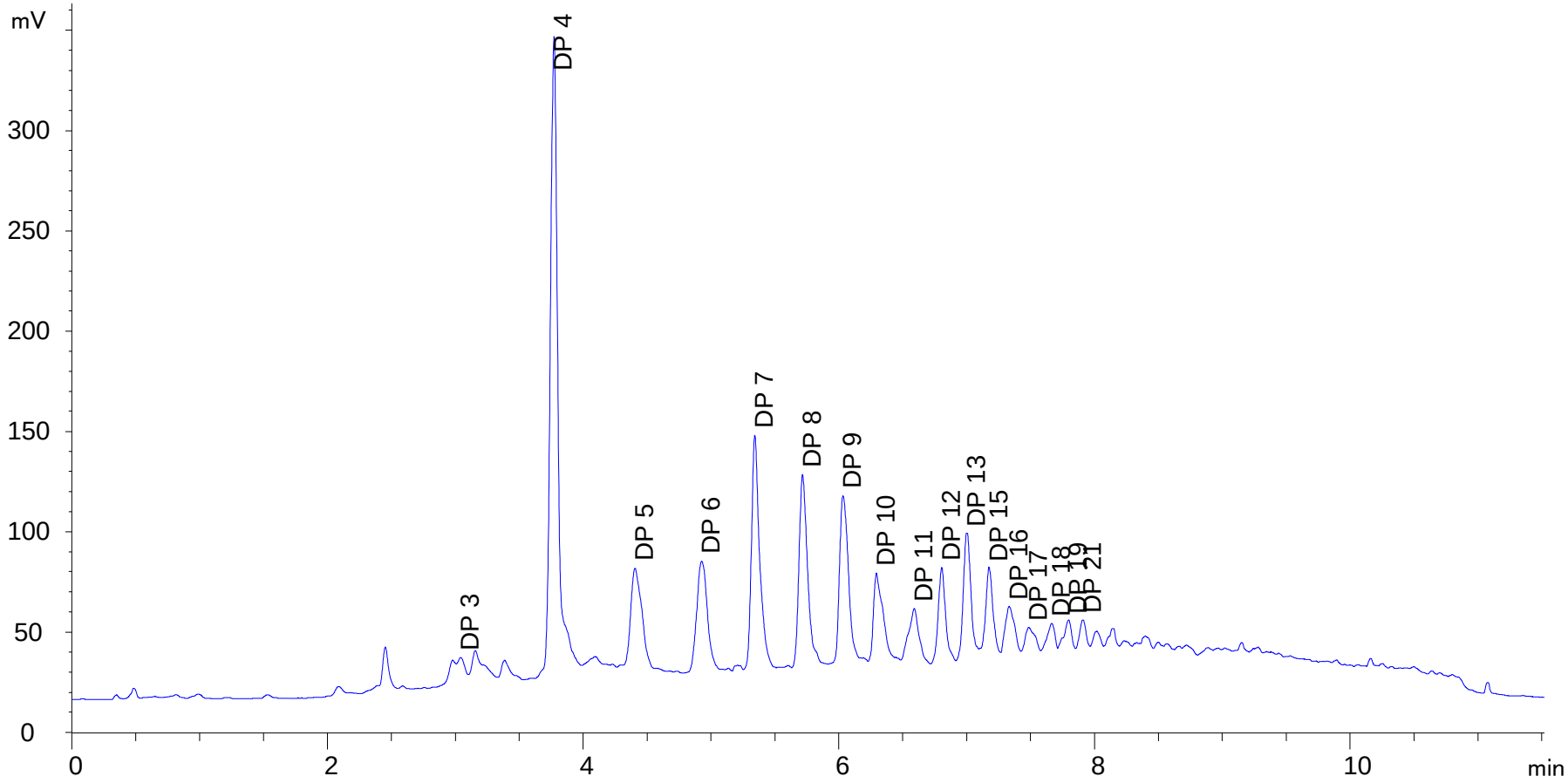
- ABV: 6.5% IBU: 75 Color: 13.5
- Aggressively hopped IPA with aromas of citrus, fruit, pine. A subtle malt sweetness with notes of caramel.



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 µL of H_3PO_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!

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

Stephanie Schuster, Ph. D.

Thomas J. Waeghe, Ph.D.

Michael Woodman

HALO®

TECHNICAL REPORT: AMT-TRFB0621-2

TITLE: FAST SCREENING OF OLIGO- AND POLY-SACCHARIDES IN BEER

MARKET SEGMENT: FOOD/BEVERAGE

AUTHOR:

Conner McHale, Technical Support Specialist

Data Courtesy of: Merlin K. L. Bicking, Ph. D., Senior Analytical Scientist (ACCTA, INC)

ABSTRACT

A fast screening of oligo- and poly- saccharides in beer is performed during various parts of the brewing process using evaporative light scattering detection. This includes samples throughout the mashing process, fermentation, and the finished product. Monitoring the sugar composition can signal the status of fermentation and aid in quality control. Hydrophilic interaction liquid chromatography (HILIC) mode was selected for the best resolution and speed using superficially porous particle technology (SPP). Analyzing beer sugar profiles using high pressure liquid chromatography can significantly help brewers with lot-to-lot repeatability, quality of their product, and troubleshooting techniques.

INTRODUCTION

Beer is one of the most widely consumed beverages in the world. This beverage is simply made up of water, malted grains (malted barley, wheat, corn, sorghum, rye), hops, and yeast. As simple as this may sound, beer has a very complex chemical composition and has a wide range of flavors. Monitoring yeast behavior, hop profiles, sugar composition, and water purity are just a few of the important components to make a delicious beer. Common instrumentation used to measure these components includes UV-Vis spectrophotometers, gas chromatographs, high pressure liquid chromatography (HPLC), and mass spectrometry. Using these instruments are not required, however, they can in aid quality assurance significantly improving repeatability, taste, and yields.

A fast screening of oligo- and poly-saccharides in beer is performed throughout various parts of the brewing process including the final product. This can help brewers better understand the sugar behaviors in their beer and to know when the fermentation process is complete.

EXPERIMENTAL

Fast screenings of oligo- and poly-saccharides in beer are performed using HPLC coupled with an evaporative light scattering detector. The evaporative light scattering detector (ELSD) is a type of LC detector. The detector is very useful when the compounds of interest do not have UV-Vis chromophores. This includes many types of compounds such as sugars, lipids, and polymers.

- How does it work?

After the eluent passes through the column, it reaches the ELSD. The eluent passes through the heated nebulizer, mixes with the nebulizer gas (nitrogen or air), and then forms an aerosol. Once nebulized, the eluent heads through a heated drift tube and the mobile phase

KEY WORDS:

oligosaccharides, polysaccharides, HILIC, superficially porous particles, beer, HPLC

evaporates. The solid particles travel through a flow cell containing a light source and a photomultiplier for detection. It is important to note that the ELSD can only detect compounds that are less volatile than the mobile phase used. A representation of a common ELSD detector can be seen in figure 1. The ELSD has the benefit of increased sensitivity and the ability to run in gradient mode compared to a refractive index detector. This gives an advantage when trying to choose the proper detection for sugar analysis. Hydrophilic interaction liquid interaction

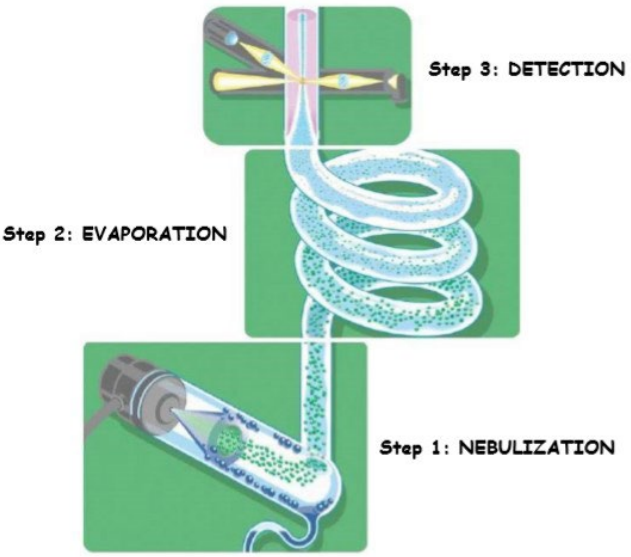


Figure 1: image from SEDEX Model 90LT ELSD manual

chromatography (HILIC) mode along with ion exchange are the preferred modes used for sugar analysis due to the polarity of the compounds. These columns can produce a wide variety of selectivity and retention due to their differences in the stationary phases. HILIC is an alternative HPLC mode primarily used to separate polar compounds. This is also known as aqueous normal phase liquid chromatography. The HALO 90 Å Penta-HILIC, 2.7 µm, 4.6 x 50mm column produces fast, high resolution results for oligo- and poly-saccharides in beer. This HILIC stationary phase works very well with these compounds due to the interactions with the -OH groups and utilizes superficially porous particle technology (SPP), allowing fast run times with high efficiency. The Penta-HILIC ligand can be seen in figure 2.

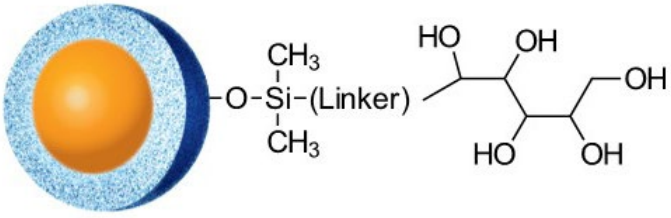


Figure 2: The HALO® Penta-HILIC ligand structure

There are some challenges when performing HPLC sugar analysis. This includes anomer splitting for certain compounds which will increase peak widths along with long retention times. Because of this a shorter column dimension is recommended along with a shorter column diameter in order to improve sensitivity and also reduce solvent consumption. Higher column oven temperatures (65˚C) have also shown a significant improvement in peak shapes compared to lower temperatures (35˚C). For example, figure 3 shows a separation of dextrose equivalent (DE) sugars which were ran at two different temperatures. Degree of polymerization (DP) increases as retention times increase. The 65 °C temperature significantly improves anomer splitting allowing for sharper peak shapes and faster retention times.

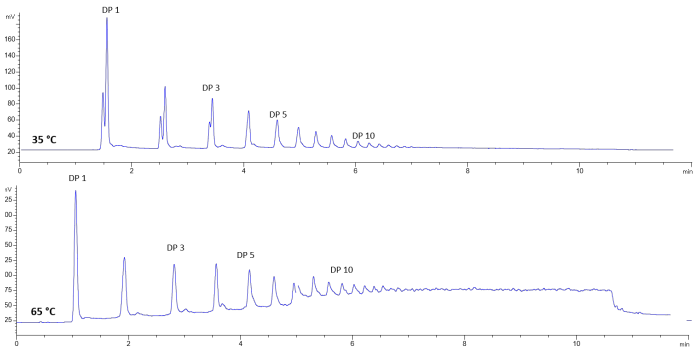


Figure 3: Dextrose equivalent sugars ran at two different temperatures to avoid anomer peak splitting. DP indicates the degree of polymerization (e.g., the number of glucose units).

TEST CONDITIONS
Column: HALO 90 Å Penta-HILIC, 2.7 µm, 3.0 x 50 mm
Part Number: 92813-405
Mobile Phase A: Water
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.)

An Agilent 1290 HPLC with diode array detection (DAD) and evaporative light scattering detector (ELSD) were used with HPLC grade acetonitrile (B) and deionized (DI) water (A). Two gradients were used for analysis: 92-42 %B in 10 minutes and 92-54 %B in 8 minutes. A flow rate of 0.75 mL/min with a column oven temperature at 65 ˚C using a 2 µL injection was implemented for all runs. The ELSD used a 10 Hz data rate, 2 sec filter, 40 ˚C, at 45 psi.

SAMPLE PREPARATION

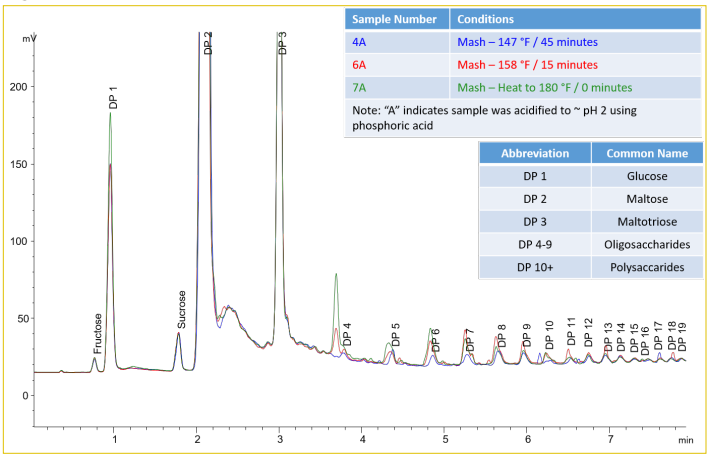
Beer samples were collected through collaboration with 3rd Act Craft Brewery (Woodbury, MN). Samples were collected throughout various parts of the brewing process and stored cold. Samples were adjusted to pH 2 with phosphoric acid in order to increase stability. Samples were then centrifuged

and the supernatant was removed. With HPLC it is best practice to remove any particulates in the sample in order to avoid plugging and contamination. Because of this, samples were then filtered through a 0.2 µm filter.

Dilution with water and 40% organic solvent (1:1 acetonitrile: methanol) was found to give the best results in terms of sensitivity. A 1:25 dilution was made with mashed samples, 1:10 or 1:5 for fermented samples, and 1:10 or1:5 or undiluted for finished product samples.

RESULTS:

Several mash samples were analyzed throughout the mashing process. This process is a pre-fermentation step that involves combining a mixture of grains and steeping them in water for a period of time at elevated temperatures, similar to making a cup of tea. Mashing allows the enzymes in the malt to break down the starch in the grain into sugars, typically maltose, to create a malty liquid called wort.1 The chromatographic overlay can be seen in figure 4. The first sample (4A) is collected during the initial mash process at 147˚F, second sample (6A) at 158˚F mid-mash, and 180˚F at the end of the process. (7A) Sugar concentrations will increase overtime as demonstrated in figure 4.



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

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

Figure 4: Mashing process of malted grains monitored

Once the mash process is complete, fermentation takes place, which is when yeast reacts with the sugars converting them to ethanol. Fructose and sucrose were added to the beer before fermentation took place to aid with fermentation. After the first day of fermentation, sucrose is completely converted to carbon dioxide and ethanol while maltose and other fermentable sugars are also decreasing in intensities. This can be seen in figure 5. A plot of the fermentable sugars is seen in figure 6, showing decreasing concentrations over time.

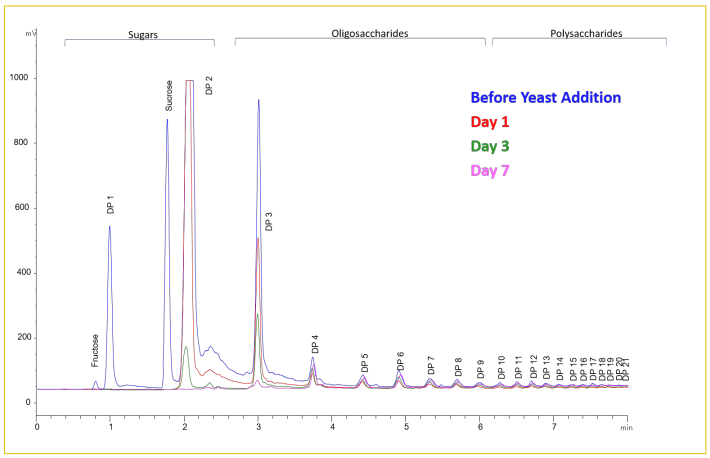


Figure 5: Oligo-and poly-saccharides are monitored throughout the beer fermentation process

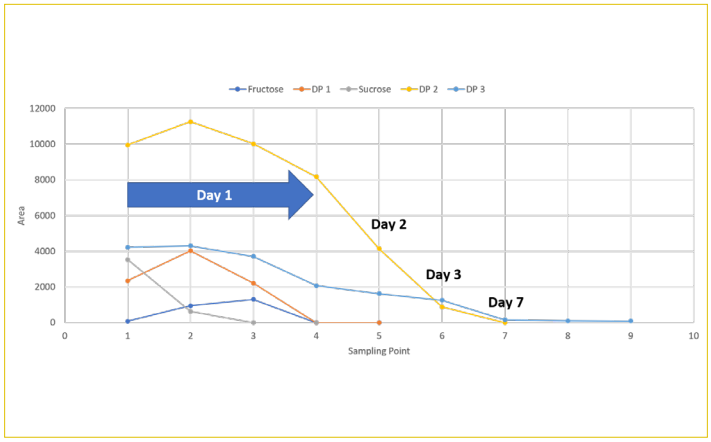


Figure 6: A plot of fermentable sugars decreasing over time during the beer fermentation process

After fermentation the beer is bottled, canned, or kegged and is then ready for consumption. Monitoring the oligo- and poly-saccharides in the finished product can give brewers information of flavor profiling and signs of batch-to-batch repeatability. The completeness of the fermentation process is easily evaluated with this technique, allowing the brewer to determine if they have used up all the available fermentable sugars. In the example below, a blueberry cream ale is analyzed in figure 7 (see next page). Fructose is present from the real blueberries added to the beer after fermentation.

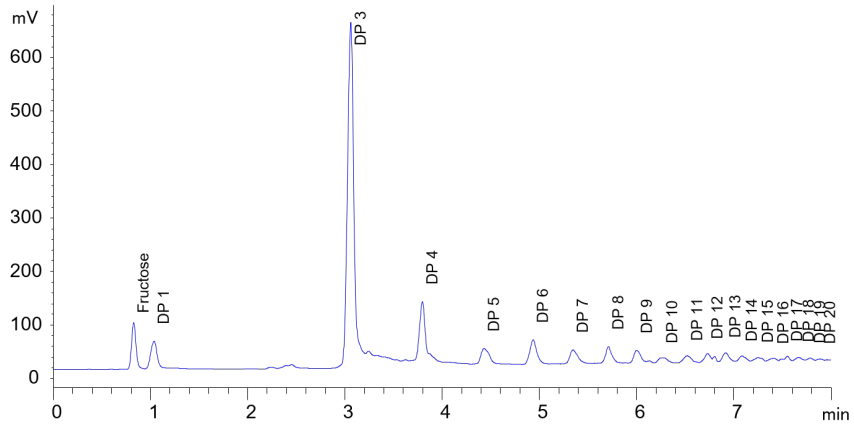


Figure 7: Analysis of blueberry cream ale from 3rd Act Brewery. Note that all the maltose (DP 2) has been fermented. Only small amounts of glucose remain.

CONCLUSION

A fast screening of oligo- and poly- saccharides in beer is performed with a HALO® Penta-HILIC column paired with an ELSD. The SPP particle technology along with the columns HILIC properties allows for fast and efficient separations. Screening these compounds provides information on fermentable sugars within the beer making process and can be very useful for the brewer. Cold sample storage at pH 2 preserves both mash and fermentation samples for later analysis. After several different sample preparation techniques, dilution in an aqueous-organic mixture provided the best results for sensitivity.

ACKNOWLEDGEMENTS:

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2. 3rd Act Craft Brewery (Woodbury, MN)

REFERENCES:

1. Audrey Ensminger (1994). Foods and Nutrition Encyclopedia. CRC Press. p. 188. ISBN 978-0-8493-8980-1.